potravinárstvo®

Vol. 18 2024

Slovak Journal of Food Sciences

ISSN 1337-0960 (online)

Scientific peer-reviewed OPEN ACCESS journal focussed on food science

potravinarstvo.com

Potravinarstvo Slovak Journal of Food Sciences
Vol. 18, 2024

potravinárstvo®

Slovak Journal of Food Sciences

• Journal title: ***Potravinarstvo Slovak Journal of Food Sciences*** • Volume: 18, Year 2024 • Evidence no. of the Ministry of Culture of Slovak Republic: 3771/09 • ISSN 1337-0960 (electronic version, online) • Publisher: HACCP Consulting, Slivková 12, 951 01 Nitrianske Hrnčiarovce, Slovakia, European Union • Publisher cooperates with SLP London, the Scientific Literature Publisher, 3rd Floor, 86-90 Paul Street, London EC2A 4NE, United Kingdom • The article XML conversion is provided by the contractual partner JatsTech, Rok Society Plot # R-278 near Jamali road Sch 33, Karachi, Pakistan • Editor-in-Chief: doc. Ing. Peter Zajác, PhD. • Periodicity: Articles are published continuously in the Volume during the year • Form of the publishing: electronic online • Journal language: English • Journal website: www.potravinarstvo.com • Address of Editor office: Potravinarstvo Slovak Journal of Food Sciences, Slivková 12, 951 01 Nitrianske Hrnčiarovce, Slovakia, European Union • E-mail: editor@potravinarstvo.com • Tel.: +421908164361, +421904138562 • Linguistic editing: Published articles undergone language editing by English proof-reader, authors are fully responsible for grammar in the articles • Graphic design: HACCP Consulting • Price: not for sale • Distributor: HACCP Consulting at www.potravinarstvo.com • Place of publication: Nitra, Slovakia, European Union • Legal information and authors rights: authors and advertisers are legally responsible for information published in articles or advertisement. Potravinarstvo Slovak Journal of Food Sciences, Editor and Editorial Board are not responsible for any third-party parties' damages or claims for compensation arising out of or in connection with published articles or advertisements in Potravinarstvo Slovak Journal of Food Sciences or journal website www.potravinarstvo.com • Journal is indexed in: SCOPUS, CrossRef, DOAJ Directory of Open Access Journals, EBSCO Host, CAB Abstracts and CABI Full text, FSTA – The Food Science Resource, UIUC OAI registry, AGRIS/FAO, DRJI Directory of Research Journals Indexing, Google Scholar, OCLC The OAIster database, INDEX Copernicus, UlrichsWeb Global Serials Directory, MENDELEY, EZB Electronic Journals • Name and abbreviation of the journal used for indexation in international databases: ***Potravinarstvo Slovak Journal of Food Sciences*** (***Potr. S. J. F. Sci.***). Former Slovak journal title: Potravinarstvo®.

HACCP CONSULTING

SLP London

Scope of the Potravinarstvo Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences is an open access scientific journal intended for the food industry and scientists working in this field. Articles published in the journal are peer-reviewed by two reviewers and available free of charge online on the journal website. Journal covers areas including food hygiene, food safety, food quality, food microbiology, food laws and regulations, ingredients and ingredient functionality, nutraceuticals, product formulation, sensory science and sensory statistical analysis, process control and its contribution to food processing operations, food chemistry, food toxicology, food engineering, food technology and biotechnology, nourishment, public health, primary production of food, food adulteration, food economics and marketing, the environmental effect on food and food machinery.

Open Access

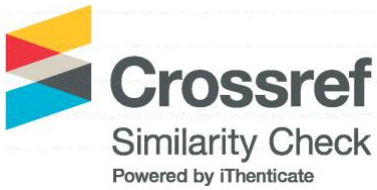
We are providing free online access to all published articles in our journal. You are free to:

- Share — copy and redistribute the material in any medium or format.
- Adapt — remix, transform, and build upon the material for any purpose, even commercially.
- The licensor cannot revoke these freedoms as long as you follow the license terms.

If you decide to use articles published in our journal you have to:

- Make statement (citation), that article was published first time in our journal according to the example presented below in the copyright.

https://creativecommons.org/licenses/by/4.0/



Plagiarism Policy

Potravinarstvo Slovak Journal of Food Sciences does not allow any form of plagiarism. Plagiarism is considered to be a serious breach of scientific ethics by the Journal. We are using Corssref Similarity Check Powered by iThenticate to protect authors rights.

Journal Metrics

Powered by Scopus

SJR 2020: 0.259 SJR 2021: 0.228 SJR 2022: 0.278 SJR 2023: 0.232

CiteScore 2020: 1.6 CiteScore 2021: 1.9 CiteScore 2022: 2.5 CiteScore 2023: 2.5

prof. MVDr. Jozef Nagy, PhD.
University of Veterinary Medicine in Košice, Slovakia

doc. Ing. Jolana Karovičová, CSc.
The Slovak Technical University in Bratislava, Slovakia

prof. Ing. Róbert Toman, Dr.
The Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Slovakia

prof. Dr. Teresa Fortuna, DSc.
Agricaltural University of Cracow, Poland

prof. Tadeusz Trziszka, Ph.D., DSc.
University of Wroclav, Poland

Ing. Roman Labuda, PhD.
Institute of Agrobiotechnology, Tuln, Austria

Asso. Prof. (Dr.) Anil Kumar, PhD.
Delhi Technolocal

Dr. Oksana Belous
State Research Institute of Xisco, Russia

Ana Carla Marques Pinheiro, Ds.
UFLA Lavras, Brazil

prof. Gábor Géczi
Szent István University, Hungary

MVDr. Ing. Václav Trojan, PhD.
Mendel University in Brno, Czech Republic

prof. Dr. Peter Raspor, Dr.h.c.mult., Uni. dipl. eng.
University of Primorska, Slovenia

M.Sc Mohammad Ali Shariati
Orel State Agrarian university, Russia

prof. M.Sc. Salam, A., Ibrahim, PhD.
North Carolina A&T State University, Greensboro, NC, USA

prof. Dr. Julijana Tomovska
University “St. Kliment Ohridski” – Bitola, North Macedonia

Ing. MVDr. Ján Buleca, PhD.
Technical University in Košice, Slovakia

Pharm Dr. Maciej Bilek, PhD.
University of Rzeszow, Faculty of Biology and Agriculture, Department of Food and Agriculture Production Engineering, Poland

doc. Ing. Radovan Kasarda, PhD.
Slovak University of Agriculture in Nitra, Faculty Agrobiology and Food Resources, Slovakia

prof. Ing. Ľudmila Nagyová, PhD.
Slovak University of Agriculture in Nitra, Faculty of Economics and Management, Slovakia

Prof. Ing. Vladislav Sukhenko, Dr.Sc.
National University of Life and Environmental Sciences of Ukraine, Ukraine

Tatevik Avetisyan, PhD.
Michigan State University, Department of Community Sustainability, USA

List of Reviewers, 2024:

Name	Country
Abdula Elbashir	Sudan
Achinna Poshadri	India
Adegbola Dauda	Nigeria
Ahmad Zubir Ibrahim	Malaysia
Aigul Kozhakhmetova	Kazakhstan
Aigul Tayeva	Kazakhstan
Ainur Kharzhau	Kazakhstan
Akeem Raji	Nigeria
Alexander Fehér	Slovakia
Alizadeh Behrooz	Iran
Alona Shuliar	Ukraine
Amelia Knight	USA
Ammar B. Altemimi	Iraq
Anar Daniyarova	Kazakhstan
Anatolii Novikov	Ukraine
Andrey Bratsikhin	Russia
Andriy Kalunka	Ukraine
Anna Cierniak-Emerych	Poland
Antonella Pasqualone	Italy
B. S. Khatkar	India
Baiyi Lu	China
Belle Christoffers	Argentina
Chang Kong	China
Daniel Granato	Finland
Dmytro Kucher	Ukraine
Dwiyati Pujimulyani	Indonesia
Elfi Anis Saati	Indonesia
Eunok Choe	Korea
Fridrikh Sholcz	Germany
Galia Zamaratskaia	Sweden
Gregoria S, Suhartati Djarkasi	Indonesia
Halina Tkaczenko	Poland
Hao Zhang	China
Herianus J. D. Lalel	Indonesia
Hülya GÜL	Turkey
Irwandi Jaswir	Malaysia
Jagadish Chandra Joardar	Bangladesh
Ján Buleca	Slovakia
Jan Twardone	Poland
Jana Tkáčová	Slovakia

Name	Country
Jaroslav Jedlička	Slovakia
Jordi Rovira	Spain
Jozef Čapla	Slovakia
Jozef Čurlej	Slovakia
Jozef Golian	Slovakia
Khaswar Syamsu	Indonesia
Kulbaram Bayazitova	Kazakhstan
Lengel Svitlana	Hungary
Libor Kalhotka	Czechia
Liviu Gaceu	Romania
Ľubomír Lopašovský	Slovakia
Lucas Cook	USA
Lucia Benešová	Slovakia
Małgorzata Kus-Liskiewicz	Poland
Mária Angelovičová	Slovakia
Marija Zheplinska	Ukraine
Marina Aleksandrovna Vajtanis	Russia
Maroš Jakab	Ireland
Marta Laranjo	Portugalo
Maryam Mizani	Iran
Maryna Kryvtsova	Ukraine
Maryna Mardar	Ukraine
Mihaiela Cornea-Cipcigan	Romania
Miroslav Jovanovic	Switzerland
Mohammad Jafar	Iran
Muktharudin Muchsiri	Indonesia
Nataliia Grynevych	Ukraine
Natasa Poklar Ulrih	Slovenia
Nikolaj Zaharez	Ukraine
Nor Samsinar Kamsi	Malaysia
Nora Denissova	Latvia
Nurul Huda	Malaysia
Oday Hasan Ali AL-Jammaas	Iraq
Oksana Shkromada	Ukraine
Orazbayev Batyr Bidaibekovich	Kazakhstan
Patrícia Martišová	Slovakia
Peter Timmer	USA
Peter Zajác	Slovakia
Petr Rzevskii	Poland
Rauan Zholmyrzayeva	Kazakhstan

Name	Country
Roland Kozdrowski	Poland
Roman Khajsetovich Kandrokov	Russia
Rose Ferreira	Finland
Rui Costa	Portugal
Saltanat Suimbayeva	Kazakhstan
Saverio Mannino	Italy
Şenol KÖSE	Turkey
Sergey Shahov	Russia
Sri Agustini	Indonesia
Stepan Tcimbaluk	Ukraine
Tetiana Sylchuk	Ukraine
Tetyana Kalna-Dubinyuk	USA
Usman Pato	Indonesia
Vesna Đorđević	Serbia
Vladimir Ivanov	USA
Vladimír Vietoris	Slovakia
Yundo Li	China
Yuriy Skliarenko	Ukraine
Zhienbaeva Saule Turganovna	Kazakhstan

Potravinarstvo Slovak Journal of Food Sciences, Vol. 18, 2024

TABLE OF CONTENT

Articles doi links:

1.	1-19	https://doi.org/10.5219/1909
2.	20-35	https://doi.org/10.5219/1925
3.	36-49	https://doi.org/10.5219/1950
4.	50-65	https://doi.org/10.5219/1938
5.	66-76	https://doi.org/10.5219/1941
6.	77-96	https://doi.org/10.5219/1947
7.	97-108	https://doi.org/10.5219/1945
8.	109-121	https://doi.org/10.5219/1898
9.	122-138	https://doi.org/10.5219/1933
10.	139-156	https://doi.org/10.5219/1935
11.	157-173	https://doi.org/10.5219/1948
12.	174-184	https://doi.org/10.5219/1934
13.	185-206	https://doi.org/10.5219/1949
14.	207-222	https://doi.org/10.5219/1930
15.	223-238	https://doi.org/10.5219/1937
16.	239-250	https://doi.org/10.5219/1953
17.	251-267	https://doi.org/10.5219/1951
18.	268-280	https://doi.org/10.5219/1944
19.	281-296	https://doi.org/10.5219/1956
20.	297-312	https://doi.org/10.5219/1959
21.	313-330	https://doi.org/10.5219/1960
22.	331-346	https://doi.org/10.5219/1957
23.	347-365	https://doi.org/10.5219/1962
24.	366-385	https://doi.org/10.5219/1961
25.	386-397	https://doi.org/10.5219/1946
26.	398-407	https://doi.org/10.5219/1983

27.	408-424	https://doi.org/10.5219/1939
28.	425-438	https://doi.org/10.5219/1952
29.	439-452	https://doi.org/10.5219/1954
30.	453-467	https://doi.org/10.5219/1958
31.	468-479	https://doi.org/10.5219/1967
32.	480-496	https://doi.org/10.5219/1965
33.	497-511	https://doi.org/10.5219/1991
34.	512-522	https://doi.org/10.5219/1970
35.	523-546	https://doi.org/10.5219/1972
36.	547-569	https://doi.org/10.5219/1963
37.	570-587	https://doi.org/10.5219/1978
38.	588-604	https://doi.org/10.5219/1971
39.	605-618	https://doi.org/10.5219/1987
40.	619-632	https://doi.org/10.5219/1989
41.	633-653	https://doi.org/10.5219/1942
42.	654-665	https://doi.org/10.5219/1955
43.	666-682	https://doi.org/10.5219/1981
44.	683-696	https://doi.org/10.5219/1976
45.	697-718	https://doi.org/10.5219/1998
46.	719-740	https://doi.org/10.5219/1995
47.	741-754	https://doi.org/10.5219/1992
48.	755-775	https://doi.org/10.5219/1997
49.	776-791	https://doi.org/10.5219/1985
50.	792-806	https://doi.org/10.5219/1964
51.	807-818	https://doi.org/10.5219/2000
52.	819-833	https://doi.org/10.5219/2004
53.	834-846	https://doi.org/10.5219/2003

We support science and research.

www.haccp.sk

potravinárstvo®
OPEN ACCESS
Received: 13.8.2023
Revised: 19.10.2023
Accepted: 23.11.2023
Published: 5.1.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 1-19
https://doi.org/10.5219/1909
ISSN: 1337-0960 online
www.potravinarstvo.com

Effects of milk type, pasteurization, and in-container heating on Nabulsi cheese yield, chemical composition, and texture

Khaled Abu-Alruz

ABSTRACT

Nabulsi cheese is a white-brined cheese made from different types of milk using different processing methods. This research aimed to investigate the effects of the following three factors on the quality of Nabulsi cheese: milk type (cow's, goat's, and sheep's milk), milk pasteurization (unpasteurized vs. pasteurized), cheese pasteurization (traditional boiling vs. in-container heating), and their two- and three-way interactions. The effect of the first two factors on the cheese yield and those of all three factors on the chemical composition and texture profile analysis (TPA) of cheese were investigated. The type of milk significantly affected all parameters tested. Similarly, milk pasteurization affected all parameters evaluated except ash content and hardness, while cheese pasteurization affected moisture content and cohesiveness. The two-way interaction between the type of milk and milk pasteurization affected the cheese yield, chemical composition (except for protein, fat, and ash contents), and TPA. The three-way interaction significantly affected the chewiness of cheese. The type of milk had the highest effect on cheese yield, fat and ash contents, and chewiness. In comparison, the interaction between the kind of milk and milk pasteurization had the highest effect on cheese hardness. In contrast, milk pasteurization had the highest effect on the remaining tested parameters.

Keywords: white-brined cheese, Nabulsi cheese, chemical composition, yield, texture profile analysis

INTRODUCTION

White-brined Nabulsi cheese is one of the most important cheeses produced in Jordan and surrounding countries **[1]**. It is classified as a semi-hard cheese made without starter cultures **[2]**. Nabulsi cheese is usually processed from sheep's and goat's milk due to the absence of carotenoid pigments in its fat; these types of milk produce white cheese, an important attribute as its name implies **[3]**. Since Nabulsi cheese is traditionally made from sheep's milk, its production peaks in spring. Due to its seasonality and the increased demand for cheese, other types of milk, including cow's milk, are usually used **[4]**. Unpasteurized milk is used in the traditional procedure of making Nabulsi cheese **[5]**, **[6]**, **[4]**, **[7]**. The resulting cheese is subjected to heat treatment by boiling it in a brine solution (>18%), and after that, the cheese and brine are allowed to cool separately. Large enamelled metal containers of variable sizes (1-5 kg) are then filled with cheese and brine **[8]**. Nabulsi cheese is intended to have a shelf life longer than a year **[2]**. There are few scientific studies on the impact of various milk types on the quality of white-brined Nabulsi cheese.

The cheese deteriorates in quality during storage **[1]**, **[9]**, **[10]**, particularly in texture **[4]**. Deterioration manifests as surface slime, discoloration, off-flavor, and gas formation. The traditional method of Nabulsi cheese production needs to improve the following three areas: the use of unpasteurized milk in cheese processing, boiling cheese before packing, and cheese storage in large metal containers. A large proportion of the Nabulsi cheese production is small-scale or family business near the location where the milk is available. Due to the difficulty in controlling the microbial load in milk – which may impair the cheese boiling step's effectiveness – and the increased demand for large quantities of milk to produce larger amounts of cheese, milk pasteurization is

increasingly deemed necessary **[4]**. After boiling the cheese in a brine solution, there is a risk of cross-contamination from different sources, such as from unboiled brine, the surrounding environment, and cheese handlers **[11]**. Using large metal containers for storage also exposes the cheese to cross-contamination from repeated opening and closing during usage **[12]**, **[8]**, **[2]**. Several studies investigated the use of in-container heating of Nabulsi cheese using small containers as an alternative to the traditional boiling step **[5]**, **[13]**, **[2]**. On the commercial scale, some producers started to produce Nabulsi cheese with in-container heating in small containers.

Milk pasteurization and in-container heating are expected to be industrial standards in the production of Nabulsi cheese, in addition to the increased need to use different types of milk to meet the growing demand for this type of cheese. To the best of our knowledge, little information is available on the use of different kinds of milk, milk pasteurization, and in-container heating on the quality of Nabulsi cheese. Therefore, this research aimed to investigate the effects of these factors on the yield, chemical composition, and texture profile analysis (TPA) of the Nabulsi cheese.

Scientific Hypothesis

The three investigated factors (type of milk, milk pasteurization, and cheese pasteurization) and their two- and three-way interactions are expected to significantly affect the quality of Nabulsi cheese. Knowing the factors with the most significant effects will be of great importance in optimizing the process of Nabulsi cheese processing.

MATERIAL AND METHODOLOGY

Samples

Samples of cheese were prepared using the three types of milk (cow's, goat's, and sheep's milk) from the same batch. The experiment was repeated two times. Samples of milk were tested immediately for chemical analysis. For chemical analysis of cheese, cheese was tested in its salted form immediately after processing. For texture analysis of cheese, cheese was desalted first by soaking it in tap water overnight under refrigeration.

Animals, Plants, and Biological Materials

Cow's milk (moisture: 88.40%; dry matter: 11.6%; protein: 3.85%; fat: 3.3%; ash: 0.73%; specific gravity: 1.028; pH: 6.3), goat's milk (moisture: 87.94%; dry matter: 12.6%; protein: 4.02%; fat: 3.7%; ash: 0.85%; specific gravity: 1.026; pH: 6.4), and sheep's milk (moisture: 83.14%; dry matter: 16.86%; protein: 5.96%; fat: 5.00%; ash: 0.89%; specific gravity: 1.032; pH: 6.4) were obtained from a dairy plant (Faculty of Agriculture, Al-Karak, Jordan), and cheese coagulant (Rennimax 2100 Granula, Spain).

Instruments

The following equipment was used: texture analyzer (6700 TVT; Perten, Sweden), Kjeldahl (VAP 450; Gergardet, Germany), Gerber (Funke Gerber, Denmark), Oven (TR 240; Nabertherm, Germany), muffle furnace (Nat 30/65; Nabertherm, Germany), and pH meter (S400; Mettler Toledo, Switzerland).

Laboratory Methods

Chemical milk analysis: Association of Official Analytical Chemists (AOAC) methods were used to determine moisture, protein, fat, and ash percentages **[14]**. The following methods were used: Kjeldahl for protein, the Gerber method for fat, the oven method for moisture, and the muffle furnace for ash. The pH was determined using a calibrated pH meter (S400; Mettler Toledo, Switzerland). Specific gravity was determined using a lactometer.

Chemical cheese analysis: AOAC methods **[14]** were used to determine the chemical composition of the cheese samples, which were assessed immediately after processing without desalting. The following methods were used: Kjeldahl for protein, the Gerber method for fat, the oven method for moisture, and the muffle furnace for ash. The pH was determined using a calibrated pH meter (S400; Mettler Toledo, Switzerland).

Cheese yield: Following the pressing and salting steps, the cheese yield was calculated using the following equations:

$$Yield_{After\ pressing} = \text{weight of cheese after pressing} / \text{weight of milk} \times 100$$

$$Yield_{After\ salting} = \text{weight of cheese after salting} / \text{weight of milk} \times 100$$

TPA cheese: A texture analyzer (6700 TVT; Perten, Sweden) was used to measure hardness, cohesiveness, chewiness, adhesiveness, and gumminess. A 20-kg load cell and cylindrical probe made of stainless steel (45 mm × 50 mm, height × diameter) were used. The instrument was programmed using the software Texcalc (Perten) with the following program: two compression cycles, a 5-mm starting distance from the sample, 50% compression, and 1 mm/s test speed. The software calculates the TPA parameters from the distance/time and force curve. The maximum force observed during the first compression cycle was hardness. Cohesiveness was calculated by dividing the area under the second compression cycle by the area of the first compression cycle. The distance recorded during a sample's second compression compared with the distance recorded during its first

compression was used to measure springiness. The hardness, cohesion, and springiness values were multiplied to determine chewiness. The adhesiveness area was measured when the probe was pushed away from the sample during the adhesive withdrawal phase.

Description of the Experiment

Sample preparation: Nabulsi cheese samples were produced according to the steps shown in Figure 1. The milk was divided into two portions; the first was pasteurized (72 °C, 5 mins), while the second was not. Both parts were tempered to about 35 °C, and 0.02% $CaCl_2$ was added only to the pasteurized milk. The coagulant was added per the manufacturer's recommendation and left for about 30 minutes to allow curd formation. The curd was divided into small cubes and left for 10 mins. It was then placed into cheesecloth, drained for 15 mins, and pressed under a heavy weight for 2 hrs. After that, the cheese block was cut into small pieces ($3 \times 3 \times 2$ cm^3) and sprinkled with salt.

The salted pieces were left overnight at room temperature (25 °C) to drain the maximum water. The salted cheese pieces were further divided into two parts. The first part was heat-treated by in-container heating; in this method, glass jars were filled with cheese pieces and a hot 18% brine solution. The jars were immediately closed, heated in a hot water bath until the cold spot inside the jars (the center of the jar) reached 85 °C, and then immediately cooled. The jars were stored at room temperature. The second salted cheese part was heat-treated using the traditional method; in this method, the cheese pieces were boiled in an 18% brine solution until the center of the cheese pieces reached 85 °C. Then, the cheese pieces were removed from the brine solution, and both were allowed to cool separately. After cooling, glass containers were filled with the cheese pieces, and brine solution was added to cover them entirely. The glass containers were closed and stored at room temperature.

No special sample preparation was performed for the chemical analysis of milk, cheese, and cheese yield determination. For TPA, cheese samples were first desalted by soaking them in water overnight at a refrigerator temperature of 5 °C. A stainless steel cylindrical cutter cut the cheese samples with identical dimensions (15 mm × 20 mm, height × diameter). Four cheese samples from each treatment were tempered at room temperature before testing.

Number of samples analyzed: 24.

Number of repeated analyses: Four for cheese TPA and two for other tests.

Number of experiment replication: two.

Design of the experiment: Firstly, the experimental design was performed. For cheese's yield, two-way analysis of variance (ANOVA) was used to investigate the effect of the type of milk (cow's, goat's, and sheep's milk), milk pasteurization (raw versus pasteurized), and their interaction on the yield of Nabulsi cheese. For cheese's chemical analysis and TPA, three-way ANOVA was used to investigate the effects of the type of milk (cow's, goat's, and sheep's milk), milk pasteurization (raw versus pasteurized), and cheese heat treatment (traditional versus in-container heating), as well as their two-way and three-way interactions on the chemical composition and TPA of Nabulsi cheese. The whole experiment was repeated twice. Secondly, different types of milk were collected, and the cheese samples were prepared according to the steps described previously in the sample preparation section. Thirdly, cheese samples were analyzed for chemical composition and TPA. Fourthly, the data were statistically analyzed.

Statistical Analysis

The data were analyzed using Minitab 19 (Minitab Inc., USA). Backward elimination (alpha to remove = 0.05) minimizes the model. Means separation was performed using Tukey's test with $p \leq 0.05$ regarded as statistically significant. Furthermore, a residual normal probability plot was used to verify that the model satisfied the ANOVA assumptions.

RESULTS AND DISCUSSION

The two-way interaction results are presented if they are significant for yield data. If not, the results of the significant main effects are presented. When the three-way interaction effect is significant for chemical analysis results and TPA, it is presented below. When it is not, the significant results of two-way interactions are presented. Only the significant main effects are shown if no significant interactions are detected.

Yield: Both yield values (after pressing and after salting) were affected by the type of milk and milk pasteurization and the two-way interaction between them; therefore, only the results of the effects of the two-way interaction are presented. Of all the significant factors, the type of milk had the greatest standardized effect on yield values (Figure 2 and Figure 4). Figure 3 shows the significant differences between the yield after pressing for cheese made from the three milk types, whether pasteurized or raw. The highest yields were for sheep's milk

(29.95% and 26.73% for pasteurized and raw milk, respectively), followed by goat's milk (17.45% and 17.72% for pasteurized and raw milk, respectively), and the lowest values were for cow's milk (14.93% and 13.48% for pasteurized and raw milk, respectively) with significant differences between them. Toufeili and Ozer **[11]** reported comparable results for sheep's milk white cheese yield (26-28%) and goat's and cow's milk yield (15-16%). Tadjine et al. **[15]** divided the yield factors into milk quality (type of milk and protein's genetic variant) and the cheese-making process. Skeie **[16]** reviewed the factors affecting cheese yield. Milk pasteurization affected the yield depending on the type of milk used; it increased significantly in sheep's milk cheese by 12.05% and was not affected in cow's milk cheese and goat's milk cheese.

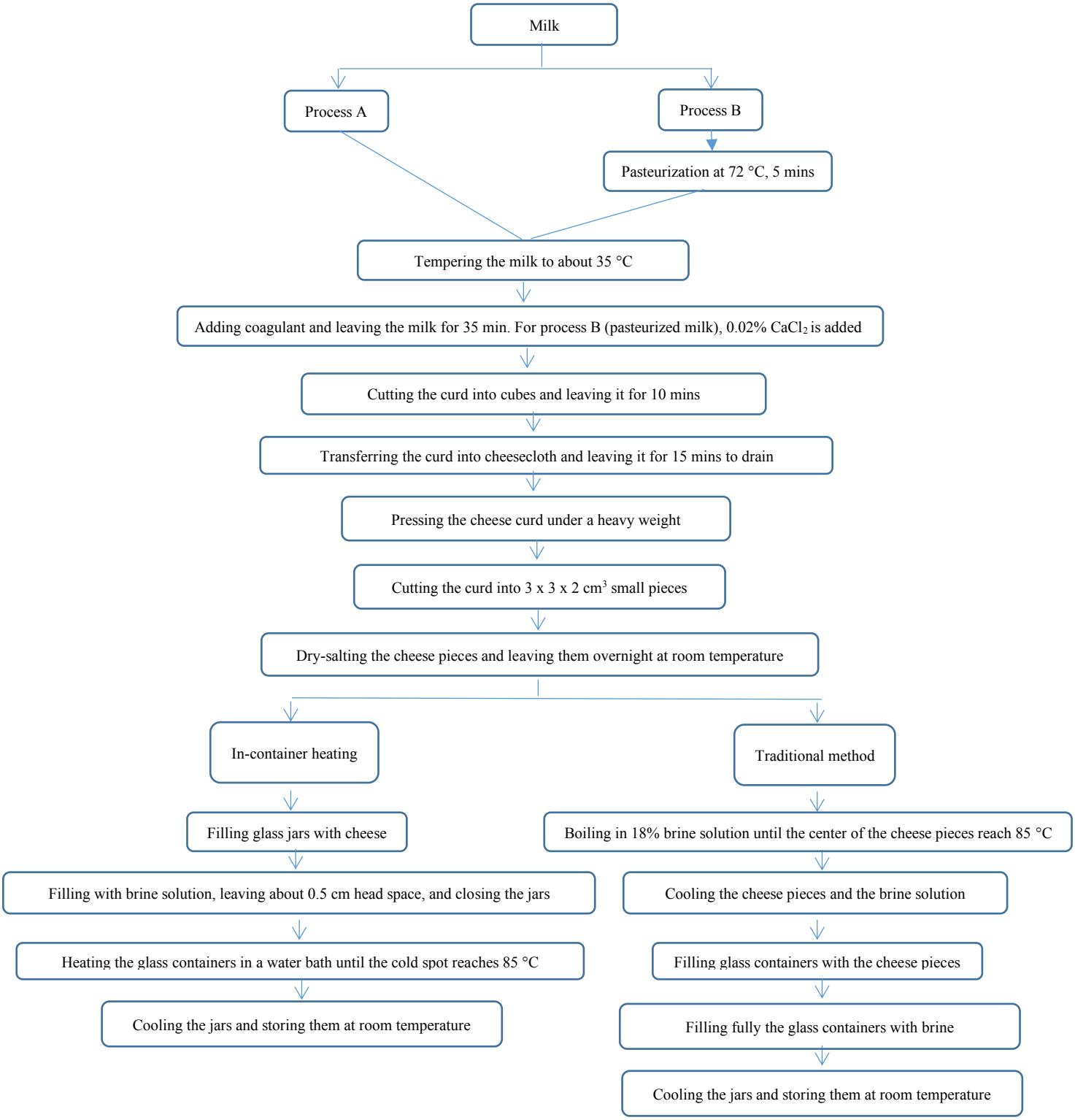


Figure 1 Flow diagram for Nabulsi cheese processing steps.

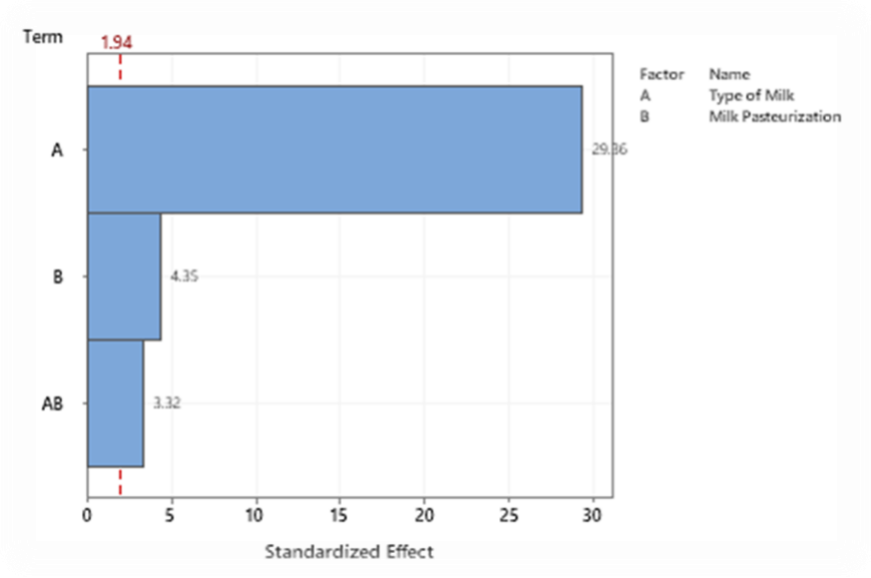


Figure 2 Standardized effects of different factors on the cheese yield after pressing.

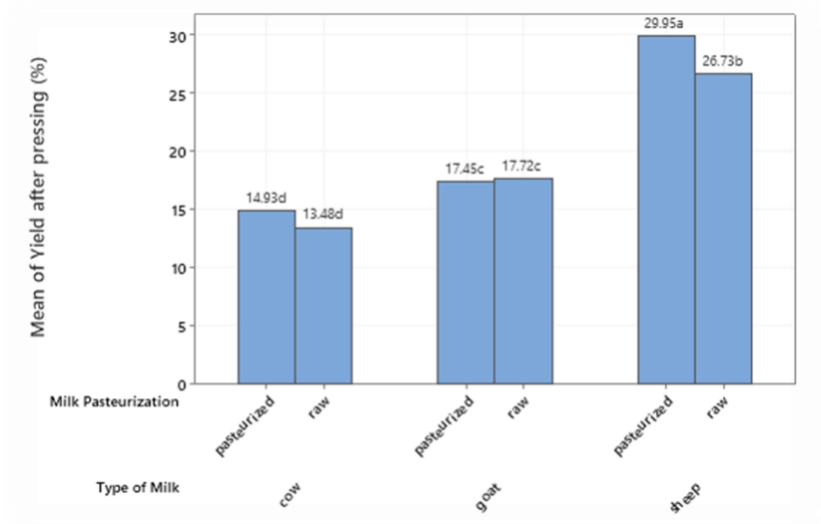


Figure 3 Effect of the two-way interaction between the type of milk and milk pasteurization on the cheese yield after pressing.

After-salting cheese yield values are presented in Figure 5. As expected, all yield values after salting were lower than after pressing due to the dry salting of the cheese pieces. The differences between different types of cheese follow almost the same pattern discussed before for the yield after pressing; the highest yields were for sheep's milk cheese, either using pasteurized milk (26.08%) or raw milk (21.85%), which were significantly higher than those for goat's milk cheese (13.70% and 12.82% using pasteurized and raw milk, respectively) and cow's milk (12.70% and 10.51% using pasteurized and raw milk, respectively). The effect of pasteurization on the cheese yield after salting depended on the type of milk used; it significantly increased in sheep's milk cheese by 19.36% and in cow's milk cheese by 20.84%, but it was not affected in goat's milk cheese.

Several studies reported the positive effect of milk pasteurization on cheese yield **[15]**, **[16]**, **[17]**, **[18]**, **[19]**, **[20]**, **[21]**, **[22]**, **[23]**. In contrast, Drake **[24]** reported no effect of milk pasteurization on cheese yield. These differences in the effect of pasteurization on cheese yield could be related to the quality of the milk, pasteurization treatment, and cheese processing method **[15]**. In our results, the effect of milk pasteurization on the percentage of yield increase after salting for cow's milk cheese was slightly higher than that for sheep's milk cheese. No agreement exists between studies on the type of milk that provides the highest yield upon pasteurization. This might be related to varied factors, including fat and protein ratio, milk composition, and the genetic variant of proteins **[15]**. The percent of yield increase after pasteurization was reported in different studies. For instance, **[15]** reported 8.1% and 13.9% for cow's and goat's milk, respectively; **[20]** reported 8.05% for cheese made from

cow's milk; and **[25]** reported 41% for cheese made from cow's milk. The increase in cheese yield after milk pasteurization is due to the increase in water retention of the cheese and the recovery of whey proteins and salt **[15]**. Makhal et al. **[23]** investigated the effect of different heat treatments on yield. However, only one heat treatment was used in this study, and the cheese yield is expected to change with different heat treatments.

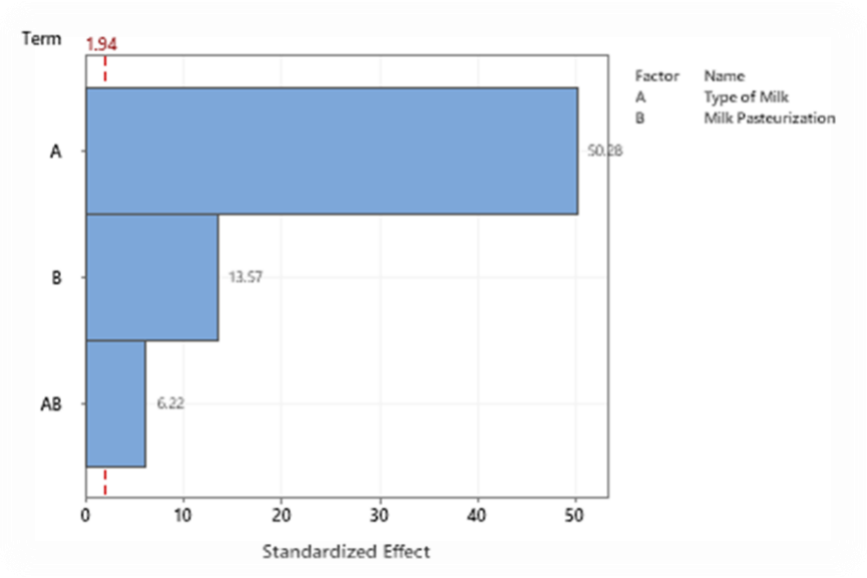


Figure 4 Standardized effects of different factors on the cheese yield after salting.

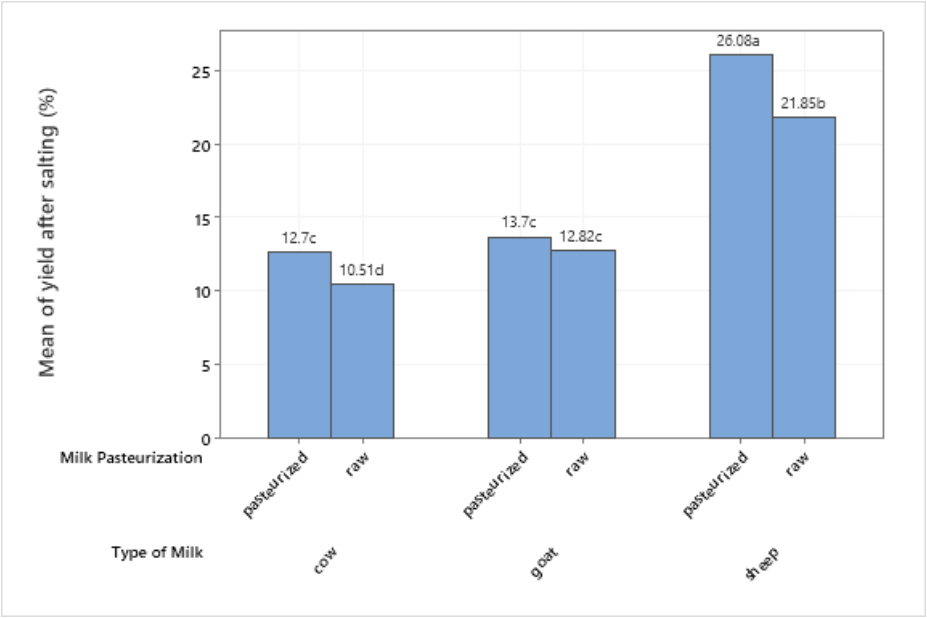


Figure 5 Effect of the two-way interaction between the type of milk and milk pasteurization on the cheese yield after salting.

In this study, it seems that the differences in cheese yield between raw and pasteurized milk were not just related to the pasteurization but also to the addition of 0.02% $CaCl_2$, which was confirmed to affect the cheese yield in previous studies **[22]**, **[23]**. Ocak et al. **[22]** relate the increase in yield caused by the addition of $CaCl_2$ to the rapid curd formation rate, which results in increased rigidity at an early stage of curd formation that, in turn, lowers the syneresis, consequently increasing the cheese yield. Makhal et al. **[23]** found that adding 0.02% $CaCl_2$ to milk without pasteurization increased the cheese yield by 4.9%, and when combined with pasteurization, the cheese yield increased to 11.18%; however, no improvement in yield when $CaCl_2$ was used in a concentration of 0.012% or 0.016% was observed. In this research, it is impossible to determine the effect of $CaCl_2$ separately from the effect of pasteurization. Therefore, the increase in yield was probably due to the combined effect of heat treatment and $CaCl_2$ addition.

Chemical Analysis: Figure 6 shows the Pareto chart for the standardized effect of different factors on moisture content. It is clear that the moisture content was significantly affected by all three main factors (type of milk, milk pasteurization, and cheese pasteurization) and the two-way interaction (type of milk*milk pasteurization), and the largest standardized effect was for milk pasteurization (7.45). Therefore, the following discussion will present the results of the two-way interaction between the milk and milk pasteurization type and the main effect of cheese pasteurization. Figure 7-a shows the main effects of cheese pasteurization (in-container heating vs. traditional boiling); in-container heating significantly increased the moisture content of cheese (40.73%) compared with traditional cheese heating (38.83%). Figure 7-b shows the effect of the two-way interaction between the type of milk and milk pasteurization. The moisture contents of cheese made from raw cow's, goat's, and sheep's milk were 35.54%, 41.11%, and 36.95%, respectively. The moisture content in cheese from raw goat's milk was significantly higher than in raw cow's or sheep's milk. Pasteurization of milk significantly increased the moisture content of cheese made from cow's and sheep's milk by 14.83% and 14.53%, respectively. However, pasteurization did not affect the moisture content in cheese made from goat's milk.

The cheese protein content was affected by the milk and milk pasteurization type without interaction effects, and the largest standardized effect was for milk pasteurization (Figure 8). Therefore, the following discussion will be limited to the significant main effects (type of milk and milk pasteurization). Milk pasteurization significantly reduced the protein content to 18.69% compared with no pasteurization, which was 21.14% (Figure 9-a). Cheese made from cow's and goat's milk had the highest protein content (21.09% and 20.31%, respectively), which differed significantly from that of cheese made from sheep's milk (18.35%) (Figure 9-b).

The fat content of cheese was significantly affected by the type of milk and milk pasteurization with no interaction effects, and the highest standardized effect was for the type of milk (6.83) (Figure 10); therefore, the following discussion will be limited to the significant main effects (type of milk and milk pasteurization). Cheese made from cow's and sheep's milk had the highest fat content (25.56% and 27.69%, respectively), significantly higher than that of cheese made from goat's milk (21.31%) (Figure 11-a). Cheese made from pasteurized milk had a significantly lower fat content (23.96%) than cheese made from raw milk (25.75%) (Figure 11-b).

The ash content of cheese was only affected by the type of milk (Figure 12). Cheese made from goat's milk had the highest ash content (15.31%), which was significantly higher than that of cheese made from cow's and sheep's milk (13.34% and 12.49%, respectively) (Figure 13).

The salt content of cheese was affected by the milk type and milk pasteurization and the interaction between them, with milk pasteurization having the highest standardized effect (4.92) (Figure 14). Therefore, the following discussion will consider only the two-way interaction between the type of milk and milk pasteurization. Cheese made from pasteurized cow's and goat's milk had significantly higher salt content (12.91% and 12.79%, respectively) than of cheese made from pasteurized sheep's milk (11.69%) (Figure 15). Milk pasteurization significantly increased the salt content in cheese made from goat's milk (12.79%) compared with that made from raw goat's milk (10.23%). However, it did not affect the salt content in other types of cheese (Figure 15). The differences in salt content derived from the milk type used might be related to variations in salt diffusion coefficient in distinct types of cheese **[26]**. The effect of milk pasteurization on salt content in goat's milk cheese might be explained by the changes in whey protein structure, affecting the salt diffusion coefficient.

As illustrated in Figure 16, the pH value of cheese was significantly affected by the milk and milk pasteurization type and the two-way interaction between them; therefore, only the two-way interaction between milk type and milk pasteurization will be presented. The type of milk had the highest standardized effect (9.16) on the pH of cheese (Figure 16). Milk pasteurization significantly increased the pH values of cow's and goat's milk cheese. However, it did not affect the pH of cheese made from sheep's milk (Figure 17).

Summarizing the chemical analysis results, the milk type significantly affected the cheese's chemical composition. Cheese made from cow's and goat's milk (either raw or pasteurized) had significantly higher protein content (21.09% and 20.31%, respectively) (Figure 9) and salt levels when using pasteurized milk (12.91% and 12.79%, for cow's and goat's milk, respectively) (Figure 15). Cheese from cow's and sheep's milk had significantly higher fat content (25.56% and 27.69%, respectively) (Figure 11-a), whereas cheese from goat's milk had significantly higher ash content (15.31%). Pasteurization of milk significantly lowered the fat (Figure 11-b) and protein contents (Figure 9-a) in the cheese and significantly increased the cheese's moisture (Figure 7-b), salt content (Figure 15), and pH (Figure 17), depending on the type of milk used. In-container heating of cheese significantly increased the moisture content of cheese.

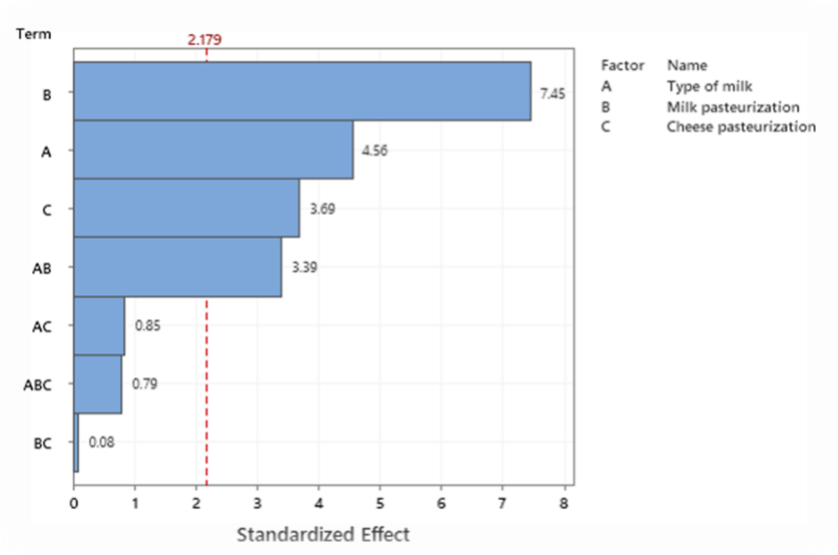
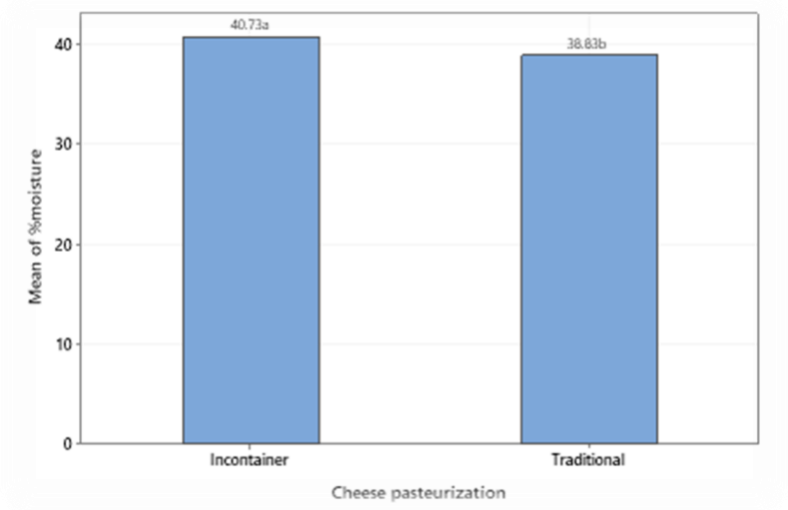


Figure 6 Standardized effects of different factors on the moisture in Nabulsi cheese.

A. Cheese pasteurization



B. Two-way interaction between the type of milk and pasteurization

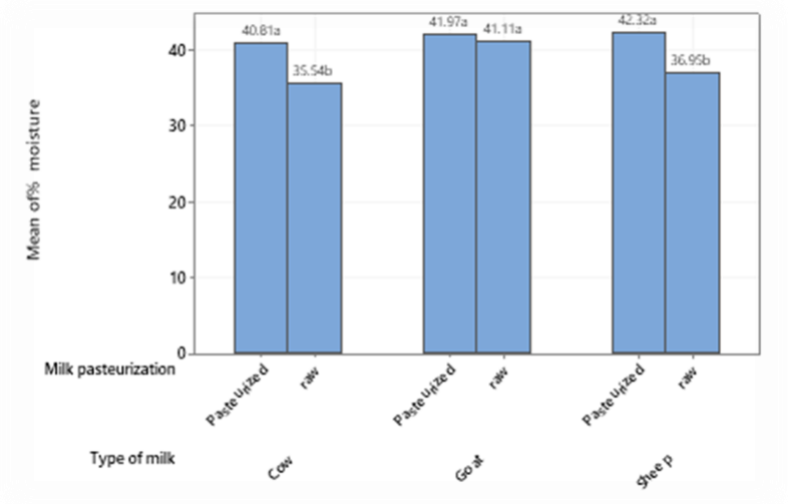


Figure 7 Effects of cheese pasteurization (A) and two-way interaction between the type of milk and milk pasteurization (B) on moisture in Nabulsi cheese.

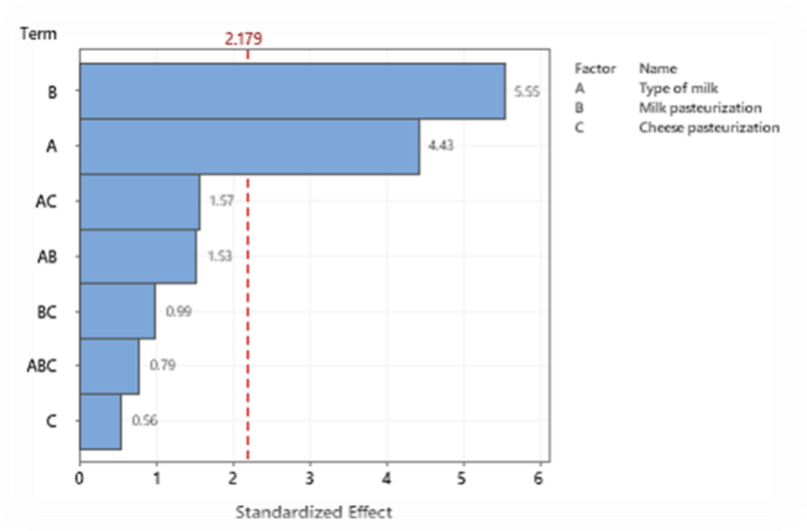
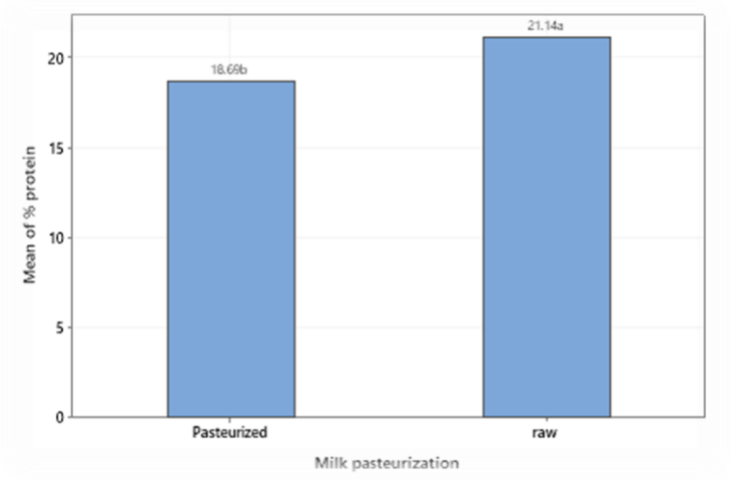


Figure 8 Standardized effects of different factors on the protein content in Nabulsi cheese.

A. Milk pasteurization



B. Milk type

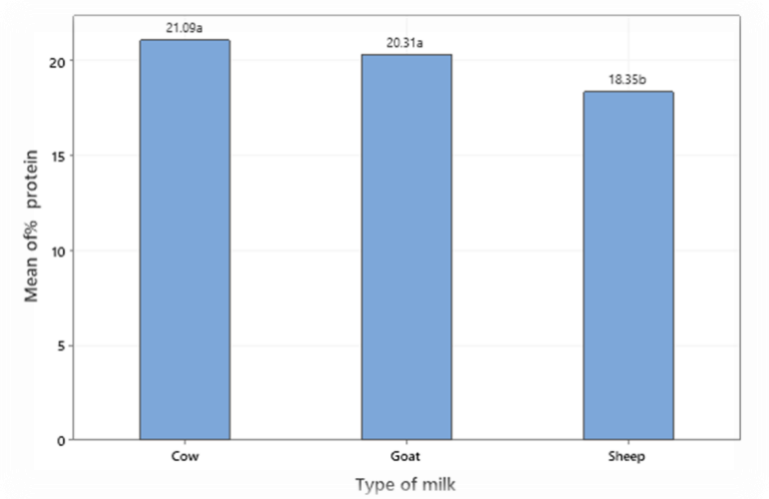


Figure 9 Effects of milk pasteurization (A) and type of milk (B) on the protein content in Nabulsi cheese.

In this study, the chemical composition of cheese made from raw cow's milk was lower than those of **[27]** for moisture, pH, and protein content and higher for fat and ash contents. Compared with the results reported by **[28]**, it has lower moisture content and higher protein, fat, and ash contents. Raw sheep's milk cheese it contains lower

moisture and higher protein, fat, and ash contents compared with that reported by **[5]**. The chemical composition of cheese was attributed to the milk type and source, cheese manufacturing methods, seasonal fluctuations, cattle diet, curd pressing degree, and whey drainage **[28]**.

The effects of milk pasteurization on cow's and sheep's milk cheese chemical analysis agreed with those for white cheese found by **[20]**, **[29]**. The effect of milk pasteurization on the chemical composition of cheese might be related to the water and salt retention by cheese related to the pasteurization of milk and to $CaCl_2$ addition, discussed previously in the yield section.

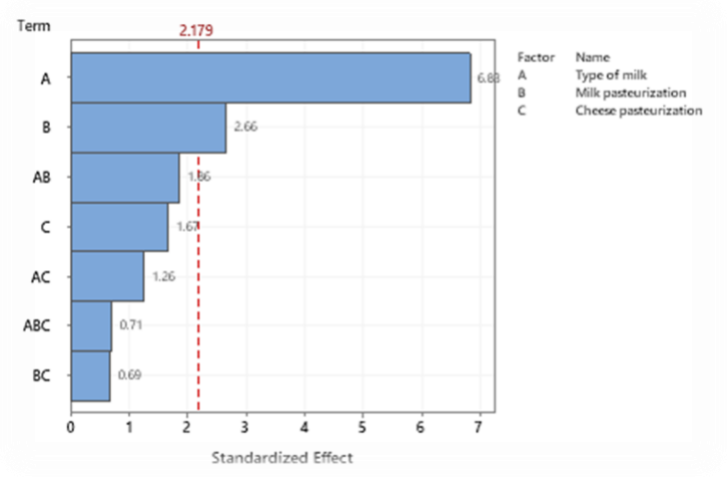
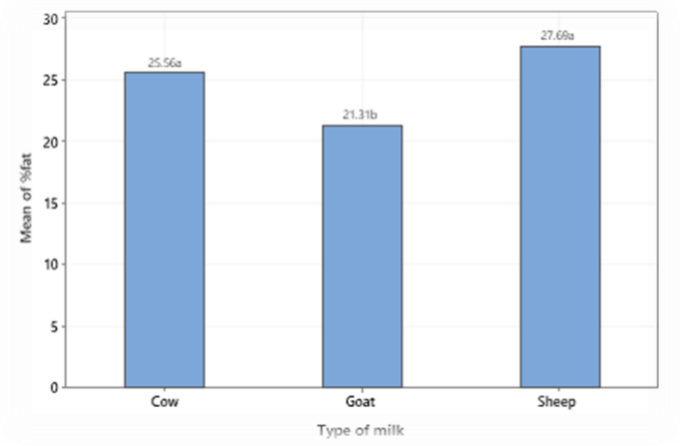


Figure 10 Standardized effects of varied factors on the fat content in Nabulsi cheese.

A. Type of milk



B. Milk pasteurization

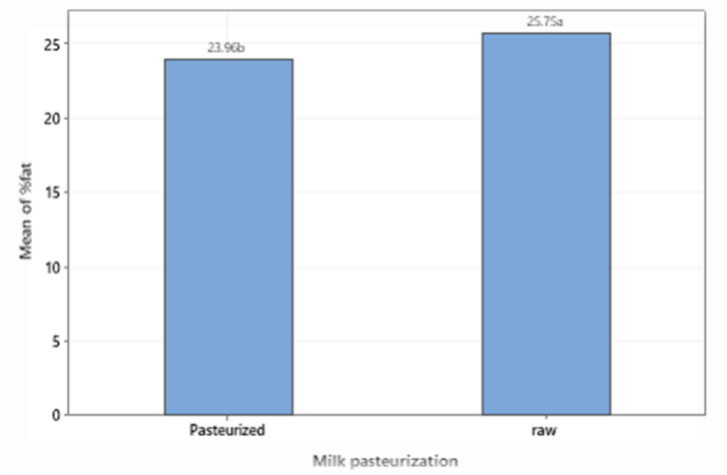


Figure 11 Effects of the type of milk (A) and milk pasteurization (B) on the fat content in Nabulsi cheese.

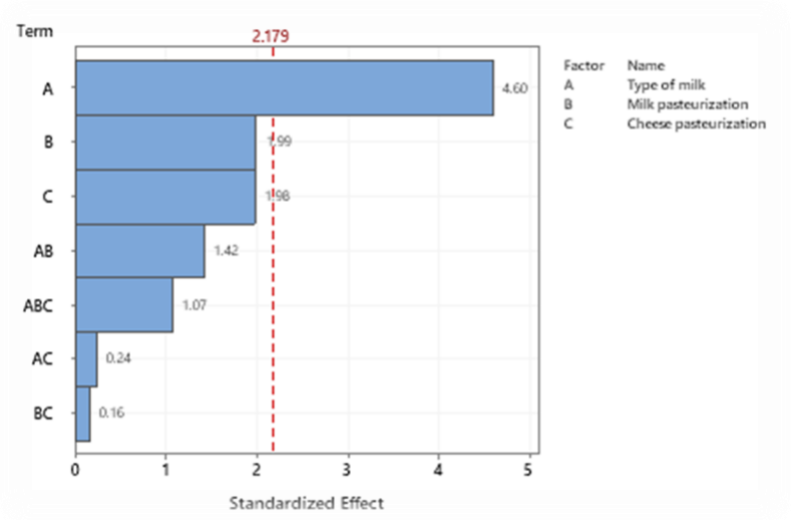


Figure 12 Standardized effects of distinct factors on the ash content in Nabulsi cheese.

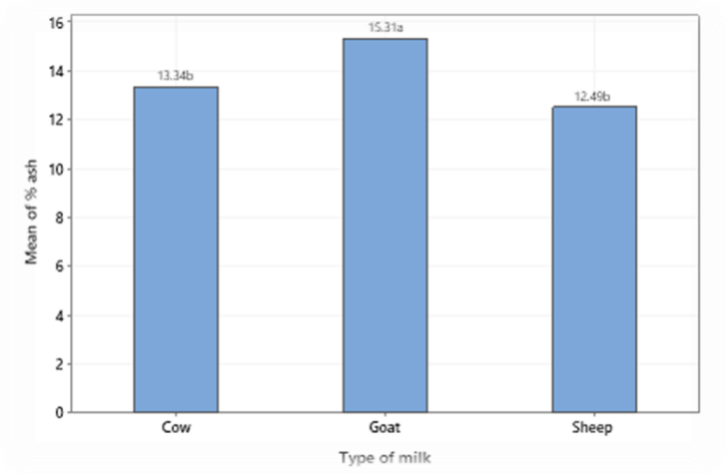


Figure 13 Effect of the type of milk on the ash content in Nabulsi cheese.

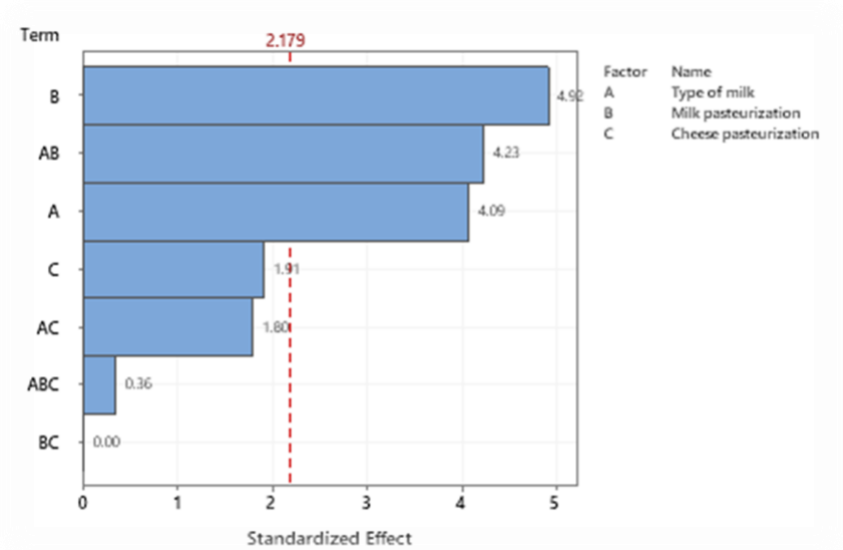


Figure 14 Standardized effects of distinct factors on the salt content in Nabulsi cheese.

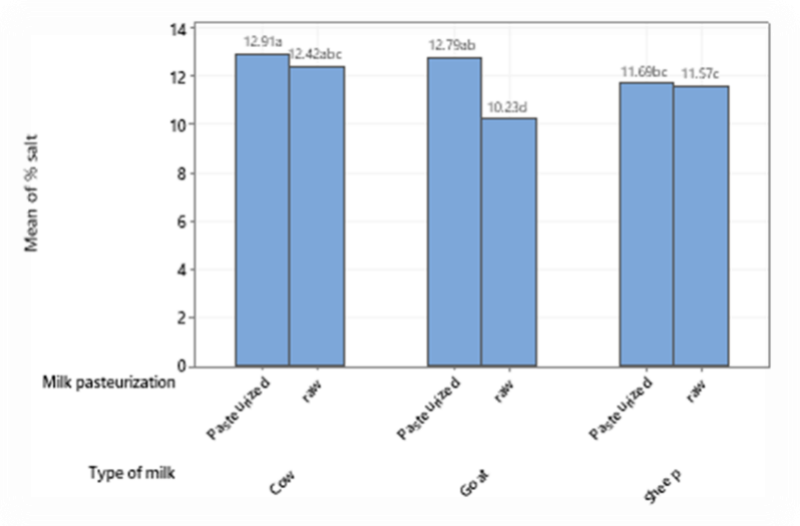


Figure 15 Effects of the two-way interaction between the type of milk and milk pasteurization on the salt content in Nabulsi cheese.

The effect of in-container heating in increasing the moisture content of cheese might be related to the prevention of water evaporation from cheese, which is encountered during the traditional boiling step; this is commercially made in water baths open to the surrounding atmosphere. This evaporation was prevented during in-container heating, which resulted in the cheese's increased moisture content.

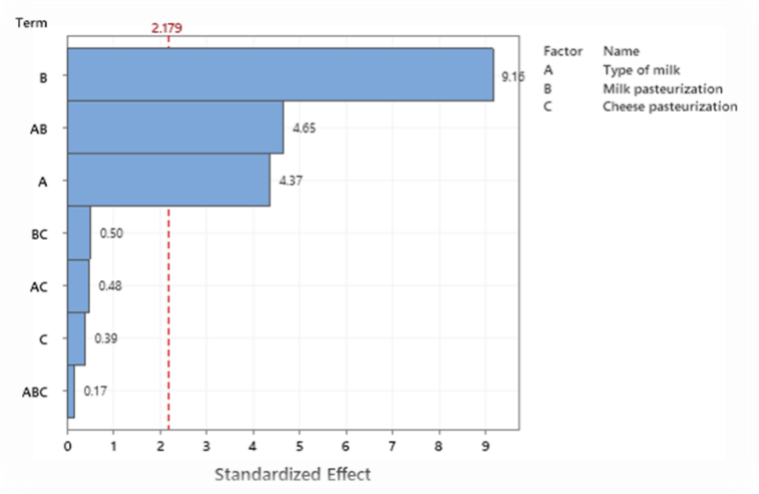


Figure 16 Standardized effects of distinct factors on the pH of Nabulsi cheese.

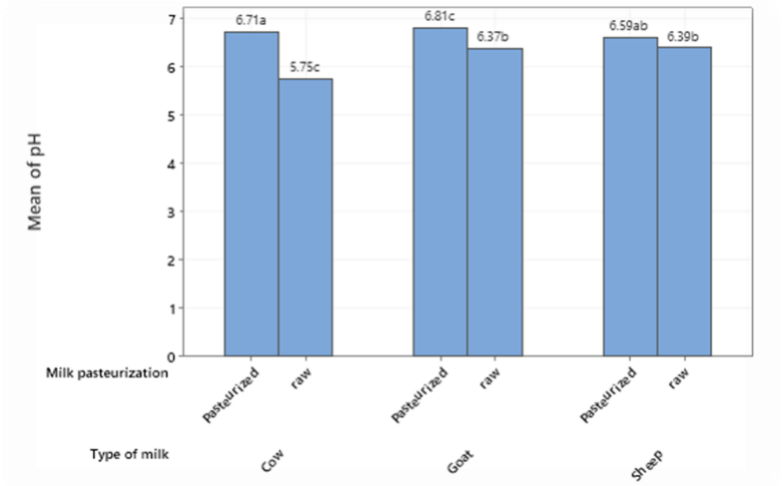


Figure 17 Effects of the two-way interaction between the type of milk and milk pasteurization on the pH of Nabulsi cheese.

TPA: In the following discussion of TPA parameters, the springiness values were not presented because they were close to 1 and not significantly affected by the factors investigated in this study. Similarly, the adhesiveness results were not presented because they were too small to be of practical importance and were not affected by the factors investigated in this study.

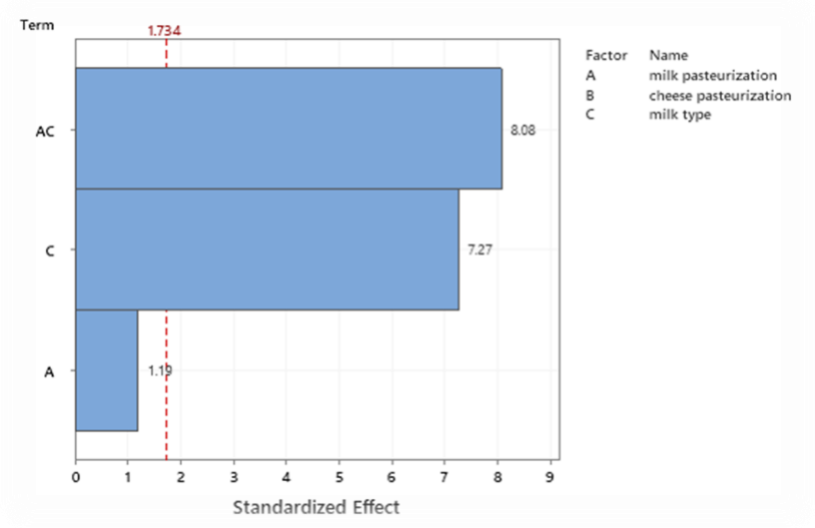


Figure 18 Standardized effects of different factors on the hardness of Nabulsi cheese.

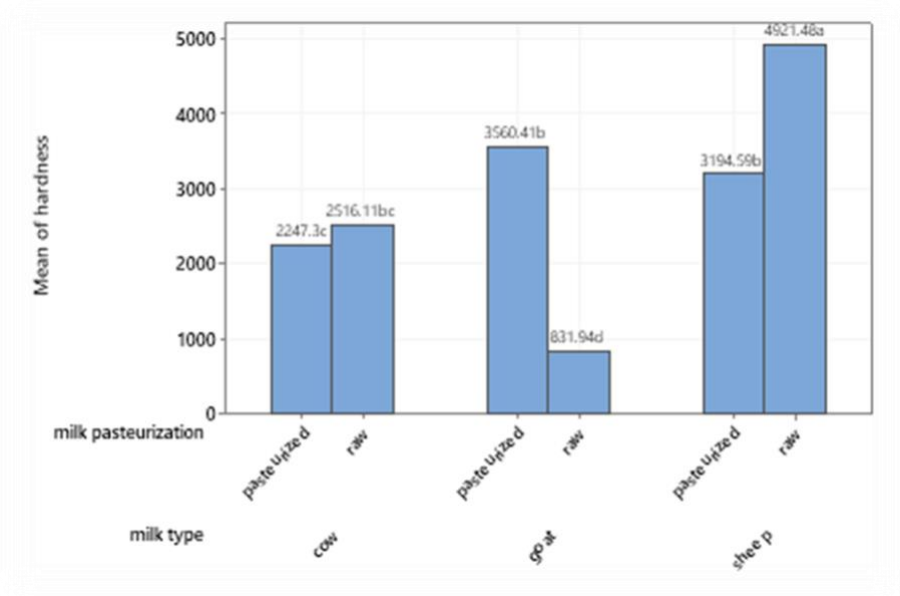


Figure 19 Effects of the two-way interaction between the type of milk and milk pasteurization on the hardness of Nabulsi cheese.

Hardness was affected by the type of milk and the two-way interaction between the type of milk and milk pasteurization (Figure 18); therefore, the following discussion will be limited to the significant two-way interaction (type of milk*milk pasteurization). The hardness values (Figure 19) for cheese made from raw cow's, goat's, and sheep's milk (2516.11, 831.94, and 4921.48 g, respectively) differ significantly from each other, with the highest value for cheese made from sheep's milk and the lowest for cheese made from goat's milk. These findings agreed with previous studies showing that sheep's milk cheese had higher hardness values than cow's milk cheese **[30]**, **[31]**, **[32]**. Milk pasteurization affected the hardness of Nabulsi cheese depending on the type of milk used. For cow's milk, pasteurization did not significantly affect the hardness (2247.3 g), whereas, for goat's milk, pasteurization significantly increased hardness to 3560.41 g.

In contrast, sheep's milk was significantly reduced to 3194.59 g. The decrease in hardness values due to milk pasteurization was previously reported **[34]**, **[29]**. On the contrary, **[33]** reported increased hardness for cheese made from pasteurized milk. The difference in the effect of pasteurization on the hardness of cheese made from different types of milk might be related to the protein structure **[26]**, extent of denaturation upon heating **[33]**, and

severity and type of heat treatment **[29]**. The decrease in hardness upon milk pasteurization might be related to the protein's increased moisture-holding capacity **[34]**, **[29]**. The significant increase in moisture in cheese made from sheep's milk upon pasteurization (Figure 7-b) and the subsequent decrease in hardness (Figure 19) supports the previous hypothesis. Conversely, the increase in hardness upon milk pasteurization might be related to the denaturation of whey protein and complex formation with casein micelles **[33]**.

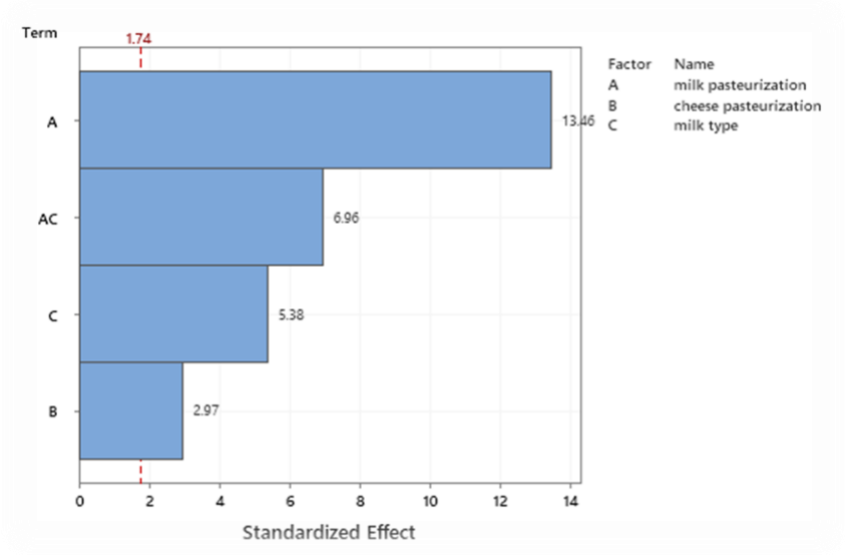
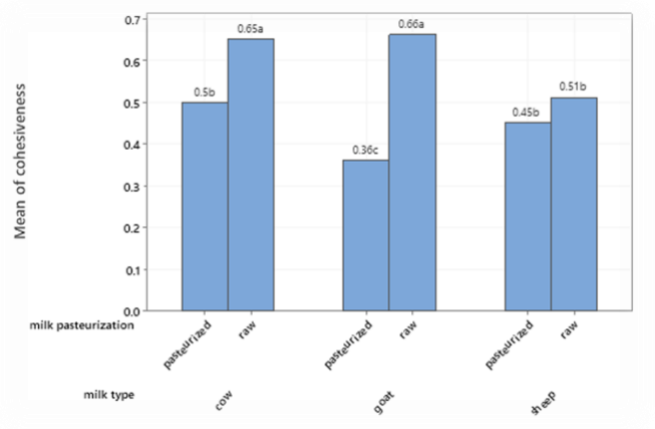


Figure 20 Standardized effects of distinct factors on the cohesiveness of Nabulsi cheese.

A. Two-way interaction between the type of milk and milk pasteurization



B. Cheese pasteurization

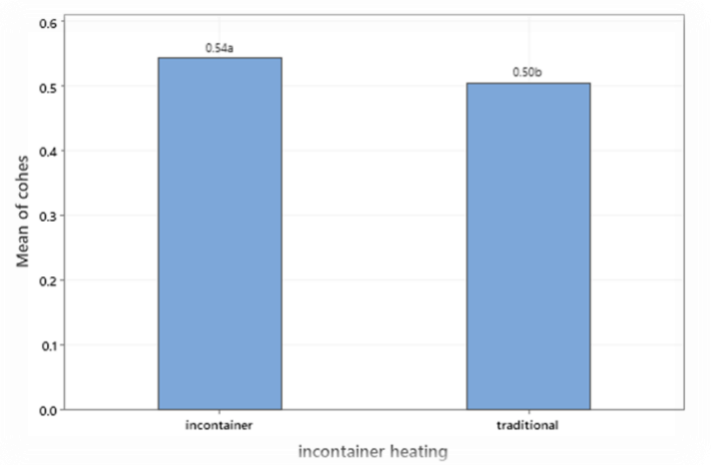


Figure 21 Effects of the two-way interaction between the type of milk and milk pasteurization (A) or cheese pasteurization (B) on the cohesiveness of Nabulsi cheese.

Cohesiveness is the strength of the internal bonds that comprise the product's body **[33]**. The cohesiveness values of Nabulsi cheese were affected by the three studied factors and by the two-way interaction between the type of milk and milk pasteurization (Figure 20); therefore, the results of the main effect of cheese pasteurization and the two-way interaction type of milk*milk pasteurization will be presented.

As shown in Figure 21-a, the cohesiveness of cheese made from raw cow's and goat's milk (0.65 and 0.66, respectively) did not differ significantly. However, they were significantly higher than cheese made from raw sheep's milk (0.51). Milk pasteurization significantly reduced the cohesiveness values of cheese made from cow's and goat's milk (0.5 and 0.36, respectively), with significant differences between them. However, pasteurization did not significantly affect the cohesiveness of cheese made from sheep's milk (0.45). In-container heating of cheese significantly increased cohesiveness to 0.54 compared with the traditional method (0.50) (Figure 21-b), which may be related to the higher moisture content of cheese heated in-container. This is in line with the results reported by **[27]**, who found that cheese cohesiveness increased with increasing moisture content. The differences in cohesiveness values from different types of cheese made from different types of milk might be related to the changes in protein–protein and protein–water interactions.

Chewiness is the amount of energy required to chew a solid food until it is ready to swallow and is calculated as the product of hardness, cohesiveness, and springiness **[33]**. As shown in Figure 22, the chewiness values were significantly affected by the type of milk and milk pasteurization, two-way interaction (type of milk*milk pasteurization), and three-way interaction (type of milk*milk pasteurization*heat treatment of cheese); therefore, the results of the three-way interaction will be presented. The three-way interaction is illustrated in Figure 23; in-container heating of cheese did not significantly affect chewiness compared with traditional heating of cheese regardless of the type of milk used or whether the milk used was raw or pasteurized. Milk pasteurization did not affect chewiness compared with no pasteurization for cheese made from cow's and goat's milk. However, milk pasteurization significantly affected chewiness in cheese made from sheep's milk. Cheese made from pasteurized milk and heated in-container had the lowest value (1192.07 g), which differed significantly from other cheese made from raw sheep's milk and heated either by in-container heating or using the traditional method. The lowest chewiness values were for cheese made from raw goat's milk whether heated in-container or using the traditional method (517.45 and 385.35 g respectively), and the highest values were for cheese made from raw sheep's milk whether heated in-container or using the traditional method (2620.45 and 2341.86 g, respectively). Chewiness is a secondary parameter calculated from primary parameters (hardness, cohesiveness, and springiness) and therefore affected by factors affecting these parameters.

A sample of the produced Nabulsi cheese is shown in Figure 24.

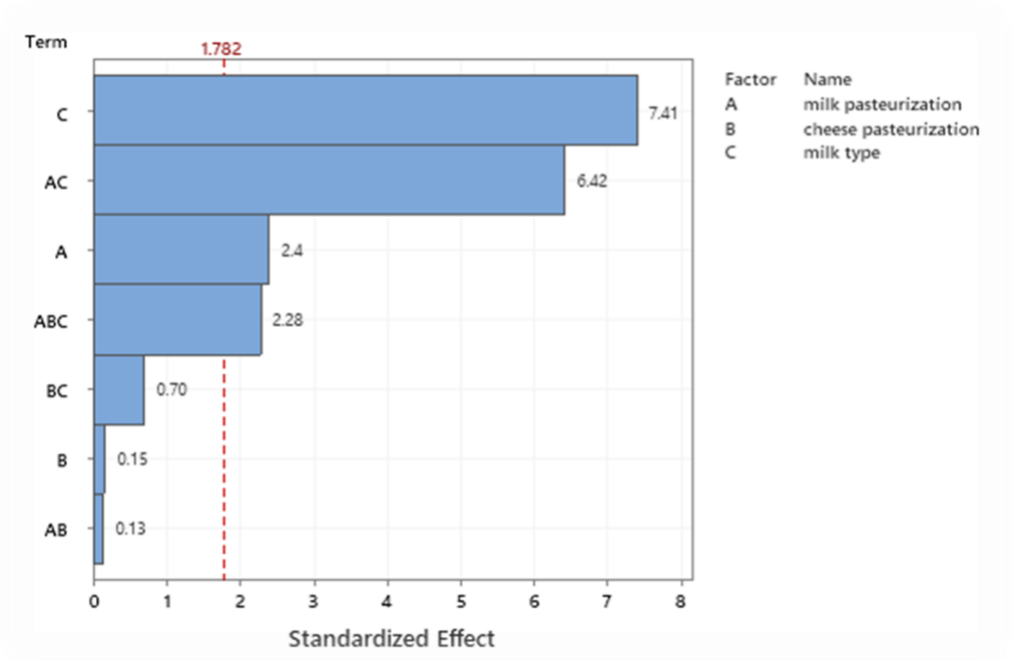


Figure 22 Standardized effects of distinct factors on the chewiness of Nabulsi cheese.

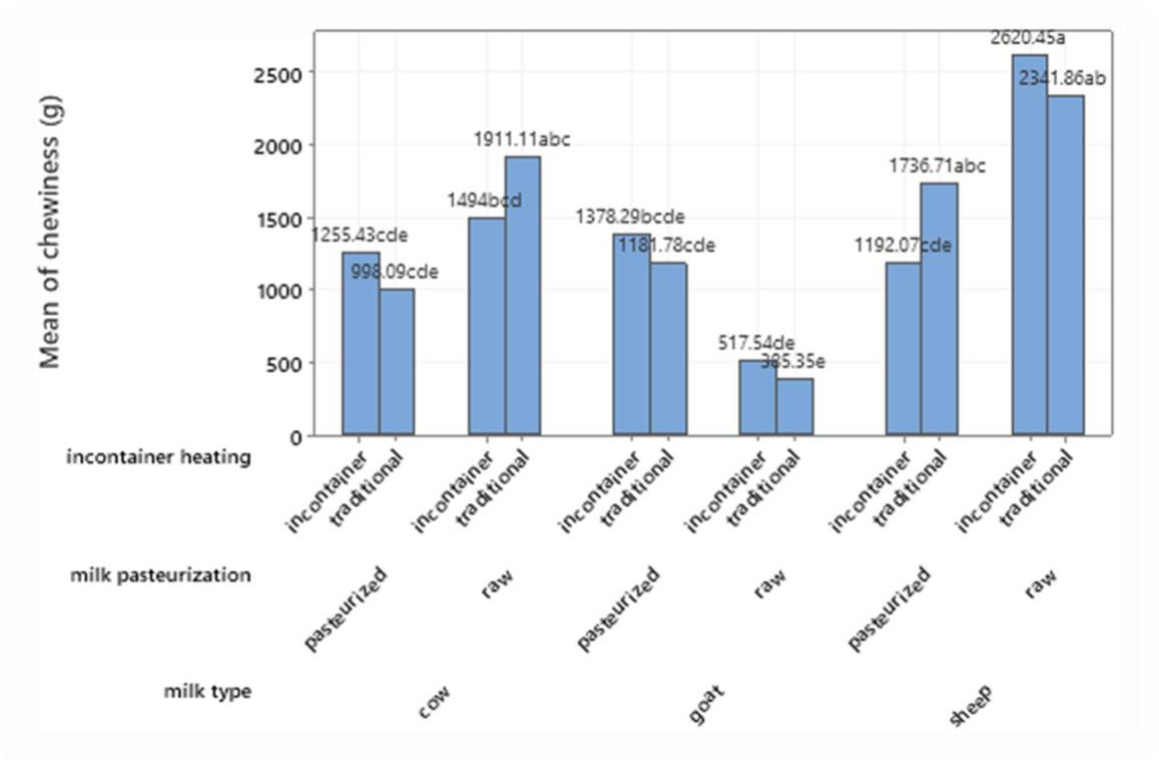


Figure 23 Effects of the three-way interaction (type of milk*milk pasteurization*cheese in-container heating) on the chewiness of Nabulsi cheese.

Figure 24 Nabulsi cheese.

CONCLUSION

White-brined Nabulsi cheese producers increasingly adopt milk pasteurization, $CaCl_2$ addition, and in-container heating as industry standards, which indicates the importance of investigating their effects on the yield, chemical composition, and TPA of cheese produced from different types of milk. Cheese made from sheep's milk had the highest yield after pressing or salting, followed by goat's and cow's milk. Pasteurization of milk significantly increased the yield (after pressing and salting) of cheese made from cow's and sheep's milk but did not affect the yield of cheese made from goat's milk. The type of milk significantly affected the chemical composition of cheese. Cheese made from cow's and goat's raw or pasteurized milk had significantly higher protein content (21.09% and 20.31%, respectively), and when using pasteurized milk, had higher salt (12.91% and 12.79%, respectively) than cheese made from sheep's milk. Cheese from cow's and sheep's milk had significantly higher fat content (25.56% and 27.69%, respectively), whereas cheese from goat's milk had significantly higher ash content (15.31%). Pasteurization of milk significantly lowered the fat and protein contents of cheese. It significantly increased the cheese's moisture, salt levels, and pH depending on the type of milk used. In-container heating of cheese significantly increased the moisture content of cheese. There were significant differences in the hardness of cheese made from raw milk, with the highest value for sheep's milk and the lowest for goat's milk. Cheese made from sheep's milk had significantly lower cohesiveness than cow's or goat's milk. Cheese made from raw sheep's milk had the highest chewiness value, which significantly differs from other types of cheese. The literature contains almost no information regarding the effects of the factors investigated in this study on the quality of Nabulsi cheese; therefore, the results of this research will give cheese producers an insight into the impact of adopting the new processes investigated in this research on the quality of Nabulsi cheese and the cost of production as it is affected by the cheese yield. In addition, the results give information about the factors affecting the quality attributes of Nabulsi cheese measured in this study that could be used for future studies to optimize the process of Nabulsi cheese production.

REFERENCES

1. Yamani, M. I., Al-Nabulsi, A. A., Haddadin, M. S., & Robinson, R. K. (1998). The isolation of salt-tolerant lactic acid bacteria from ovine and bovine milks for use in the production of nabulsi cheese. In International Journal of Dairy Technology (Vol. 51, Issue 3, pp. 86–89). Wiley. https://doi.org/10.1111/j.1471-0307.1998.tb02643.x
2. Al-Dabbas, M. M., Al-Ismail, K., & Al-Abdullah, B. M. (2011). Effect of chemical composition on the buffering capacity of selected dairy products. In Jordan Journal of Agricultural Sciences (Vol. 7, Issue 4, pp. 600–700). Deanship of Scientific Research (DSR), The University of Jordan.
3. Alichanidis, E., & Polychroniadou, A. (2008). Characteristics of major traditional regional cheese varieties of East-Mediterranean countries: a review. In Dairy Science and Technology (Vol. 88, Issues 4–5, pp. 495–510). Springer Science and Business Media LLC. https://doi.org/10.1051/dst:2008023
4. Haddadin, M. S., Shahin, R. M. A., & Son, R. K. R. (1995). The influence of the casein:fat ratio in sheep's milk on the chemical composition and sensory properties of nabulsi cheese. In International Journal of Dairy Technology (Vol. 48, Issue 3, pp. 71–75). Wiley. https://doi.org/10.1111/j.1471-0307.1995.tb02471.x
5. Humeid, M. A., Tukan, S. K., & Yamani, M. (1990). In-bag steaming of white brined cheese as a method for preservation. In Milchwissenschaft-milk Science International (Vol. 45, Issue 8, pp. 513–516). Hochschule Hannover – University of Applied Sciences and Arts Fakultaet 2.
6. Mallatou, H., Pappas, C. P., & Voutsinas, L. P. (1994). Manufacture of feta cheese from sheep's milk, goats' milk or mixtures of these milks. In International Dairy Journal (Vol. 4, Issue 7, pp. 641–664). Elsevier BV. https://doi.org/10.1016/0958-6946(94)90039-6
7. Sabbah, M., Di Pierro, P., Dell'Olmo, E., Arciello, A., & Porta, R. (2019). Improved shelf-life of Nabulsi cheese wrapped with hydrocolloid films. In Food Hydrocolloids (Vol. 96, pp. 29–35). Elsevier BV. https://doi.org/10.1016/j.foodhyd.2019.05.010
8. Al-Ismail, K. M., & Humied, M. A. (2002). Effect of processing and storage of brined white (Nabulsi) cheese on fat and cholesterol oxidation. In Journal of the Science of Food and Agriculture (Vol. 83, Issue 1, pp. 39–43). Wiley. https://doi.org/10.1002/jsfa.1278
9. Khaled. (2009). Effect of Proteases on Meltability and Stretchability of Nabulsi Cheese. In American Journal of Agricultural and Biological Sciences (Vol. 4, Issue 3, pp. 173–178). Science Publications. https://doi.org/10.3844/ajabssp.2009.173.178
10. Mazahreh. (2009). The Effect of adjusting PH on Stretchability and Meltability to White Brined Nabulsi Cheese. In American Journal of Applied Sciences (Vol. 6, Issue 4, pp. 543–550). Science Publications. https://doi.org/10.3844/ajassp.2009.543.550

11. Toufeili, I., & Özer, B. (2006). Brined Cheeses from the Middle East and Turkey. In Brined Cheeses (pp. 188–210). Wiley. https://doi.org/10.1002/9780470995860.ch6
12. Haddadin, M. S. Y., & Shahin, R. M. A. (1995). Nabulsi: A Jordanian cheese. In Dairy industries international (Vol. 60, Issue 7, pp. 33–34). Bell Publishing Ltd.
13. Abu-Alruz, K. (1999). A study of in-can heating preservation of nabulsi cheese with emphasis on halotolerant bacteria [master thesis, University of Jordan, Amman, Jordan].
14. AOAC (Association of Official Analytical Chemists). (2019). Official methods of analysis.
15. Tadjine, D., Boudalia, S., Bousbia, A., Khelifa, R., Mebirouk Boudechiche, L., Tadjine, A., & Chemmam, M. (2020). Pasteurization effects on yield and physicochemical parameters of cheese in cow and goat milk. In Food Science and Technology (Vol. 40, Issue 3, pp. 580–587). FapUNIFESP (SciELO). https://doi.org/10.1590/fst.13119
16. Skeie, S. (2007). Characteristics in milk influencing the cheese yield and cheese quality. In Journal of Animal and Feed Sciences (Vol. 16, Issue Suppl. 1, pp. 130–142). The Kielanowski Institute of Animal Physiology and Nutrition, PAS. https://doi.org/10.22358/jafs/74164/2007
17. Lau, K. Y., Barbano, D. M., & Rasmussen, R. R. (1990). Influence of Pasteurization on Fat and Nitrogen Recoveries and Cheddar Cheese Yield. In Journal of Dairy Science (Vol. 73, Issue 3, pp. 561–570). American Dairy Science Association. https://doi.org/10.3168/jds.s0022-0302(90)78703-6
18. Salwa, A. A., & Galal, E. A. (2002). Effect of milk pretreatment on the keeping quality of Domiati cheese. *In* Pakistan Journal of Nutrition (Vol. 1, Issue 3, pp. 132–136). Science Alert. https://doi.org/10.3923/pjn.2002.132.136
19. San Martín-González, M. F., Rodríguez, J. J., Gurram, S., Clark, S., Swanson, B. G., & Barbosa-Cánovas, G. V. (2007). Yield, composition and rheological characteristics of cheddar cheese made with high pressure processed milk. In LWT - Food Science and Technology (Vol. 40, Issue 4, pp. 697–705). Elsevier BV. https://doi.org/10.1016/j.lwt.2006.03.022
20. Sulieman, A. M. E.-H. (2007). Effect of Pretreatment of Milk Quality Characteristics of Jibna-Beida (White Cheese). In International Journal of Food Engineering (Vol. 3, Issue 4). Walter de Gruyter GmbH. https://doi.org/10.2202/1556-3758.1123
21. Abdalla, M. O. M., & Ahmed, O. I. (2010). Effect of heat treatment, level of sodium chloride, calcium chloride on the chemical composition of white cheese. In Conference: Research Journal of Animal and Veterinary Sciences (Vol. 5, pp. 69–72). Science Publications.
22. Ocak, E., Andiç, S., & Tunçtürk, Y. (2014). The Effect of Homogenization, CaCl2 Addition and Pasteurization on White Cheese and Whey Composition. In Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi (Vol. 24, Issue 1, pp. 70–78). Yuzuncu Yil Universitesi Tarim Bilimleri Dergisi. https://doi.org/10.29133/yyutbd.235918
23. Makhal, S., Kanawjia, S. K., & Giri, A. (2013). Role of calcium chloride and heat treatment singly and in combination on improvement of the yield of direct acidified Cottage cheese. In Journal of Food Science and Technology (Vol. 52, Issue 1, pp. 535–541). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-013-1028-5
24. Drake, M. A., Harrison, S. L., Asplund, M., Barbosa-Canovas, G., & Swanson, B. G. (1997). High Pressure Treatment of Milk and Effects on Microbiological and Sensory Quality of Cheddar Cheese. In Journal of Food Science (Vol. 62, Issue 4, pp. 843–860). Wiley. https://doi.org/10.1111/j.1365-2621.1997.tb15468.x
25. Abdalla, M., & Salih, H. M. A. (2020). Effect of heat treatment of milk on the physicochemical, microbiological and sensory characteristics of white cheese (Gibna bayda). In GSC Advanced Research and Reviews (Vol. 3, Issue 3, pp. 020–028). GSC Online Press. https://doi.org/10.30574/gscarr.2020.3.3.0044
26. Aminifar, M., Hamedi, M., Emam-Djomeh, Z., & Mehdinia, A. (2012). The effect of ovine and bovine milk on the textural properties of Lighvan cheese during ripening. In International Journal of Dairy Technology (Vol. 66, Issue 1, pp. 45–53). Wiley. https://doi.org/10.1111/1471-0307.12009
27. Ayyash, M., & Shah, N. P. (2011). The effect of substituting NaCl with KCl on Nabulsi cheese: Chemical composition, total viable count, and texture profile. *Journal of Dairy Science*, *94*(6), 2741–2751. https://doi.org/10.3168/jds.2010-3976
28. Al-Dabbas, M. M., Saleh, M., Abu-Ghoush, M. H., Al-Ismail, K., & Osaili, T. (2014). Influence of storage, brine concentration and in-container heat treatment on the stability of white brined Nabulsi cheese. In International Journal of Dairy Technology (Vol. 67, Issue 3, pp. 427–436). Wiley. https://doi.org/10.1111/1471-0307.12139
29. Hamdy, S., Abdelmeged, D., & Abd Elmontaleb, H. (2022). Influence of Different Heat Treatments on the quality characteristics of Edam Cheese. In Egyptian Journal of Food Science (Vol. 0, Issue 0, pp. 0–0). Egypts

Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/ejfs.2022.118954.1119

30. Ponce de Leon-Gonzalez, L., Wendorff, W. L., Ingham, B. H., Thomas, D. L., Jaeggi, J. J., & Houck, K. B. (2002). Influence of Ovine Milk in Mixture with Bovine Milk on the Quality of Reduced Fat Muenster-Type Cheese. In Journal of Dairy Science (Vol. 85, Issue 1, pp. 36–42). American Dairy Science Association. https://doi.org/10.3168/jds.s0022-0302(02)74050-2
31. Pappa, E. C., Kandarakis, I., & Mallatou, H. (2007). Effect of different types of milks and cultures on the rheological characteristics of Teleme cheese. In Journal of Food Engineering (Vol. 79, Issue 1, pp. 143–149). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2006.01.038
32. Lteif, L., Olabi, A., Kebbe Baghdadi, O., & Toufeili, I. (2009). The characterization of the physicochemical and sensory properties of full-fat, reduced-fat, and low-fat ovine and bovine Halloumi. In Journal of Dairy Science (Vol. 92, Issue 9, pp. 4135–4145). American Dairy Science Association. https://doi.org/10.3168/jds.2009-2070
33. Lepesioti, S., Zoidou, E., Lioliou, D., Moschopoulou, E., & Moatsou, G. (2021). Quark-Type Cheese: Effect of Fat Content, Homogenization, and Heat Treatment of Cheese Milk. In Foods (Vol. 10, Issue 1, p. 184). MDPI AG. https://doi.org/10.3390/foods10010184
34. AWAD, S. (2006). Texture and flavour development in Ras cheese made from raw and pasteurised milk. In Food Chemistry (Vol. 97, Issue 3, pp. 394–400). Elsevier BV. https://doi.org/10.1016/j.foodchem.2005.05.012

Funds:

This work was supported by a grant from the Deanship of Scientific Research at Mutah University.

Acknowledgments:

The author would like to thank the Deanship of Scientific Research at Mutah University for their financial support.

Conflict of Interest:

The author declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Khaled Abu-Alruz**, Mutah University, Faculty of Agriculture, Department of Nutrition and Food Technology, Mutah, 61710, Al-Karak, Jordan,
E-mail: kabu_ruz@mutah.edu.jo; khaledabualruz@yahoo.com
ORCID: https://orcid.org/0000-0001-7788-9723

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 22.9.2023
Revised: 10.11.2023
Accepted: 14.11.2023
Published: 9.1.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 20-35
https://doi.org/10.5219/1925
ISSN: 1337-0960 online
www.potravinarstvo.com

Mathematical modelling and optimization of the granulation process of loose compound feed for broilers

Alma Yermukanova, Georgii Stankevych, Leonid Podobed, Saule Zhiyenbayeva, Eva Mrkvicová

ABSTRACT

The article presents the results of studying the indicators of the crumbling of granules and the specific energy costs of experimental batches of granulated feed for broilers. The process of granulation of compound feed for broilers was studied using the statistical planning method of a multifactorial experiment using a granulation line, including a granulator press. The dependence of the crumbling of granules and the energy consumed on the selected factors has been established. The moisture content of the feed mixture W (%) and steam pressure P (MPa) were chosen to optimise the granulation process. A matrix plan of the experiment was compiled. With the optimal values of the factors obtained in the study using the experimental planning method, an experimental batch of granulated feed was developed. At the selected levels of factors, the calculated value of the crumbling of granules was 20.11%, which fits into the optimum according to the standard of the crumbling of granulated feed for poultry (no more than 22%). The specific electricity consumption was 9.23 kWh/ton. Experiments have shown that the discrepancies between the experimental and calculated data are insignificant and within these indicators' experimental error determination. Mathematical modelling of the granulation process of loose compound feed for broilers made it possible to solve an important practical problem – to optimize the granulation mode, which ensures the production of good quality granules with minimal energy consumption. The obtained optimal granulation parameters can serve as the basis for producing granulated feed for broilers.

Keywords: granulation, crumbability, granular feed, mathematical modeling, broiler

INTRODUCTION

Nature did not endow the bird with teeth; as a result, it is not able to hold and grifted taste, and the bird's actual intake stance left its mark on the nature of the choice and perception of the taste of the feed and on the actual intake of the bird. Moreover, birds are characterized by a more pronounced reaction of food intake to its physical and mechanical characteristics than mammals **[1]**.

For broiler chickens in the first seven days of their life, the physical structure of the feed is of particular importance. At this age, they are more willing to consume small granules, which contributes to the most intensive growth and development of the intestine: an increase in its length, mass, number of villi and microvilli, nutrient absorption surface, synthesis, and secretion of digestive enzymes. At an early age, the small intestine also grows intensively in chickens, in which the main absorption of nutrients and biologically active substances occurs. Subsequently, the growth of the gastrointestinal tract continues with less intensity **[2]**. It is believed that the physical form of the feed (loose, granules, or semolina) and size (particle or granule size) have a significant impact on broiler growth and feed intake **[3]**, **[4]**. In research Mingbin, feed form had a greater effect on broiler growth performance and the digestive tract than feed particle size.

Granular feed has several advantages over loose feed **[5]** since the separation of components **[6]** during transportation and distribution is excluded. At the same time, the bird does not have the opportunity to select individual particles, which eliminates the violation of the balance of feeding and reduces losses due to scattering and dust. Biologically active substances (especially carotene and vitamins) are better preserved in granules; during the granulation process, due to pressure and temperature, the availability of nutrients increases, and anti-nutritional factors are destroyed. The digestibility of organic substances of granulated feed is increased by 2.2-3.0%. As a result of granulation, the feed volume is reduced, which contributes to a more economical use of vehicles and storage facilities, provides a longer preservation of nutrients, and improves the sanitary condition of the feed **[7]**, **[8]**.

The quality of the granulation process can be assessed according to such criteria as energy costs for the formation of granules and their quality. Hydrothermal treatment has a significant influence on them. Under the influence of warm wet steam, loose mixed fodder undergoes, on the one hand, structural and mechanical changes, and on the other hand, biochemical changes, as a result of which a product of viscosity necessary for pressing is obtained. The component composition determines its colloidal porous structure. When moisture is absorbed, colloids swell and change. With an increase in temperature, the swelling is more intense, while the plastic properties of the product increase. The fat released from plant and animal cells uniformly envelops the warm and moist surface of the feed particles. When steaming grain components, starch gelatinizes and passes into a soluble form, more accessible to the action of enzymes. The dextrins and simple sugars formed as a result contribute to the adhesion of the feed particles to each other.

The most important negative of granulation is a very serious increase in energy consumption for its production compared to the production of loose feed. On average, this increase can lead to an increase in feed cost by 25-35% **[9]**.

Hancock **[10]** asserts that poor physical pellet quality (PPQ) produces more fine particles during feed transportation from feed mills to poultry house feed lines. Pellets are submitted to friction, impact, and pressure during storage, transport, and dispatch from the feed mill to the farms **[11]**, **[12]** and poor-quality pellets disintegrate, resulting in a feed consisting of a few pellets and fines. The geometric mean diameter (GMD) of fine particles is equal to or lower than that of mash diets, and these particles may cause a nutritional imbalance in feed chemical composition, which may negatively affect animal performance.

Some researchers **[13]**, **[14]**, **[15]** report that poor PPQ negatively changed the feed intake pattern of broilers.

Pellet quality is the ability to resist fragmentation and abrasion during handling without breaking up and reach feeders without generating high fines **[16]**. The pellet durability index (PDI) is one of the main parameters used to determine pellet quality, as it indicates the percentage of pellets that remain intact after being submitted to mechanical forces.

In the Republic of Kazakhstan, the crumbling of feed pellets is determined. The crumbling of granules is a qualitative indicator that characterizes the degree of cohesion of the particles that make up the granules. When transporting feed, especially over long distances, and if reloading from one mode of transport to another is forthcoming, or if transportation is carried out on roads of unsatisfactory condition, the granules can collapse, losing consumer qualities and decreasing volume.

The index of crumbling of compound feed for poultry affects the loss of feed nutrients and safety during transportation and distribution.

The crumbling of granules is usually determined according to GOST 28497-2014. The essence of the method lies in destroying the analysed product's granules, separating undestroyed granules from fines and crumbs by sifting, weighing them, and then calculating the crumbling **[17]**.

Thus, granulated complete feed has many advantages since it contains nutrients in its composition in a concentrated form, is easily distributed, and helps to maintain microbiological purity in the poultry house. With higher productivity rates, pelleting can significantly reduce unproductive feed losses and save on their total consumption per unit of product received. Granulation reduces the dispersion of compound feed in the poultry house, positively affecting its microclimate.

Given the above, using mathematical modeling, it is important to control the physical and mechanical characteristics of granulated feed and establish modes of compound feed production with the minimal crumbling of granules and specific energy costs. Therefore, it is advisable to optimize the granulation process when developing compound feed recipes by developing a mathematical model, considering its specific energy consumption, without losing the quality of the final product. This will reduce feed production costs and increase profitability **[18]**. Granulation of mixed fodder with optimal parameters will allow granules that meet the standard's requirements to be obtained.

Scientific Hypothesis

Drawing up a mathematical model of the granulation process based on experimental data will make it possible to determine optimal granulation modes that ensure minimum specific energy consumption with normalized granule crumbliness.

MATERIAL AND METHODOLOGY

Samples

Granulated feed for broiler chickens (13-28 days of growing). They are cylindrical granules of yellow-green color with a diameter of 3.5 mm and a length of 5 mm. The composition contains raw materials of plant origin (wheat, corn, corn germ, soybean meal, flaxseed cake, soybean oil), limestone flour, premix, and the mineral vermiculite.

Chemicals

No chemicals were used.

Instruments

CAS SW-2 bench scales (CAS Corporation, Seoul, South Korea), Model N: MW-113000, is used for weighing test samples. Model U17-EKG, (Zernotekhnika, Moscow, Russia) is installed to determine feed pellets crumbling. Round laboratory sieves with a stainless-steel shell with a cell size of – 3.0 mm and a diameter of – 300 mm (IP Sedov A. B., Moscow, Russia) are used to separate destroyed granules from unresolved ones.
Glass container for pouring the analyzed sample and weighing.

Laboratory Methods

The crumbling of granules was determined by GOST 28497-2014 “Feed, mixed feed.

The specific power consumption was determined using a wattmeter device.

Description of the Experiment

Sample preparation: 16 samples were analyzed, with two repeated analyses and two experiment replications for each sample.

Number of samples analyzed: 16.

Number of repeated analyses: 2.

Number of experiment replication: 2.

Design of the experiment: The development of experimental compound feed for broilers according to the developed recipes using optimal granulation modes was carried out at the Agrofit LLP plant in Konaev, Almaty region. Complete set of the granulation line at the plant from the Chinese company "HENAN RICHI MACHINERY CO.LTD". A press granulator with a vertical ring die was used for granulating the feed. The diameter of the holes in the matrix of the press granulator was 3.5 mm.

The technological process of granulation was carried out in the following sequence. The finished mixture was fed into the dispenser of the granulator press. It was the dosing of the amount of the mixture by changing the speed of the screw. Next, the mixture entered the mixer. Here, it is thermally treated with steam. The prepared mixture was fed into the press unit. In the press unit, the matrix rotated, and the press rollers rotated on their axis each. The mixture was drawn into the gap between the matrix and the rollers and pressed into the holes in the matrix. As a result, the formation of granules occurred. The size of the holes in the matrix determined the granule diameter. The length of the granules was adjustable. The granules were cut to the desired length with a shear knife. The process of granule formation took place at a temperature of 80 °C.

Data processing and all necessary calculations were done using the PLAN sequential regression analysis program developed at the Odesa National Technological University.

The essence of sequential regression analysis is that the least squares method, implemented in matrix form, calculates the regression coefficients, checks by Student's criterion t and x significance, the coefficient with the minimum ratio of its magnitude to the critical value is removed from the insignificant ones, and then the regression coefficients are recalculated. This cyclic procedure ends when only significant regression coefficients remain in the equation. Then, according to the Fisher criterion F, the adequacy of the obtained regression equation to the experimental data is checked **[19]**.

To determine the dispersion of reproducibility (experimental errors), 3 parallel experiments were carried out in the center of the experiment.

The calculations of the regression coefficients were carried out using matrices in natural dimensions; accordingly, the equations themselves were also obtained in natural dimensions. The adequacy of the mathematical model was tested based on the Fisher dispersion coefficient **[20]**. Fisher's test is an important statistical tool for model validation and analysis of variance.

Compound feeds for broilers are high in protein and high in energy and, therefore, must contain highly digestible components at a fat level of no more than 8%, with a higher percentage of the presence of fatty substances; granules with sufficient strength cannot be prepared.

The content of fats in raw materials influences the feed granulation process and results. The pressure exerted on the particles of plant materials during granulation leads to fats and oils moving to their surface **[21]**. The surface layer of lipids acts as a lubricant, reducing the friction in the spinneret and thereby reducing the pressure of granulation and energy costs **[22]**. Fat reduces the contact of raw materials with the walls of the die channel, facilitating the passage of feed through it and thereby reducing its compaction.

Therefore, pelleted feed should contain a certain amount of fat – at least 2% [**23]**. Such raw materials are meal, cake, wheat, and corn gluten.

The composition of the complete compound feed for broilers developed by us contains grain crops (up to 60%), linseed cake, meal, and oils (up to 35%), mineral raw materials (up to 5%), oils were applied to the granules by spraying after the granules left the granulator.

The crumbling of granules was determined by GOST 28497-2014 “Feed, mixed feed. Method for determining the crumbling of granules "on the device U17-EKG. The grinder is a two-chamber box with dividers that affect the feed sample during its rotation. To do this, two portions of 500.0 ±0.1 g of finished granular feed were placed in the installation chamber. After that, the device was turned on, and the camera was rotated.

The pellet chamber is rotated for 10 minutes at 50 rpm. Then, the device automatically turns off; the contents are poured and weighed with an error of ±0.1 g.

The crumbling was calculated using the formula:

$$K = \frac{m_1 - m_2}{m_1} \times 100\% \quad (1)$$

Where:

m_1 is the mass of granules before testing, g; m_2 – the mass of granules after testing, g.

The arithmetic mean of the results of two parallel determinations was taken as the final test result. The specific consumption was understood as the obtained value of the cost of electricity per unit of feed granulation, determined by the formula:

$$q = \frac{w}{\mathrm{M}} \quad (2)$$

Where:

w – the actual consumption of electricity for the production of granulated feed in the amount of M (tons per hour).

The mathematical model was compiled using a multifactorial experiment. As factors were chosen: the moisture content of the feed mixture and steam pressure. For the output criteria, the indicator of the quality of the granules (crumbiness) and the specific energy consumption for granulation were taken. To obtain a mathematical model of the process of getting granules in the form of a polynomial of the second degree, a 2-factor 4 c fh-level plan was implemented.

Statistical Analysis

The obtained experimental results were processed using methods for planning multivariate experiments based on the least squares method and subsequent regression analysis, which included calculating regression coefficients, assessing their significance, and checking the adequacy of the resulting regression equation. Based on the research, regression equations were obtained that adequately (according to the Fisher criterion) describe the dependences of the above indicators of the quality of granulated feed y1 and specific energy consumption y2 on the factors W and P that affect them.

RESULTS AND DISCUSSION

The granulation process is a complex technological process, the results of which depend on the influence of many mechanical, thermal, and technological factors. Its mathematical description is also complex.

The parametric diagram of the granulation process, performed in our research is shown in Figure 1. The input factors of the X group influence the granulation process, represented by the moisture content of loose feed W and steam pressure P **[24]**, **[25]**.

As the output criteria Y, normalized indicators of the quality of granules are taken – the crumbling of granules y_1 and specific electricity costs y_2. Disturbing factors, designated as Z (mixed feed recipe, granulation temperature, steam consumption, granule diameter, etc.) **[26]**, were the same (stable) in the experimental studies.

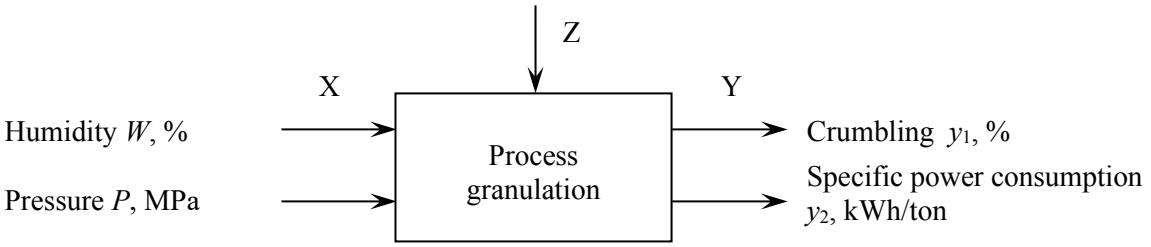


Figure 1 Parametric diagram of the granulation process.

At the first, experimental, research stage, the dependences of the main indicator of the quality of crumbling granules y_1 and the specific energy consumption for the granulation process y_2 on the granulation modes - the moisture content of loose feed W and steam pressure P were studied.

At the second, analytical, stage, based on the results of the experimental studies, regression equations were obtained that describe the change in the crumbling of granules and the specific energy consumption for the granulation process from the granulation modes.

At the third stage of research, based on the obtained mathematical models of the granulation process, an analysis was made of the influence of the studied factors W and P on the crumbling of the obtained granules (y_1) and the specific power consumption for granulating feed (y_2), and determined the optimal granulation modes that provide at minimum specific power consumption normative indicators of crumbling of granules.

Taking into account the complex dependence of the quality indicators of granules and the specific energy costs on the granulation modes, a four-level plan of experiments was drawn up, in which the moisture content of the loose feed was changed in the range of 13-19 % with a step of 2%, and the steam pressure in the range of 0.1 ... step 0.1 MPa. Such a multi-level plan will make it possible to obtain quadratic equations that describe complex nonlinear dependencies between the quality of granules and the specific energy consumption for their production, as well as to optimize granulation modes.

To reduce the influence of uncontrolled parameters on the results of experiments, the experiments were randomised using tables of random numbers **[27]**.

The general form of quadratic regression equations for 2 factors is as follows:

$$y_i = b_0 + b_1 W + b_2 P + b_{11} W^2 + b_{22} R^2 + b_{12} W P \quad (3)$$

Where:

y_i – i - th criteria of optimality: y_1 – crumbling, %; y_2 – specific electricity consumption for granulation, kW.h/t; b_0, …, b_{12} – regression coefficients determined by the least squares method based on experimental data; W – moisture content of loose feed, %; P – steam pressure, MPa.

The regression coefficients included in equation (2) characterize the influence of the first (W) and second (P) factors on the output criteria – granule crumbability y_1 and specific energy costs y_2. In this case, coefficients b_1 and b_2 reflect the linear nature of the influence of factors W and P, respectively, coefficients b_{11} and b_{22} are non-linear, and coefficient b_{12} is a joint pair interaction of the studied factors W and P. The "minus" signs in front of the coefficients indicate a decrease in the values of the output criteria y_1 and y_2 with an increase in the corresponding factors W or P, and the plus signs indicate, on the contrary, an increase in the criteria y_i with an increase in W or P. The detailed nature of the influence of factors is discussed further when analyzing the corresponding regression equations, and a clearer idea of the nature of the influence factors W and P is given in Figures 2-4.

Compiled for the studied criteria y_1 and y_2, the mathematical models allow predicting changes in feed crumbling and specific energy consumption depending on the values of the factors W and P.

W and P were formulated and solved, providing the minimum power consumption for the granulation process at a normalized value of the crumbling of granules – no more than 22%. At the same time, two-sided restrictions (limits of change) were imposed on the values of the factors W and P, equal to the conditions of the experiments in the matrix of experiments.

To visually illustrate the nature of the dependence of both optimization criteria on the factors influencing them, the resulting regression equations were presented in the form of surfaces and response isolines.

Matrix of the experiment plan with the conditions of the experiments and results of the experimental determination in each experiment of crumbling y_1 and specific energy costs for the granulation process y_2 are given in Table 1. The same table also shows the calculated values $\hat{y}_1$ and $\hat{y}_2$ obtained from adequate regression equations, which are given below in Tables 2 and Table 3.

Table 1 Conditions and results of experiments to study the dependence of the crumbling of granules and the specific energy consumption for the granulation process, depending on the modes of granulation.

Experience number	Experience conditions		Experimental results		Calculation results	
	W, %	*P*, MPa	y_1, %	y_2, kW.h/t	$\hat{y}_1$, %	$\hat{y}_2$, kW.h/t
1	13	0.1	26.42	14.41	27.03	14.67
2	15	0.1	23.89	13.68	22.16	13.00
3	17	0.1	20.63	12.84	20.68	12.23
4	19	0.1	21.49	13.02	22.59	12.35
5	13	0.2	25.80	13.86	25.99	14.23
6	15	0.2	23.08	13.17	21.12	12.56
7	17	0.2	18.45	10.46	19.64	11.79
8	19	0.2	21.08	11.75	21.54	11.91
9	13	0.3	23.56	12.91	24.94	13.50
10	15	0.3	22.33	12.74	20.07	11.84
11	17	0.3	15.70	9.35	18.59	11.06
12	19	0.3	22.36	11.08	20.50	11.18
13	13	0.4	23.19	12.57	23.90	12.48
14	15	0.4	21.73	12.03	19.03	10.82
15	17	0.4	13.03	9.08	17.55	10.05
16	19	0.4	22.04	10.89	19.45	10.17

A comparison of experimental and calculated values of granule crumbliness and specific energy consumption for their granulation shows some discrepancies (discrepancies) between them caused by the influence of some random and unaccounted factors, designated as Z in the parametric diagram (see Figure 1). However, these discrepancies are expressed by the average relative error (Mean Relation Percentage Error) is 2.51% for granule crushability and 5.67% for specific energy costs, which is quite acceptable for technical calculations.

As seen from Table 1 of the results of experimental studies, an increase in the humidity *W* of loose feed at a constant pressure *P* first leads to a decrease in granule crumbliness, and then to its increase. Thus, having a mathematical dependence of the crumbability of granules y_1 on the factors *W* and *P*, it is possible to calculate by calculation the values of *W* and *P* at which the crumbability will be minimal.

The humidity of bulk feed has a similar effect on the specific energy costs during granulation – increasing humidity at constant pressure first reduces energy costs y_2 then increases them. The presence of an equation that describes this pattern will make it possible to determine the values of *W* and *P* that provide the lowest specific energy consumption for the granulation process.

However, due to the likely presence of the effect of the mutual influence of factors *W* and *P* on both the crumbability of granules and specific energy consumption, it is possible to solve the optimization problem using the obtained mathematical models – to find such values of *W* and *P* at which there will be minimal energy consumption and the crumbability of granules will not exceed the normalized value meaning. This is an urgent task and is the goal of this work.

Using the PLAN sequential regression analysis program described above, the regression coefficients b_i, their confidence intervals ε_i, and some statistical characteristics of the equations for describing the dependence of the crumbling of granules y_1 and specific energy costs for granulation $\mathbf{y}_2$ on humidity *W* and steam pressure *P* during granulation of loose mixed fodder.

Summary data on characteristics of regression coefficients b_i and their confidence intervals ε_i are given in Table 2.

Table 2 Summary of characteristics of regression coefficients b_i and their confidence intervals ε_i.

Criteria	b_i / ε_i	Indices of coefficients b_i and their confidence intervals ε_i					
		0	**1**	**2**	**11**	**22**	**12**
y_1	**All coefficients b_i and their corresponding confidence intervals ε_i**						
	b_i	147.56	-14.618	-31.640	0.4234	0.3750	1.313
	ε_i	64.72	7.99	80.88	0.247	98.982	3.959
	Significant coefficients b_i and their confidence intervals ε_i						
	b_i	142.29	-14,290	-10.445	0.4234	-	-
	ε_i	62.56	7.931	8.853	0.2475	-	-
y_2	**All coefficients b_i and their corresponding confidence intervals ε_i**						
	b_i	47.50	- 3.847	- 10.80	0.1116	20.00	– 0.4390
	ε_i	28.84	3.56 2	36.04	0.110 3	44.11	1.7645
	Significant coefficients b_i and their confidence intervals ε_i						
	b_i	47.39	-3.956	-	0.1116	-14.55	-
	ε_i	27.87	3.534	-	0.1103	7.77	-

The confidence intervals ε_{bi} given in Table 2 were determined by the expression:

$$\varepsilon_{bi} = tS_e\sqrt{c_{ii}},$$

Where:
t – is the Student's test with a confidence level of p = 0.95; S_e – root mean square dispersion (error) of experiments; c_{ii} – diagonal elements of the dispersion matrix.

The significance of regression coefficients was assessed by observing the ratio $| b_i | \geq \varepsilon_{bi}$.

The table shows two variants of the values of regression coefficients for crumbability y_1 and specific energy costs y_2, calculated using the PLAN program. The first option shows all 6 coefficients, including the insignificant ones, and the second option shows only the significant ones, obtained after sequentially eliminating the insignificant ones and recalculating the remaining regression coefficients in the equation. From Table 2 the exclusion of insignificant coefficients leads to a change in the numerical values of both the coefficients bi and their confidence intervals ε_{bi} remaining in the equation.

Thus, the use of sequential regression analysis made it possible to obtain simplified adequate quadratic models of the granulation process. The regression equations in natural variables obtained based on processing the results of the experiments are summarized in Table 3. The same table shows the mean square errors of the experiments S_e and inadequacy $S_{n.ad}$, as well as the calculated F_p and critical F_{kp} values of the Fisher criterion, indicating that both equations obtained adequately describe the experimental data at a confidence level $p = 0.05$ (i.e., 5%).

Table 3 Regression equations in natural variables describing the dependencies of granule crumbling and specific energy consumption on granulation modes.

Regression Equations in Natural Variables	Standard deviation		Fisher's criterion	
	Experimental, S_e	**Inadequacy, $S_{n.ad.}$**	**Settlement, Fp**	**Critical F_{kp}**
Crushability of granules, %				
$y_1 = 142.29 – 14.290\ W – 10.445P – 0.4234\ W^2$	0.92	2.31	6.28	19.41
Specific electricity consumption, kW.h/t				
$y_2 = 47.39 – 3.956\ W + 0.1116\ W^2 – 14.55P^2$	0.41	0.94	5.31	19.41

Based on the obtained regression equations, the calculated crumbling values were determined, $\hat{y}_1$ and swelling $\hat{y}_2$ granules for each experience are given above in Table 3.

Analysis of the obtained regression equations shows the following. The crumbling of granules y_1 and the specific energy consumption y_2 depend on both studied factors W and P. However, in the equation for the crumbling of granules y_1, the quadratic coefficient b_{22} for the factor P and the coefficient b_{12} were statistically insignificant pairwise interactions of factors W and P.

A significant non-linear (quadratic) effect on the crumbling of granules at $p = 0.05$ is exerted by the moisture content of loose feed W (coefficients b_1 and b_{11}). The effect of pressure on the crumbling of granules is linear (coefficient b_2).

In the equation for the specific cost of electricity for granulation of loose mixed fodder y_2, the coefficient b_2 and the coefficient of pair interaction b_{12} turned out to be insignificant factors W and P.

The remaining coefficients in both equations are significant at $p = 0.05$.

Significant quadratic coefficients indicate that there are extrema – minima y_1 and y_2 for negative humidity coefficients (b_{11}) and maximum y_2 for a positive vapor pressure coefficient (b_{22}).

The non-linearity of the regression equations and the fact that they are obtained in natural variables make it difficult to unambiguously assess the degree of influence of each of the considered factors W and P on the crumbling of granules and the specific cost of electricity for granulation.

Getting a better understanding of the influence of factors W And P on the criteria y_1 and y_2 can be based on the response surfaces (Figures 2 and 3) and isolines (Figures 4 and 5) built based on the regression equations given in Table 3.

Analysis of the response surface in Figure 2 clearly shows that with an increase in the moisture content of loose feed from 13 to 19%, the crumbling of granules first decreases according to a parabolic law. Then, upon reaching a minimum (at $W = 16.87\%$), it begins to increase.

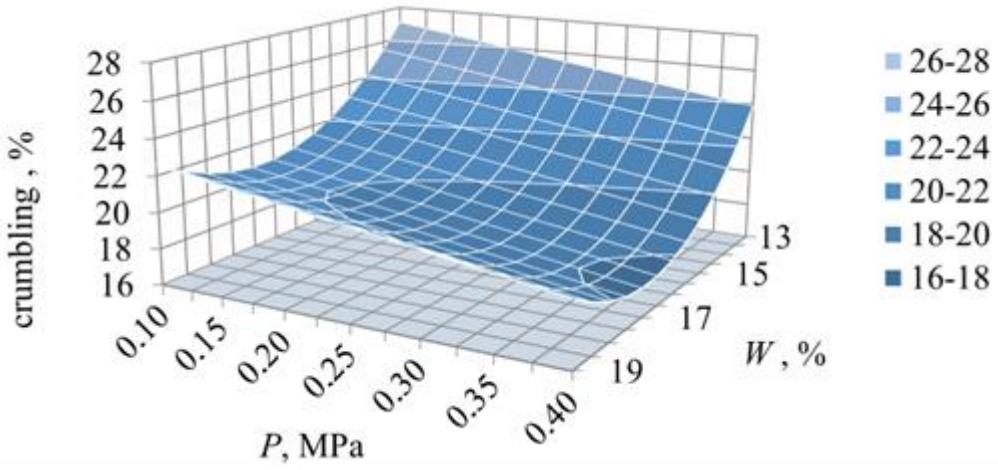


Figure 2 The response surface of the dependence of the crumbling of granules on the moisture content of loose feed and steam pressure during granulation.

It can also be seen that, regardless of the feed's moisture content, the granules' crumbling with increasing steam pressure gradually decreases according to a linear law and reaches the lowest value of 17.52% at $W = 16.87\%$ and $P = 0.4$ MPa.

The area of admissible values of the moisture content of loose compound feed and steam pressure during granulation, marked in green, is also clearly visible, in which the normalized crumbling of granules is ensured, not exceeding 22 %.

A clearer picture of the values of W and P, which provide the crumbling of granules up to 22%, can be seen in Figure 3, which shows the isolines of the dependence of the crumbling of granules on the indicated factors W and P. It is also clearly seen that at $W = 19\%$, the permissible crumbling of granules can be ensured at P not less than 0.157 MPa. On the other hand, at $P = 0.10$ MPa, the permissible crumbling of granules can be ensured at W in the range of 15.11-18.94%.

Any intermediate values of the factors W or P at which the crumbling of the granules will not exceed 22 % can be determined from the above regression equation for y_1 by setting one of the factors and calculating the other. Less accurately, the same can be determined from Figure 3.

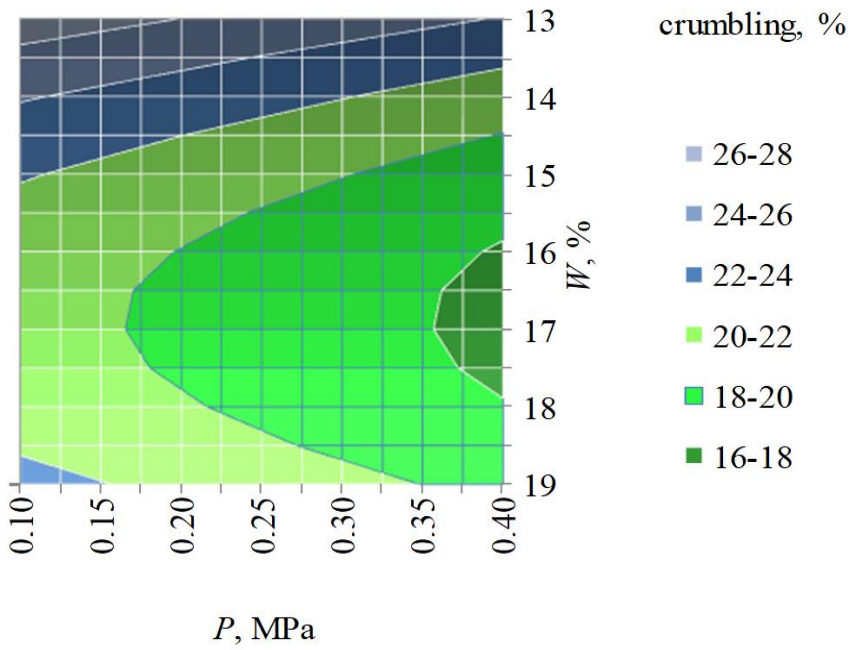


Figure 3 Isolines of the dependence of the crumbling of granules on the moisture content of loose mixed fodder and steam pressure during granulation.

Considering the response surface and isolines shown in Figure 4 and Figure 5 of the dependence of the specific energy costs for the granulation process on the moisture content of loose mixed fodder W and steam pressure during granulation P, their nonlinear (quadratic) nature is visible, determined by significant coefficients b_{11} and b_{22} in the equation for y_2.

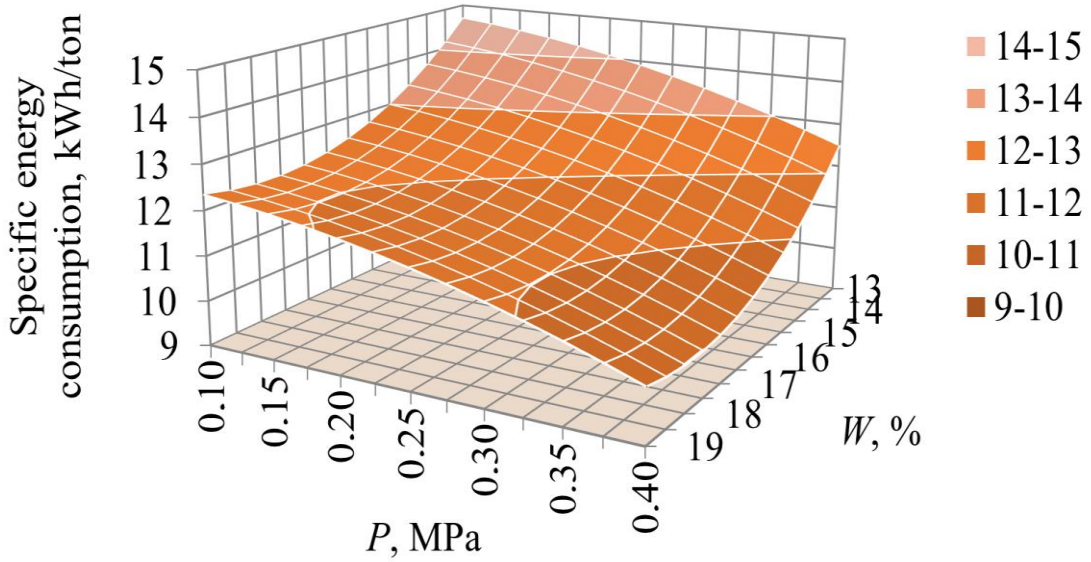


Figure 4 The response surface of the dependence of the specific energy consumption for the granulation process on the moisture content of loose feed and steam pressure during granulation.

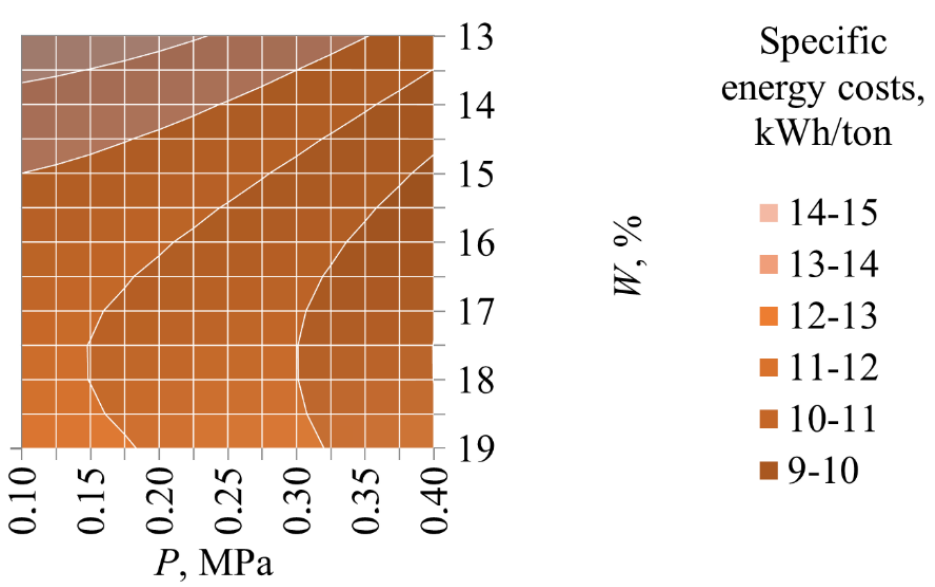


Figure 5 Isolines of the dependence of the specific energy consumption for the granulation process on the moisture content of loose feed and steam pressure during granulation.

Due to the significant negative parabolic dependence of the specific energy consumption for the granulation process y_2 on the steam pressure P, its increase, regardless of the moisture content of the loose feed W, leads to a gradual decrease *in* y_2, reaching the minimum values of energy consumption at $P = 0.4$ MPa. The same nature of the dependence of y_2 on the factors W and P is more accurately seen in Figure 5. It can be seen that the minimum energy consumption for the granulation process is provided at a vapor pressure of $P = 0.4$ MPa in the moisture range of loose mixed fodder $W = 17.50$-18.00%.

A joint analysis of the isolines in Figure 3, where green tints highlight the area of admissible values of crumbling granules $y_1 \leq 22\%$, and in Figure 5, which shows the isolines of changes in the specific energy consumption for the granulation process y_2, shows that at steam pressure $P = 0.4$ MPa and moisture content of loose feed $W = 17.50$-18.00%, the crumbling of granules will be in the acceptable range equal to 20.84-21.21%.

Using the equations of dependencies y_1 and y_2 on the granulation modes (factors W and P) given in Table 3, the optimization problem was solved to determine the optimal granulation modes W and P that provide the minimum energy costs for granulation y_2 with a crumbling of granules of not more than 22%.

To determine the technological modes of granulation of loose mixed fodder, under which the minimum energy costs will be ensured, the following equation was taken as the objective function:

$$y_2 = 47.39 - 3.956\,W + 0.1116\,W^2 - 14.55P^2 \rightarrow min \quad (4)$$

The equation of the dependence of the crumbling of granules on the modes of granulation was taken as a limitation on the normalization of the quality of the granules:

$$y_1 = 142.29 - 14.290\,W - 10.445P - 0.4234\,W^2 \leq 22\% \quad (5)$$

Restrictions on the modes of granulation of loose feed were taken equal to the ranges of changes in the conditions of experiments in the matrix of experiments:

$$13\,\% \leq W \leq 19\%;\ 0.1\ \text{MPa} \leq P \leq 0.4\ \text{MPa} \quad (6)$$

Using the obtained system of equations and inequalities (4)-(6), the optimal technological modes of granulation of loose mixed fodder were determined by the method of nonlinear programming, subject to all restrictions:

– moisture content of loose mixed fodder $W^{opt} = 17.73\%$;

– steam pressure during granulation $P^{opt} = 0.4$ MPa.

These modes provide, when granulating loose mixed fodder, the minimum energy costs equal to $y_2^{opt} = 9.985$ kWh/ton, and the normalized value of the crumbling of granules, equal to $y_1^{opt} = 20.99\%$.

An experimental batch of granulated mixed fodder was developed with the optimal values of the factors, the moisture content of loose mixed fodder of 17.73%, and the steam pressure during granulation of 0.4 MPa. The experimental value of the crumbling of the granules was 20.11 %. The specific electricity consumption was 9.23 kWh/t. The experiments showed that the discrepancies between the experimental and calculated data are insignificant and are within the error of the experiments for determining these indicators.

Many researchers report that broilers fed pelleted feed had higher body weight and better feed conversion **[28]** than broilers fed loose feed **[29]**, **[30]**, **[31]**, **[32]**, **[33]**. Compared to a loose diet, a pelleted diet improves egg production, shell strength, and egg white quality **[34]**. When using granulated feed, the digestibility of protein (by 2.7%), fat (by 0.2%), and nitrogen-free extractive substances (by 5.3%) increased, as well as the use of nitrogen,

calcium, and phosphorus **[35]**. Other benefits of granulation include reduced ingredient separation, ease of handling, improved feed flow in equipment, reduced formulation cost by incorporating alternative ingredients, and reduced caloric intake. Compared to mash, pellets improve bird performance by reducing feed wastage, facilitating selective feeding, eliminating pathogens, improving palatability, and increasing nutrient uptake. One of the disadvantages is that granulation costs about 10% more than the production of loose feed mixture **[36]**.

The positive effect of pelleting on broiler performance is partly due to improved nutrient digestibility **[37]**, increased feed consumption **[38]**, and increased broiler resting time, which favors lower energy expenditure in maintaining and increasing the availability of net energy for production **[39]**. However, this better performance can only be achieved if the pellets maintain their integrity until the birds ingest them.

Conditioning is the most critical step in manufacturing a quality pellet **[40]**. Steam conditioning represents a manipulable thermo-mechanical processing variable **[41]**. To obtain a good quality granulated feed, it is necessary to precondition the raw material, which is ensured by moisturizing it and changing its structure **[42]**. During conditioning, the hot steam breaks down the structure of the starch, causing it to gelatinize **[43]**, and allowing the feed particles to bind, resulting in strong granules. Applications of steam in animal feed manufacturing have long been recognized as a good way to produce high-quality pellets **[44]**.

During conditioning, steam enhances particle adhesion, improving pellet **[45]** quality, as documented by Buchanan and Moritz **[46]**. With the right conditioning process, the granules have high strength, the consumption of energy used for their production is reduced, and the wear of the dies is also reduced **[47]**. Heat applied during conditioning may aid in the destruction of pathogens (i.e., Salmonella) and anti-nutritive factors found in certain ingredients (i.e., trypsin inhibitor in soybean meal) **[48]**.

High dietary fat content may result in less durable pellets **[49]**. Fat reduces the contact of the meal with die-hole walls, facilitating feed passage through the die and thereby reducing feed compaction inside the die holes **[50]**. The addition of fat before conditioning causes partial encapsulation of feed particles and hinders the penetration of steam, which thus reduces starch gelatinization and weakens capillary adhesion forces **[51]**.

Understanding how to optimize pellet quality through precision thermo-mechanical processing may impact broiler performance, nutrient availability, and, thus, the cost of production **[52]**. Choosing proper conditioning process parameters could save electrical energy consumption in the pelleting process and achieve targeted pellet quality **[53]**.

If the granulation technology is violated, the feed granules can be of high humidity and temperature and easily break down. The right moisture content value will maximize the quality of the pellets and increase the value of the Pellet Durability Index (PDI). Many factors affect the moisture content in the feed, such as changing the steam pressure configuration, adding moisture to the mixing process, changing the retention time configuration, and other methods **[54]**.

The ratio of the individual feed components in terms of their ability to interact with each other is also important. Hydrophobic and gyrophilic components are poorly retained among themselves in the composition of the granules. Often, the strength of the granules is sought to be increased by using very high granulation temperatures (above 85 °C) and high steam pressure. The performance of a feed mill is often attempted to be increased by rapidly cooling the pellets **[55]**.

It should be understood that such methods of increasing productivity and increasing the strength of granules are fraught with a sharp deterioration in their quality. The granules become very dense with sharp edges. Feeding such feed leads to trauma to the bird's oral cavity and damage to the cuticle of the muscular stomach **[56]**. Such granules, as well as fine grinding of loose compound feed, sharply increase the viscosity of the feed in the intestinal contents, which leads to a decrease in digestibility and assimilation of the feed, as noted above when characterizing loose compound feed.

Koshak and Koshak **[57]** studied in detail the effect of the composition of compound feed for poultry on the specific energy intensity of the granulation process. They found that an increase in the grain content in the feed by 35.16% leads to an increase in the specific energy intensity of the process by 60.13%. An increase in the content of meals and oils in feed by 7.2% causes a decrease in specific energy consumption by 18.1%.

The formulation of a pelleted diet has always been considered to be one of the most important factors that influence pellet durability. It is also highly influential in energy consumption as many ingredients are known to improve or diminish production capacity dramatically. This can be due to the presence, or lack thereof, of lubricating factors, the ability of ingredients to scour or “polish” the pellet dies, or because the bulk density of an ingredient requires the mill to exert an excess amount of energy to compress the material before it can be extruded through the die **[58]**.

Therefore, when developing compound feed formulas, it is necessary to consider the optimal content of meals and oils, both in terms of the exchange energy of the compound feed, its nutritional value, digestibility, and in terms of the specific energy intensity of the granulation process.

With a decrease in palatability, one should first study the physical and mechanical properties of the feed and evaluate them from the point of view of optimality **[59]**. Unsatisfactory physical and mechanical characteristics of the feed mixture or compound feed can drastically reduce the rate and degree of nutrient intake **[60].** Heterogeneous grinding of food makes the bird choose first particles of food 0.5-1.4 mm in diameter, then larger ones. The bird almost does not consume flour and grain dust, finely ground to 0.2 mm feed components. Pulverity and heterogeneity of feed reduces nutrient intake in chickens by 15-19% and in adult hens by 10-14% **[61]**. Sorting the feed by the bird during its consumption negates the computer's accuracy in calculating the feed ratio **[62]**.

As a result of solving the optimization problem, the optimal values of the input parameters were obtained, which made it possible to obtain high-quality mixed fodders. As a result of a comprehensive assessment of the quality of complete granulated feed, it was found that the resulting feed met the requirements for feed for poultry.

CONCLUSION

Mathematical modeling of the granulation process of loose compound feed for broilers made it possible to solve an important practical problem of optimizing the granulation modes, which ensures the production of granules of the required quality with minimal energy consumption. With the optimal values of the factors obtained in the study using the experimental planning method, an experimental batch of granulated feed was developed. At the selected levels of factors, the calculated value of the crumbling of the granules was 20.11%. The specific electricity consumption was 9.23 kWh/ton. The experiments showed that the discrepancies between the experimental and calculated data, respectively, the crumbling of 20.99% and the specific power consumption of 9.985 kWh/ton, are insignificant and are within the error of the experiments for determining these indicators. The conducted studies and modeling of the granulation process of loose compound feed made it possible to find out the nature of its moisture content's influence on granules' quality, as well as to determine the optimal granulation modes that provide normalized crumbling of granules. Granulation of mixed fodder using the optimal granulation parameters obtained using mathematical modeling is recommended: moisten loose mixed fodder up to 17.73% and maintain steam pressure during granulation at 0.4 MPa. The obtained optimal granulation parameters, established using mathematical modeling, can serve as the basis for producing granulated feed for broilers. Numerous studies have reported that the physical form of feed significantly impacts broiler growth and feed intake. Unsatisfactory physical and mechanical characteristics of compound feed can drastically reduce the rate and degree of nutrient intake. The first and most distinct reaction to a change in the physical and mechanical properties of the feed is the reaction to a change in the rate and volume of feed eaten by birds.

REFERENCES

1. Podobed, L. I., & Ponomareva, A. I. (2020). Osnovy korrekcii kormleniya sel'skohozyajstvennoj pticy. In Prakticheskoe rukovodstvo (p. 400). Strata.
2. Podobed, L. I., Laptev, G. Yu., Kapitonova, E. A., & Nikonov, I. N. (2017). Optimizaciya pishchevareniya i proteinovoe pitanie sel'skohozyajstvennoj pticy: uchebnoe posobie dlya studentov vuzov (p. 348). RAJT PRINT YUG.
3. Dozier, W. A., Behnke, K. C., Gehring, C. K., Branton, S. L. (2010). Effects of feed form on growth performance and processing yields of broiler chickens during a 42-day production period. Journal of Applied Poultry Research (Vol. 19, Issue 3, pp. 219–226). Oxford University Press. https://doi.org/10.3382/japr.2010-00156
4. Egorov, I. (2015). Kombikorma raznoj fizicheskoj struktury v kormlenii cyplyat-brojlerov. In Kombikorma (Vol. 4, pp. 52–54). Avtonomnaya nekommercheskaya organizacziya Redakcziya zhurnala Kombikorma.
5. Blagov, D. A., Gizatov, A. Y., Smakuyev, D. R., Kosilov, V. I., Pogodaev, V. A., & Tamaev, S. A. (2020). Overview of feed granulation technology and technical means for its implementation. In IOP Conference Series: Earth and Environmental Science (Vol. 613, Issue 1, p. 012018). IOP Publishing. https://doi.org/10.1088/1755-1315/613/1/012018
6. Massuquetto, A., Panisson, J. C., Schramm, V. G., Surek, D., Krabbe, E. L., & Maiorka, A. (2020). Effects of feed form and energy levels on growth performance, carcass yield and nutrient digestibility in broilers. In Animal (Vol. 14, Issue 6, pp. 1139–1146). Elsevier BV. https://doi.org/10.1017/s1751731119003331
7. Spiridonov, I. P. (2002). Kormlenie sel'skoho-zyajstvennoj pticy ot A do YA (p. 704). Oblastnaya tipografiya.
8. Salih, F. (2019). Effects of commercial pellet and mash feed on performance of broiler chickens. In Benha Veterinary Medical Journal (Vol. 36, Issue 2, pp. 367–372). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/bvmj.2019.14876.1048
9. Podobed, L. I., & Safonov, A. P. (2022). Kormlenie sel'skohozyajstvennoj pticy s novami diagnostiki kormovyh narushenij dlya veterinarov (p. 678). Super.

10. Hancock C. J. (2010). Impact of feed form and nutrient distribution in an automated commercial broiler feeding system [Master dissertation, Kansas State University].
11. Löwe, R. (2005). Judging Pellet Stability as Part of Pellet Quality. In Feed Tech (Vol. 9, Issue 2, pp. 15–19). AFMA Matrix.
12. Mina-Boac, R. J., Maghirang, G., & Casada, M. E. (2006). Durability and Breakage of Feed Pellets during Repeated Elevator Handling. In Presented at ASABE Annual International Meeting, ASABE (Vol. 300, Issue 66044). Portland, Oregon.
13. Quentin, M., Bouvarel, I., & Picard, M. (2004). Short- and Long-Term Effects of Feed Form on Fast- and Slow-Growing Broilers. In Journal of Applied Poultry Research (Vol. 13, Issue 4, pp. 540–548). Elsevier BV. https://doi.org/10.1093/japr/13.4.540
14. Corzo, A., Mejia, L., & Loar, R. E., II. (2011). Effect of pellet quality on various broiler production parameters. In Journal of Applied Poultry Research (Vol. 20, Issue 1, pp. 68–74). Elsevier BV. https://doi.org/10.3382/japr.2010-00229
15. Lilly, K. G. S., Gehring, C. K., Beaman, K. R., Turk, P. J., Sperow, M., & Moritz, J. S. (2011). Examining the relationships between pellet quality, broiler performance, and bird sex. In Journal of Applied Poultry Research (Vol. 20, Issue 2, pp. 231–239). Elsevier BV. https://doi.org/10.3382/japr.2009-00138
16. Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2007). Feed particle size: Implications on the digestion and performance of poultry. In World's Poultry Science Journal (Vol. 63, Issue 3, pp. 439–455). Informa UK Limited. https://doi.org/10.1017/s0043933907001560
17. GOST 28497-2014. (2014). Feeds, compound feeds. Method of crumbling properties granule determination.
18. Koshak, Zh. (2009). Issledovanie energoemkosti processa granulirovaniya pri proizvodstve kombikorma dlya pticy. In Agropanorama (Vol. 2, pp. 28–30). Belorusskij gosudarstvenny`j agrarny`j tekhnicheskij universitet.
19. Ostapchuk, N. V., Kaminskij, V. D., Stankevych, G. N., & Chuchuj, V. P. (1992). Matematicheskoe modelirovanie processov pishchevyh proizvodstv. In Uchebnoe posobie (p. 175). Vishcha shkola.
20. Begimova, A., & Kuznetsova, Yu. (2017). Ob adekvatnosti matematicheskih modelei (On the adequacy of mathematical models). In Materiali IX Mejdunarodnoi studencheskoi nauchnoi konferencii. Studencheskii nauchnii forum.
21. Keysuke Muramatsu, Andréia Massuquetto, Fabiano Dahlke, & Alex Maiorka. (2015). Factors that Affect Pellet Quality: A Review. In Journal of Agricultural Science and Technology A (Vol. 5, Issue 9). David Publishing Company. https://doi.org/10.17265/2161-6256/2015.09.002
22. Stelte, W., Holm, J. K., Sanadi, A. R., Barsberg, S., Ahrenfeldt, J., & Henriksen, U. B. (2011). A study of bonding and failure mechanisms in fuel pellets from different biomass resources. In Biomass and Bioenergy (Vol. 35, Issue 2, pp. 910–918). Elsevier BV. https://doi.org/10.1016/j.biombioe.2010.11.003
23. Mohammadi Ghasem Abadi, M. H., Moravej, H., Shivazad, M., Karimi Torshizi, M. A., & Kim, W. K. (2019). Effect of different types and levels of fat addition and pellet binders on physical pellet quality of broiler feeds. In Poultry Science (Vol. 98, Issue 10, pp. 4745–4754). Elsevier BV. https://doi.org/10.3382/ps/pez190
24. Briggs, J., Maier, D. E., Watkins, B., & Behnke, K. (1999). Effect of ingredients and processing parameters on pellet quality. In Poultry Science (Vol. 78, Issue 10, pp. 1464–1471). Elsevier BV. https://doi.org/10.1093/ps/78.10.1464
25. Thomas, M., & van der Poel, A. F. B. (1996). Physical quality of pelleted animal feed 1. Criteria for pellet quality. In Animal Feed Science and Technology (Vol. 61, Issues 1–4, pp. 89–112). Elsevier BV. https://doi.org/10.1016/0377-8401(96)00949-2
26. Colovic, R., Vukmirovic, D., Matulaitis, R., Bliznikas, S., Uchockis, V., Juskiene, V., & Levic, J. (2010). Effect of Die Channel Press Way Length on Physical Quality of Pelleted Cattle Feed. In Food & Feed Research (Vol. 37, Issue 1, pp. 1–6). Poultry Science Association.
27. Ostapchuk, N. V., & Stankevych, G. N. (2007) Matematichne modelyuvannya na EOM (p. 313). Druk.
28. Lv, M., Yan, L., Wang, Z., An, S., Wu, M., & Lv, Z. (2015). Effects of feed form and feed particle size on growth performance, carcass characteristics and digestive tract development of broilers. In Animal Nutrition (Vol. 1, Issue 3, pp. 252–256). Elsevier BV. https://doi.org/10.1016/j.aninu.2015.06.001
29. McKinney, L. J., & Teeter, R. G. (2004). Predicting Effective Caloric Value of Nonnutritive Factors: I. Pellet Quality and II. Prediction of Consequential Formulation Dead Zones. In Poultry Science (Vol. 83, Issue 7, pp. 1165–1174). Elsevier BV. https://doi.org/10.1093/ps/83.7.1065
30. Amerah, A. M., Ravindran, V., Lentle, R. G., & Thomas, D. G. (2008). Influence of Feed Particle Size on the Performance, Energy Utilization, Digestive Tract Development, and Digesta Parameters of Broiler

Starters Fed Wheat- and Corn-Based Diets. In Poultry Science (Vol. 87, Issue 11, pp. 2320–2328). Elsevier BV. https://doi.org/10.3382/ps.2008-00149

31. Chewning, C. G., Stark, C. R., & Brake, J. (2012). Effects of particle size and feed form on broiler performance. In Journal of Applied Poultry Research (Vol. 21, Issue 4, pp. 830–837). Elsevier BV. https://doi.org/10.3382/japr.2012-00553
32. Abdollahi, M. R., Ravindran, V., & Svihus, B. (2013). Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. In Animal feed science and technology (Vol. 179, Issues 1–4, pp. 1–23). Elsevier BV. https://doi.org/10.1016/j.anifeedsci.2012.10.011
33. Abdollahi, M. R., Ravindran, V., & Svihus, B. (2013). Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. In Animal Feed Science and Technology (Vol. 179, Issues 1–4, pp. 1–23). Elsevier BV. https://doi.org/10.1016/j.anifeedsci.2012.10.011
34. Wan, Y., Ma, R., Khalid, A., Chai, L., Qi, R., Liu, W., Li, J., Li, Y., & Zhan, K. (2021). Effect of the Pellet and Mash Feed Forms on the Productive Performance, Egg Quality, Nutrient Metabolism, and Intestinal Morphology of Two Laying Hen Breeds. In Animals (Vol. 11, Issue 3, p. 701). MDPI AG. https://doi.org/10.3390/ani11030701
35. Kolokol`nikov, N. V., Mezenczev, I. I., Mezenczev, M. I., Chaunina, E. A., & Amiranashvili, E. I. (2019). Ispol`zovanie kombikormov raznoj fizicheskoj struktury` v kormlenii indyushat. In Vestnik Omskogo gosudarstvennogo agrarnogo universiteta (Vol. 33, Issues 1, pp. 99–105). Omskij gosudarstvenny`j agrarny`j universitet imeni P. A. Stoly`pina.
36. Jahan, M. S., Asaduzzaman, M., & Sarkar, A. K. (2006). Performance of broiler fed on mash, pellet and crumble. In International Journal of Poultry Science (Vol. 5, Issue 3, pp. 265–270). Asian Network for Scientific Information. https://doi.org/10.3923/ijps.2006.265.270
37. Zalenka, J. (2003). Effect of Pelleting on Digestibility and Metabolizable Energy of Poultry Diets. In Proceedings of European Symposium on Poultry Nutrition (Vol. 98, Issue 11, pp. 5497–5503). Elsevier. https://doi.org/10.3382/ps/pez176
38. Meinerz, C., Ribeiro, A. M. L., Penz Jr, A. M., & Kessler, A. de M. (2001). Níveis de Energia e Peletização no Desempenho e Rendimento de Carcaça de Frangos de Corte com Oferta Alimentar Equalizada. In Revista Brasileira de Zootecnia (Vol. 30, Issue 6 suppl, pp. 2026–2032). FapUNIFESP (SciELO). https://doi.org/10.1590/s1516-35982001000800011
39. Skinner-Noble, D. O., McKinney, L. J., & Teeter, R. G. (2005). Predicting effective caloric value of nonnutritive factors: III. Feed form affects broiler performance by modifying behavior patterns. In Poultry Science (Vol. 84, Issue 3, pp. 403–411). Elsevier BV. https://doi.org/10.1093/ps/84.3.403
40. Froetschner J. (2006). Conditioning Controls Quality of Pellet. In Feed Tech (Vol. 10, Issue 6, pp. 12–15). AFMA Matrix.
41. Leaver, R. H. (1988). In The pelleting process. SproutBauer.
42. Behnke, K. (1994). Factors Affecting Pellet Quality. In Proceedings of the Maryland Nutrition Conference. (pp. 44–54). Feed Ind. Counc. and Univ.
43. Massuquetto, A., Durau, J. F., Schramm, V. G., Netto, M. V. T., Krabbe, E. L., & Maiorka, A. (2018). Influence of feed form and conditioning time on pellet quality, performance and ileal nutrient digestibility in broilers. In Journal of Applied Poultry Research (Vol. 27, Issue 1, pp. 51–58). Elsevier BV. https://doi.org/10.3382/japr/pfx039
44. Sredanović, S., Lević, J., & Đuragić, O. (2005). Identification of feed raw material hazard properties. In Journal on Processing and Energy in Agriculture (former PTEP), (Vol.9, Issue 5, pp. 120–123). National Society of Processing and Energy in Agriculture.
45. Sredanović, S., & Lević, J. (2000). Conditioning: An important step in feed production. Časopis za procesnu tehniku i energetiku u poljoprivredi. In Journal on Processing and Energy in Agriculture (Vol.4, Issue 3, pp. 82–84). National Society of Processing and Energy in Agriculture.
46. Buchanan, N. P., & Moritz, J. S. (2009). Main effects and interactions of varying formulation protein, fiber, and moisture on feed manufacture and pellet quality. In Journal of Applied Poultry Research (Vol. 18, Issue 2, pp. 274–283). Elsevier BV. https://doi.org/10.3382/japr.2008-00089
47. Braginets, S. V., Bakhchevnikov, O. N., & Deev, K. A. (2023). Influence of various parameters on the vegetable raw material pelleting process and pellets quality (review). In Agricultural Science Euro-North-East (Vol. 24, Issue 1, pp. 30–45). FARC of the North-East named N.V. Rudnitskogo. https://doi.org/10.30766/2072-9081.2023.24.1.30-45
48. Thomas, M., & van der Poel, A. F. B. (1996). Physical quality of pelleted animal feed 1. Criteria for pellet quality. In Animal Feed Science and Technology (Vol. 61, Issues 1–4, pp. 89–112). Elsevier BV. https://doi.org/10.1016/0377-8401(96)00949-2

49. Moritz, J. S., Cramer, K. R., Wilson, K. J., & Beyer, R. S. (2003). Feed Manufacture and Feeding of Rations with Graded Levels of Added Moisture Formulated to Different Energy Densities. In Journal of Applied Poultry Research (Vol. 12, Issue 3, pp. 371–381). Elsevier BV. https://doi.org/10.1093/japr/12.3.371

50. Fahrenholz, A.C. (2012) Evaluating Factors Affecting Pellet Durability and Energy Consumption in a Pilot Feed Mill and Comparing Methods for Evaluating Pellet Durability [Dissertation theses, Kansas State University] (p. 92).

51. Fairfield, D. A. (2003). Pelleting for Profit Part 1. Feed and Feeding Digest, National Grain and Feed Association Part 1, No. 6. Accessed August 2015.

52. Cutlip, S. E., Hott, J. M., Buchanan, N. P., Rack, A. L., Latshaw, J. D., & Moritz, J. S. (2008). The Effect of Steam-Conditioning Practices on Pellet Quality and Growing Broiler Nutritional Value. In Journal of Applied Poultry Research (Vol. 17, Issue 2, pp. 249–261). Elsevier BV. https://doi.org/10.3382/japr.2007-00081

53. Djuro, V., Ivanov, D., Radmilo, C., & Bojana, K. (2010). Effect of steam conditioning on physical properties of pellets and energy consumption in pelleting process. In Journal on Processing and Energy in Agriculture (Vol. 14, Issue 2, pp. 106–108). Centre for Evaluation in Education and Science.

54. Rivai Suhendra F, & Iskandar, I. (2021). Analysis of Retention Time and Steam Pressure Variations in the Conditioning Process on the Moisture Content of Feed in the Packaging Process. In Journal of Mechanical, Civil and Industrial Engineering (Vol. 2, Issue 2, pp. 01–16). Al-Kindi Center for Research and Development. https://doi.org/10.32996/jmcie.2021.2.2.1

55. Podobed, L. I. (2021). Kormovye patologii sel'skohozyajstvennoj pticy Monografiya (p. 201). Akvatoriya.

56. Podobed, L. I. (2020). Kormlenie sel'skohozyajstvennoj pticy pri minimizacii primeneniya antibiotikov (p. 319). Akvatoriya.

57. Koshak, Zh., & Koshak, A. (2012). Vliyanie sostava kombikormov na udel`nuyu e`nergoyomkost` proczessa granulirovaniya. In Kombikorma (Vol. 2, pp. 63–64). Avtonomnaya nekommercheskaya organizacziya Redakcziya zhurnala Kombikorma.

58. Behnke, K. C. (2006). The Art (Science) of Pelleting. In Technical Report Series: Feed Technology (pp. 5–9). American Soybean Assn. International Marketing Southeast Asia.

59. Muramatsu, K., Maiorka, A., Dahlke, F., Lopes, A. S., & Pasche, M. (2013). Impact of particle size, thermal processing, fat inclusion and moisture addition on pellet quality and protein solubility of broiler feeds. In Journal of Agricultural Science and Technology (Vol. 3, Issue 12 A, pp. 1017–1028). CMV Verlag.

60. Svihus, B., & Zimonja, O. (2011). Chemical alterations with nutritional consequences due to pelleting animal feeds: a review. In Animal Production Science (Vol. 51, Issue 7, p. 590). CSIRO Publishing. https://doi.org/10.1071/an11004

61. Podobed, L. I. (2017). Aminokisloty v pitanii sel'skohozyajstvennyh zhivotnyh i pticy. In Monografiya (p. 279). Akvatoriya.

62. Koshak, Zh., & Ivanov, A. (2009). Issledovanie e`nergoemkosti proczessa granulirovaniya pri proizvodstve kombikorma dlya pticzy. In Agropanorama. (Vol. 2, pp. 28–30). Belorusskij gosudarstvenny`j agrarny`j tekhnicheskij universitet.

Funds:

This research was funded by Alel Agro.

Acknowledgments:

-

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Alma Yermukanova**, Almaty Technological University, Department of Bread Technology and Processing Industries, Furkat str., 348/4, 050012, Almaty, Republic of Kazakhstan,
Tel.: +7 775 964 19 90
E-mail: apple_kst@mail.ru
ORCID: https://orcid.org/0000-0003-0821-9835

Podobed Leonid, The Animal Husbandry Institute of the National Academy of Sciences of Ukraine, Department of Nutrition, Physiology of Nutrition and Feed Production, Tvarinnikiv str., 1A, 61026, Kharkiv, Ukraine,
Tel.: +38 067 998 73 15
E-mail: lpodobed1961@gmail.com
ORCID: https://orcid.org/0000-0001-7684-6289

Stankevych Georgii, Odesa National University of Technology, Department of Grain and Feed technology, Kanatna str., 112, 65039, Odesa, Ukraine,
Tel.: +38 066 295 49 98
E-mail: georgii.stn@gmail.com
ORCID: https://orcid.org/0000-0002-0583-8174

Saule Zhiyenbayeva, Almaty Technological University, Department of Bread Technology and Processing Industries, Furkat str., 348/4, 050012, Almaty, Republic of Kazakhstan,
Tel.: +7727396733, +7 707 577 83 87
E-mail: sauleturgan@mail.ru
ORCID: https://orcid.org/0000-0002-2003-8909

Eva Mrkvicová, Mendel University, Department of Animal Nutrition and Forage Production, Zemědělská str., 1665/1, 61300, Brno, Czech Republic,
Tel.: +420 731 454 367
E-mail: eva.mrkvicova@mendelu.cz
ORCID: https://orcid.org/0000-0002-2504-5024

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 10.10.2023
Revised: 20.12.2023
Accepted: 2.1.2024
Published: 9.1.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 36-49
https://doi.org/10.5219/1950
ISSN: 1337-0960 online
www.potravinarstvo.com

Characterization of soy curd residue and full-fat soy flour as protein-based food ingredients

Emmanuel Duah Osei, Abigail Ataa Pokuah, Richard Atinpoore Atuna, Sam Eudes Faisal, Anthony Amotoe-Bondzie, Abdul-Mateni Yussif, Fortune Akabanda, Francis Kweku Amagloh

ABSTRACT

The study investigated the soy curd residue and full-fat soy flour as potential protein-based food ingredients. Standard protocols were used to determine proximate parameters, functional properties, markers of oxidative stability under shelf storage, colour (CIE L* a* b*), and microbial quality of the flours. Commercial *Afayak* soybean varieties were used to prepare soy curd residue flour and two differently treated soy flours, namely full-fat soy flour and cold-water extracted full-fat soy flour. Findings from the study indicate that processing treatment and storage time significantly ($p<0.001$) affected the parameters measured. Cold-water extraction of full-fat soy flour resulted in a significantly ($p<0.001$) higher protein content denoting 1.0, and 1.2-fold than full-fat soy flour and soy curd residue, respectively. Full-fat soy flour showed the highest peroxide, acid, and p-anisidine ($p < 0.001$) under processing and storage conditions. Soy curd residue was the most oxidatively stable among the samples; however, it was noted that cold-water extraction of full-fat soy had better oxidative stability than full-fat Soy flour. After 12 weeks of storage, peroxide and acid values were below the acceptable limit of 10 mEq/Kg and 0.6 mg/KOH/g, respectively. The study supports the hypothesis that the proximate composition, physicochemical properties, and oxidative stability of soy-based flours are affected by the sample processing method and storage time. The study concludes that the samples characterized in this study are oxidatively stable, protein and energy-rich and may be ideal ingredients for food product development with desirable functional properties.

Keywords: soybean, full-fat soy, soy curd residue, oxidative stability, soy protein

INTRODUCTION

In sub-Saharan Africa (SSA), protein-energy malnutrition, iron, zinc, vitamin A, and iodine deficiency diseases are the most common diet-related problems **[1]**. The over-dependence on fast-digesting carbohydrate staple foods in Ghana has led to a greater prevalence of stunting in Ghana **[2]**. Reducing undernutrition among young children has made significant strides. The prevalence of under-five stunting decreased from 28% to 18% between 2008 and 2018 **[3]**.

One possible way to increase the nutritional value of local staples in SSA is to produce low-cost nutritious diets using locally accessible and underutilized cereals, legumes, roots, and tubers **[4]**. Among the legumes, soybean is an inexpensive and high-quality protein source that could increase the level and quality of protein in cereals and other starch-based foods. Soybean is high in vitamins and minerals and contains approximately 40-

45 % protein and 18-22 % oil **[5]**. For instance, soy protein provides important functional qualities and complete digestibility in food systems **[6]**.

Ghana produces an average of 50,000 metric tonnes of soybeans annually, yet only 15 metric tons are consumed **[7]**. Soy curd residue (SCR) is a gluten-free residue of soybean obtained during soybean and tofu processing after extracting aqueous fractions **[8]**. When 1 kg of soybean grains is processed into tofu, approximately 1.2 kg SCR is produced **[9]**. This by-product contains a moisture content, making its disposal an environmental problem due to its susceptibility to putrefaction **[10]**.

In Ghana, most companies that produce soymilk and tofu use SCR, popularly known as okara in Japanese, as animal feed or discard it, contributing to food waste **[11]**. However, SCR is a relatively cheap source of protein known for its good nutritional qualities, including its richness in isoflavones and phenolic compounds, quality protein, and ideal functional properties **[12]**. The protein efficiency ratio was higher in SCR than in other soy products, for example, 2.71 vs 2.11 in soymilk **[13]**.

Soy protein products such as defatted soy flour, tofu, soymilk, full-fat soy flour (FFSF), and SCR are of interest for food applications to improve the nutritional quality of staple foods in SSA. However, soy products contain higher polyunsaturated fatty acids (PUFA) (indicate the concentration), making them easily susceptible to oxidative rancidity and off-flavours, negatively affecting quality and shelf-life **[14]**.

FFSF is more accessible to obtain, but its high content of PUFAs renders it susceptible to oxidative rancidity **[15]**. FFSF-based products have a shorter shelf life, especially when subjected to environmental conditions of high temperatures, sunlight, and relative humidity. However, SCR, as reported by **[16]**, is associated with lower PUFAs that could improve the shelf stability of its products compared with FFSF. Therefore, this study investigated the proximate parameters, functional properties, microbial quality, and oxidative stability of soy curd residue flour and full-fat soy flour as potential protein-based food ingredients.

Scientific Hypothesis

The proximate composition, physicochemical properties, and oxidative stability of soy-based flours used in this study will depend on the sample processing method and storage duration. Protein content and oxidative stability are mainly expected to be significantly affected.

MATERIAL AND METHODOLOGY

Samples

Commercial soybean (*Afayak variety grains) was obtained from a commercial farmer in the* Tamale central market in the Northern region of Ghana for the study.

Chemicals

All reagents used in this study were of analytical grades with high purity. They were obtained from Sigma-Aldrich; Merck KGaA, Darmstadt, Germany.

Animals, Plants, and Biological Materials

Animal and biological materials were not used in this research. The plant material used for this study was *Glycine Max (L.) Merr.*

Instruments

Chroma Meter (CR-400 KONICA MINOLTA INC.; JAPAN), Hot-air dryer, electric grinder (F E 05 High-Speed grinder, China), UV-spectrophotometer (Biobase VK-1000), FOSS the Soxtec™ 8000 extraction unit, FOSS Kjeltec™ 9 Analyser.

Laboratory Methods

Proximate analysis of flour samples

Compositional analysis of the samples was conducted in Ghana at the Food and Nutrition Analytical Laboratory, SARI. The methods described in the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC 2005) International were used to determine moisture content (AOAC 945.39) with slight modification by drying the samples at 105 °C overnight for approximately 12 h instead of 24 h, crude protein (AOAC 945.39), ash (923.03), and crude fat (AOAC 922.06) **[17]**. Total carbohydrate was computed following the formula (1):

Total carbohydrate = 100 – [moisture + crude protein + total ash + crude fat]. (1)

The energy content was calculated using the Atwater factors **[18]**.

Functional properties

Water absorption capacity (WAC)

Two grams of each sample were measured into a 50 mL centrifuge tube, and 10 mL of distilled water was added. The suspension was allowed to stand for 30 min. The suspension was centrifuged at 3500 x g for 30 min (Hettich Zentrifugen model, Rotofix 32 A). The supernatant was discarded, and droplets were cleaned with cotton wool. The weight change was reported as WAC based on the original sample weight, formula (2).

$$\text{WAC (\%)} = \frac{\text{Weight of water absorbed}}{\text{Weight of dry sample used}} \times 100\ \% \quad (2)$$

Oil absorption capacity (OAC)

Two grams of oil were weighed into 50 mL centrifuge tubes, and 10 ml vegetable oil with a specific gravity of 0.99 was added. The mixture was shaken to mix completely, allowed to stand for 30 min, and centrifuged at 3500 x g for 30 min (Hettich Zentrifugen model, Rotofix 32 A). The supernatant was discarded, and droplets were cleaned from the tube. The weight change was computed and reported as OAC, formula (3).

$$\text{OAC (\%)} = \frac{\text{Weight of oil absorbed x specific gravity of oil}}{\text{Weight of dry sample used}} \times 100\ \% \quad (3)$$

Bulk density (BD)

The method described by **[19]** was adopted. Fifty grams (50) g of flour was measured into a 100 m measuring cylinder and tapped to a constant volume, following formula (4).

$$\text{BD} = \frac{\text{Weight of sample}}{\text{Volume of sample after tapping}}\ (\text{g/mL}) \quad (4)$$

Swelling index (SI)

The method prescribed by **[20]** was used to determine the swelling capacity. A graduated cylinder (100mL) was filled to the 10 mL mark with 4g of sample. Distilled water was added to make a total volume of 50 ml. By inverting the cylinder, the head of the graded cylinder was tightly covered and mixed. After 2 minutes, the suspension was inverted again and allowed to stand for 8 min. After 8 min, the sample's volume was measured. The swelling index was computed as below, following formula (5).

$$\text{SI \%} = \frac{\text{Volume after swelling} - \text{volume before swelling}}{\text{Volume before swelling}} \times 100 \quad (5)$$

Foaming capacity (FC)

The approach described by **[21]** was adopted to determine foam capacity (FC). Distilled water (25 mL) was added to 1 g of flour in a falcon tube. The suspension was shaken for 5 min to foam. The volume of foaming after 30 s whipping was calculated using formula (6).

$$\text{FC \%} = \frac{\text{Volume after whipping} - \text{volume before whipping}}{\text{Volume before whipping}} \times 100\% \quad (6)$$

Oxidative stability analysis

Extraction of crude soy Oil from samples and oxidative analysis

The crude oil was extracted from the flours using the method described by **[22]** with modification in hexane expelling time from 1 hr to 2 hr (Figure 1). Oil from respective flours (10 g) was extracted with 30 mL hexane in a 500 mL conical flask using a shaking incubator at 230 rpm for 1 hr after 1 min-long vortexing. After centrifuging the mixture at 3500 g for 5 mins, the supernatant was poured into a clean and labeled flask. The hexane was evaporated from the supernatant in a water bath at 60 °C for 2 h. The oil was extracted from the fresh samples before storage, and further extractions were carried out bi-weekly from the flour samples on shelved storage for oxidative stability studies.

Oxidative stability of the oils was carried out bi-weekly for 12 weeks. Official methods were used to determine oxidative parameters **[23]**. AOCS Cd 8b-90 for peroxide value (PV), AOCS Cd 18–90 for *p*-Anisidine (*p* AV), and AOCS Cd 3d-63 for acid value (AV). Total oxidation was computed using the equation below, formula (7) **[24]**.

TOTOX = ((2 x PV) + p AV). (7)

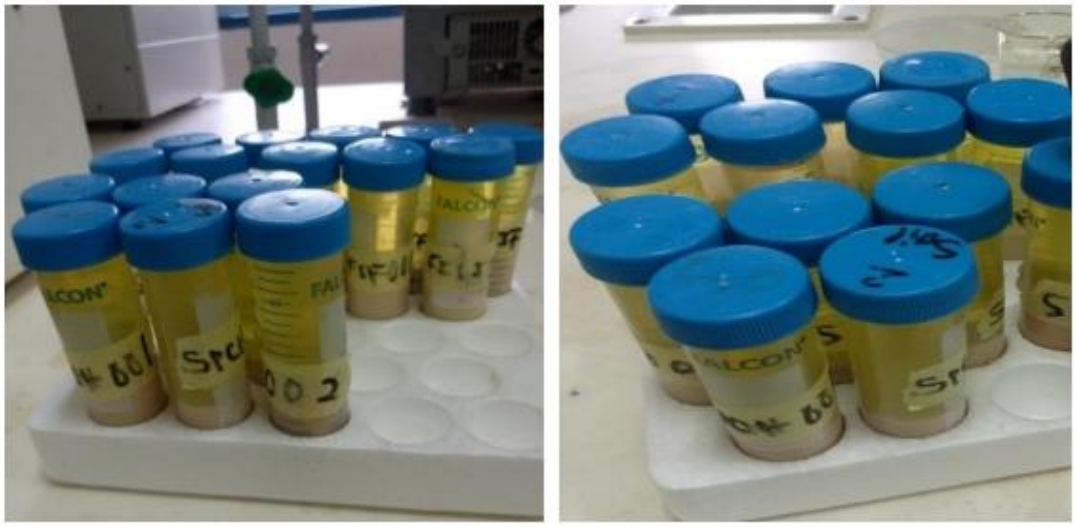


Figure 1 Oil extracted from respective flour samples.

Instrumental colour analysis

Flour samples (30 g each) were measured in a clean petri dish. The color characteristics (L* and chromaticity coordinates) of samples were determined using a Chroma Meter (CR-400 KONICA MINOLTA INC.; JAPAN) as shown in (Figure 2). The browning index was calculated as below using formula (8) **[25]**.

$$BI = 100 \text{ x } \left(\frac{X-0.31}{0.17}\right) \quad (8)$$

$$\text{Where: } X = \frac{(a* +1.75L)a}{(5.645L+a* -3.012b*)} \quad (9)$$

a* = redness or greenness
L* = lightness or darkness
b* = yellow or blue
BI = browning index

Total color difference (ΔE) was computed as below using formula (10) provided by **[26]**.

$$\Delta E = \sqrt{(L*t0 - L*t12)^2 + (a*t0 - a*t12)^2 + (b*t0 - b*t12)^2} \quad (10)$$

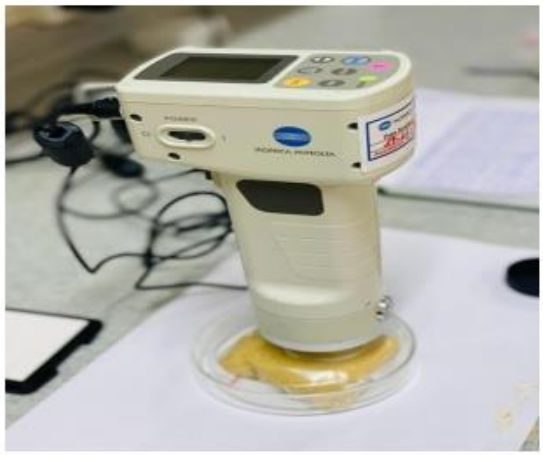


Figure 2 Colour determination of samples using Chromameter *(CR-400 KONICA MINOLTA INC.; JAPAN).*

Microbiological analysis

Ten grams of each sample was homogenized in 90 ml sterile diluent (0.1% peptone, 0.8% NaCl, pH 7.2). Tenfold serial dilutions (10^{-1} to 10^{-9}) were made with the same diluent, and 0.1ml was Spread-plated in duplicates on various media to enumerate isolates. Total viable counts and Coliform bacteria were enumerated on Nutrient agar (NA) and Membrane Faecal Coliform (mFC) media **[27]**, respectively, and incubated at 37 °C for 24 h. Yeasts and Molds were enumerated on Potato Dextrose Agar 9(Merck HG00C100) using a spread plate **[28]**. Plates were incubated for 72 hours at 25 °C. All visible colonies were counted and recorded in cfu/g.

Description of the Experiment

Sample preparation

Soy curd residue flour (SCRF)

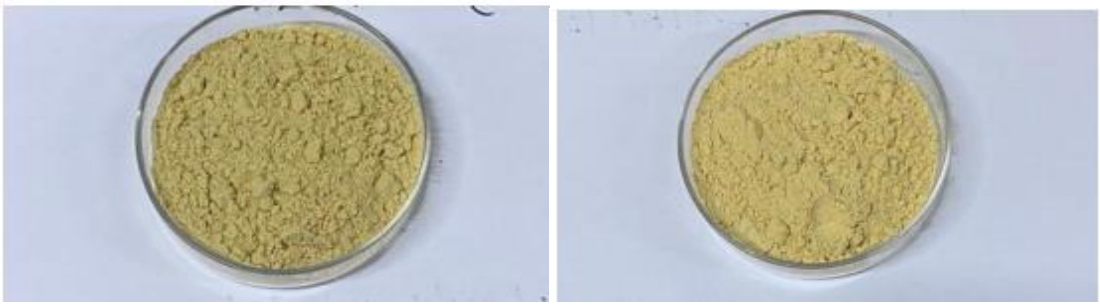
SCRF was produced according to the method described by **[29]** with modifications in soaking time and amount of water used for soaking. Cleaned and sorted soybean grains (7.5 g) were soaked in water (1:4 parts) for 8 h and wet milled with an electric grinder (F E 05 High-Speed grinder, China). The milk was collected, and the residue was oven-dried at 60°C for 12 h. The dried sample (2.9 Kg) was milled into flour (F

Full-fat soy flour (FFSF)

Sorted soybeans (5 Kg) were soaked in water for 8 h, dehulled, and oven-dried at 60°C for 12h. The dried soybeans (3.25 Kg) were winnowed to remove the hulls and milled into full-fat soy flour (FFSF) with a lab-scale grinding mill. The sample was packaged in an air-tight plastic bag, labeled, and stored at room temperature.

Cold-water extracted full-fat soy flour (CFFSF)

Sorted soybeans (10 Kg) were soaked in water for 8 h, dehulled, dried at 60°C for 12 h in a hot air oven, winnowed, and milled into flour (6.6 Kg). The flour was soaked in 1:5 parts of cold water for 5 h. The supernatants were drained off, and the residue was pressed in a cheesecloth and oven-dried for 12 h at 60 °C. The dried sample was milled and sieved to 355 µm using a laboratory sieve to yield cold-water full-fat soy flour (CFFSF). The flour was packaged in an air-tight plastic bag, labeled, and stored at room temperature.



SCRF FFSF

CFFSF

Figure 3 Flour samples.

Note: FFSF = full-fat soy flour, CFFSF = cold-water extracted full-fat soy flour, SCRF = soy curd residue flour.

Number of samples analyzed: 3.

Number of repeated analyses: Except for microbial analysis, which was carried out in duplicate, all other analyses were carried out in triplicate.

Number of experiment replications: 3 reps for each variant.

Design of the experiment:

Soybeans were purchased from the Tamale central market. The soybean was processed into the flour samples described in the sample preparation section. Proximate composition, microbial quality, and functional properties of the samples were determined using standard laboratory protocols. The flour samples were stored on the shelves for 12 weeks. In storage, samples were taken on every second week for colour determination. The flour samples were taken bi-weekly for oil extraction, and oxidative stability analysis was carried out for 12 weeks. The results obtained from the analysis were subjected to statistical analysis, and the validity of our hypothesis was verified.

Statistical Analysis

GenStat Statistical Software Edition 12.0 was used to analyze all the data generated. Data on proximate and functional properties were subjected to a one-way analysis of variance (ANOVA). Oxidative stability and colour data were analyzed using a two-way analysis of variance standardized test was used to separate the means, and a significant difference was determined at $p < 0.05$.

RESULTS AND DISCUSSION

Proximate analysis

Flour samples had moisture contents below 10% (Table 1) and were within levels prescribed by **[30]**. This may be attributed to low water activity. The result suggests that the samples may be shelf-stable since moisture which is an important medium for microbial growth **[31]** was very low in the flour samples.

The ash content of FFSF was significantly higher ($p < 0.001$) by 1.33, and 1.13-fold than CFFSF, and SCRF, respectively. It has been reported by **[32]** that soaking caused a loss of ash content. This might account for the lowest ash content recorded by CFFSF. The higher ash content of the samples suggests that they may be a good source of minerals **[31]**.

Processing treatment also resulted in significantly higher fat content ($p < 0.001$) in CFFSF, which was almost 1.0, and 1.2-fold higher than in FFSF, and SCRF, respectively. The fat content of SCRF aligns with the fat content of 22.3 ± 1.5 g/100 g reported by **[33]**. Higher fat content may contribute to energy density and act as a flavour enhancer **[34]**, however, the higher fat content of the flour samples could affect product stability as unsaturated fatty acids present in the sample are more liable to oxidative rancidity as reported **[35]**.

The protein content of CFFSF was 1.1, and 1.3 times higher ($p < 0.001$) than that of FFSF, and SCRF, respectively (Table 1). The higher protein content of CFFSF may be attributed to the discarding of soluble carbohydrates after soaking the flour in cold water for 5 h and dehulling, which leads to an increase in protein content and a reduction in fiber content **[36]**. The low protein content observed for SCRF may be attributed to the soymilk that was extracted from the raw material during its preparation. The high protein contents of the samples suggest their usefulness for improving the protein content of food products and ameliorating protein-energy malnutrition **[31]**.

Total carbohydrate was significantly higher ($p < 0.001$) in SCRF representing 2.1, and 2.7 times than FFSF, and CFFSF respectively. The high carbohydrate content of SCRF may be due to the non-dehulling of the soybeans during sample preparation. This suggests that SCRF could be used in managing protein-energy malnutrition, as there is a substantial quantity of carbohydrates from which energy can be derived to spare the protein for its primary function **[34]**. In contrast, dehulling and soaking might be the reason for the low total carbohydrate content in FFSF and CFFSF. However, the high carbohydrate content of SCRF derived from the seed hulls might reduce protein digestibility because it contains indigestible fibers that affect the digestibility of protein **[36]**.

CFFSF had the highest total energy, which was 1.0 and 1.1 times higher than FFSF, and SCRF respectively. The energy content of the samples meets the recommended energy values of 360-400 kcal (CODEX), suggesting their suitability as an energy source **[37]**.

Table 1 Proximate composition and energy value.

Flour	Proximate Composition (g/100 g)					
	Total ash	Crude fat	Moisture (%)	Crude protein	Total carbohydrates	Total Energy (kcal/100g)
FFSF	4.98±0.06^{c}	27.55±0.46^{bc}	7.370±0.19^{b}	48.39±0.02^{b}	11.71±0.02^{b}	488.3±0.12^{b}
CFFSF	3.74±0.06^{a}	27.85±0.13^{c}	6.267±0.15^{a}	53.33±0.02^{c}	8.81±0.02^{a}	499.2±0.09^{c}
SCRF	4.40±0.42^{b}	23.22±0.08^{a}	7.041±0.21^{b}	41.22±0.02^{a}	24.12±0.03^{c}	470.3±0.11^{a}
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Values are means ± SD; n = 3. All values are on a dry matter basis. Means with different letters (a, b, c) in the same column represent a significant difference (Tukey's LSD; p <0.05). FFSF = full-fat soy flour, CFFSF = cold-water extracted full-fat soy flour, SCRF = soy curd residue flour.

Functional properties

The water absorption capacity of the flours significantly varied (p<0.001) with SCRF having the highest WAC denoting 1.5, and 1.2 times than FFSF and CFFSF respectively (Table 2). The highest WAC of SCRF might be related to its higher total carbohydrate or fiber content, which favours swelling, water holding, and retention capacities **[38]**. The results show that the flour samples can be useful in the bakery industry, which requires hydration to improve handling qualities.

FFSF had significantly ($p < 0.001$) higher OAC which is 1.1 and 1.2 times than CFFSF and SCRF respectively. High OAC of the samples might improve mouthfeel and flavour retention when the samples are incorporated into food formulations **[36]**.

Generally, the bulk density of the samples was significantly low. FFSF had the lowest bulk density and differed significantly ($p < 0.001$) from CFFSF, and SCRF. Low bulk density is known to promote easy digestibility of food products. The low bulk density of the samples indicates that they may be ideal for complementary food preparations, as reported by **[39]**.

The swelling index differed significantly ($p < 0.001$), with SCRF recording the highest and FFSF the lowest. The lowest swelling index of FFSF may be attributed to dehulling, which reduces water imbibition. The high swelling index recorded for SCRF may be due to its high carbohydrate content. SCRF has been reported to be a good source of dietary fiber, which can increase water-holding capacity **[40]**. A high swelling index has been reported to be ideal for food products that require swelling **[38]**.

The foaming capacity of the samples varied markedly ($p < 0.001$). CFFSF had the highest foaming capacity, SCRF had the highest, and SCRF had the lowest. Overall, the low foaming capacity (5.63% to 11.20%) may be ascribed to the relatively high-fat contents of the samples, as reported by **[38]**. Foaming capacity is preferred in baking and as an active constituent in food preparations **[41]**.

Table 2 Functional properties.

Flour	Functional properties				
	WAC (%)	OAC (%)	BD (g/mL)	SI (mL)	FC (%)
FFSF	141.5±0.46^{a}	117.4±0.01^{c}	0.36±0.07^{a}	1.50±0.05^{a}	9.43±0.01^{b}
CFFSF	167.9±0.21^{b}	106.6±0.02^{b}	0.40±0.04^{b}	1.73±0.02^{b}	11.20±0.18^{c}
SCRF	201.8±0.03^{c}	97.5±0.02^{a}	0.41±0.05^{b}	1.91±0.02^{c}	5.63±0.17^{a}
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

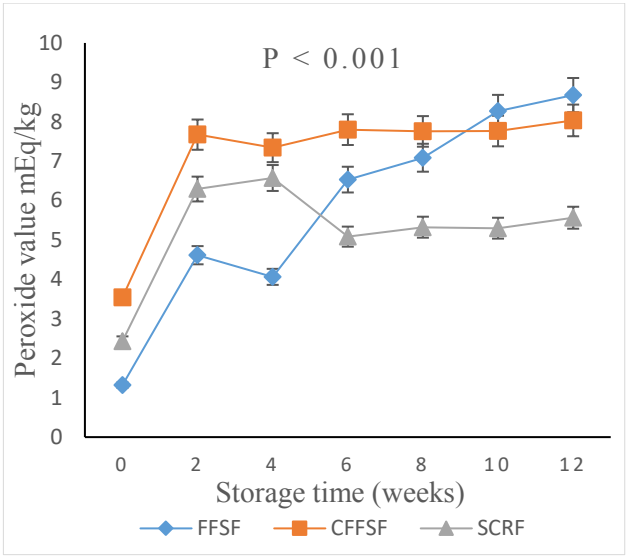
Note: Values are means ± SD; n = 3. Means with different letters (a, b, c) in the same Comparison by column represent a significant difference (Tukey's LSD; P <0.05). FFSF = full-fat soy flour, CFFSF = cold-water extracted full-fat soy flour, SCRF = soy curd residue flour, WAC = water absorption capacity, OAC = oil absorption capacity, BD = bulk density, SI = swelling index, and FC = foaming capacity.

Oxidative stability

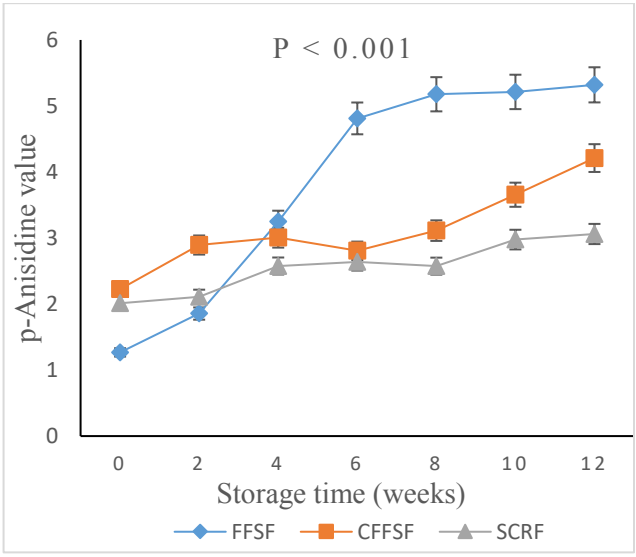
A highly notable difference ($p < 0.001$) was observed in the peroxide value (PV) of the samples, time-dependently. An overall increase in PV was generally observed for all samples (Figure 4a). Before storage, the PV of all samples was below 3.60 mEq/Kg. This initial PV recorded might be due to heat generation during

sample milling. The PV declined from week 4 to 6, which was expected due to the decomposition of hydroperoxides. All samples had PV below the limit (10 mEq/Kg) for oil specified in CODEX-STAN 210-1999 at the end of the study **[42]** FFSF had the highest PV (8.677 mEq/Kg), which was 1.07, and 1.55-times higher than CFFSF, and SCRF respectively at the end of week 12. According to O'Brien's categorization of oxidation concerning PV, all samples in this study can be considered moderately oxidized (5 < PV < 10) at the end of the analysis **[43]**.

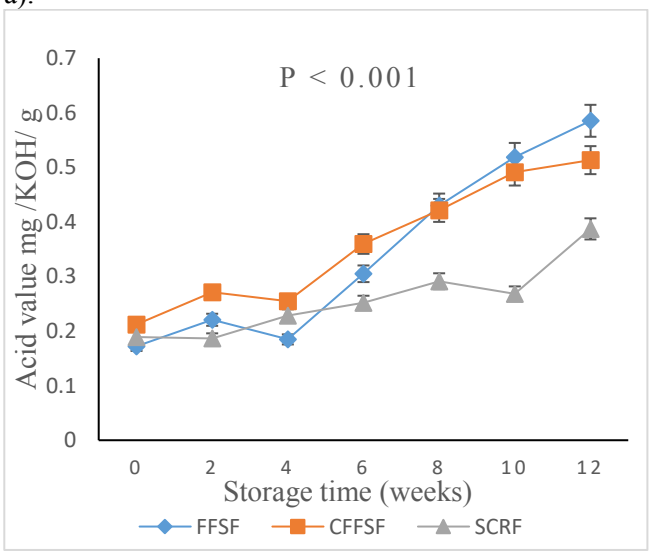
p-anisidine value (*p*-AV) varied significantly (p <0.001) among the samples. A marginal rise in *p*-AV was observed among the samples from week 0 to week 4. A noticeable and time-dependent increase in *p*-AV was observed from week 6 to week 12 for all samples except SCRF, which recorded a steady rise (Figure 4b). The general increase in *p*-AV for all samples was primarily due to hydroperoxide decomposition **[44]**. At the end of week 12, FFSF had the highest *p*-AV denoting 1.26 and 1.73-fold than CFFSF and SCRF, respectively. The *p*-AV of all samples corroborates with **[45]**, who reported low oxidized soybean oil to have a *p*-AV of 4-10. Low *p*-AV indicates low rancidity, which may be ascribed to soaking that can diminish lipid oxidation as suggested by **[46]**.



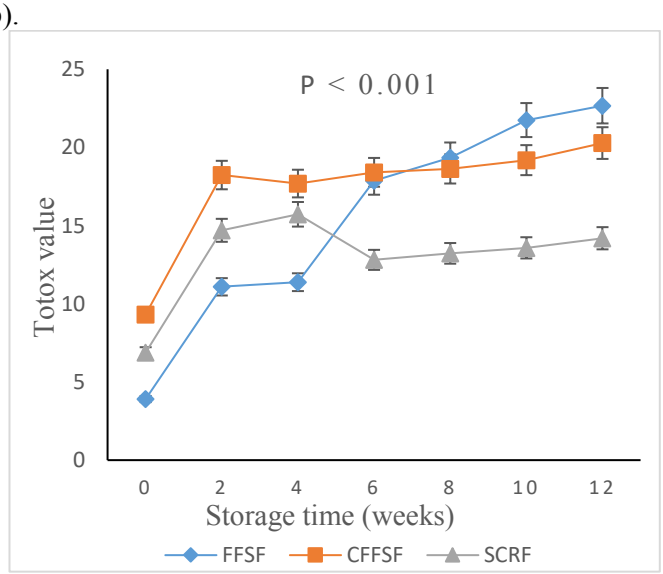
a).



b).



c).



d).

Figure 4 Changes in oxidative stability markers of samples during 12 weeks of storage.

Note: Values are means of triplicate. Error bars represent the standard deviations. A significant difference was observed at P < 0.05. FFSF = full-fat soy flour, CFFSF = cold-water extracted full-fat soy flour, SCRF = soy curd residue flour

The acid value (AV) of the samples differed significantly ($p < 0.001$). Initial acid values were below 0.2 mg KOH/g; however, an increased AV was observed with time for all samples, with FFSF recording the highest AV (Figure 4c). At week 12, the AV for all samples was below 0.6 mg/KOH complying with the permissible acid value of 0.6 mg/KOH/g for oil **[42]**. Given that free fatty acids are typically produced during the breakdown of triglycerides, AV is used as an indicator of oil rancidity, where high numbers indicate the oil or fats deterioration. The results suggest that the samples have acceptable odor intensity after 12 weeks of shelve storage **[47]**.

TOTOX was significantly ($p < 0.001$) affected by processing treatment and storage duration. Based on the PV and AV of oil, the TOTOX value is used to empirically evaluate oxidative degradation.
With time, a general increase was observed in all samples (Figure 4d). From week 6, CFFSF and FFSF observed an accentuated rise in TOTOX. After peaking at week 4, SCRF showed a decline in week 6 and a steady rise until the final week of storage. The highest TOTOX was recorded for FFSF and the lowest by SCRF. The TOTOX ranged from 14.19 to 22.67 at the end of the study. The lower TOTOX indicates a better oil quality **[48]**.

Instrumental colour analysis

Results indicated that storage duration significantly ($p < 0.001$) influenced the colour markers of the samples (Table 3).

Table 3 Colour parameters.

Colour parameter							
Week	Sample	L*	a*	b*	C	h	BI
0	CFFSF	69.92±0.03^{a}	7.04±0.11^{c}	22.32±0.01^{c}	23.41±0.11^{b}	72.49±0.09^{a}	1299.89±0.07^{c}
	FFSF	79.13±0.04^{c}	4.76±0.12^{b}	22.44±0.08^{b}	23.23±0.13^{b}	77.95±0.01^{c}	822.96±0.11^{b}
	SCRF	75.86±0.10^{b}	4.54±0.02^{a}	21.64±0.06^{a}	22.11±0.12^{a}	78.17±0.10^{b}	761.0±0.09^{a}
2	CFFSF	72.11±0.19^{a}	6.50±0.06^{c}	21.57±0.01^{a}	25.86±0.04^{c}	73.22±0.08^{a}	1185.19±0.08^{c}
	FFSF	80.70±0.06^{c}	4.92±0.06^{b}	23.68±0.13^{c}	24.18±0.14^{b}	78.12±0.12^{b}	870.56±0.12^{b}
	SCRF	78.10±0.77^{b}	4.28±0.17^{a}	22.33±0.14^{b}	22.73±0.13^{a}	79.16±0.11^{c}	716.18±0.09^{a}
4	CFFSF	72.11±0.31^{c}	6.60±0.16^{c}	20.61±0.12^{c}	21.63±0.09^{c}	72.32±0.66^{a}	1406.35±0.11^{c}
	FFSF	68.32±0.12^{b}	3.47±0.22^{b}	14.94±0.09^{b}	5.34±0.05^{a}	76.90±0.08^{b}	1038.93±0.08^{b}
	SCRF	58.35±0.15^{a}	2.89±0.11^{a}	12.79±0.11^{a}	13.13±0.05^{b}	77.26±0.09^{b}	832.68±0.07^{a}
6	CFFSF	66.99±0.11^{a}	7.87±0.06^{c}	27.06±0.11^{b}	28.18±0.11^{b}	73.78±0.36^{a}	1570.67±0.30^{c}
	FFSF	68.85±0.14^{b}	6.29±0.15^{b}	25.94±0.01^{a}	26.70±0.15^{a}	76.37±0.09^{b}	1203.24±0.07^{b}
	SCRF	74.88±0.13^{c}	5.14±0.02^{a}	26.60±0.08^{a}	27.09±0.10^{a}	79.10±0.09^{c}	927.52±0.08^{a}
8	CFFSF	65.60±0.04^{a}	7.67±0.03^{b}	26.27±0.01^{a}	27.37±0.29^{a}	73.73±0.26^{a}	1515.72±0.21^{c}
	FFSF	74.76±0.06^{b}	5.55±0.06^{a}	27.89±0.11^{b}	28.65±0.37^{b}	76.79±0.33^{b}	1270.31±0.20^{b}
	SCRF	74.40±0.07^{b}	5.17±0.10^{a}	26.55±0.06^{a}	27.06±0.31^{a}	78.97±0.33^{c}	945.39±0.31^{a}
10	CFFSF	63.88±0.33^{a}	7.91±0.30^{c}	26.24±0.33^{a}	27.40±0.03^{a}	73.32±0.05^{a}	1517.28±0.05^{c}
	FFSF	79.71±0.05^{c}	6.81±0.08^{b}	27.94±0.66^{b}	28.76±0.08^{b}	76.30±0.04^{b}	1228.19±0.07^{b}
	SCRF	72.18±0.13^{b}	5.32±0.12^{a}	26.59±0.15^{a}	27.12±0.08^{a}	78.68±0.06^{c}	942.43±0.09^{a}
12	CFFSF	69.04±0.05^{b}	6.86±0.08^{b}	27.91±0.08^{b}	27.43±0.13^{a}	76.29±0.16^{b}	1553.60±0.16^{c}
	FFSF	63.58±0.04^{a}	7.83±0.06^{c}	26.58±0.04^{a}	28.77±0.16^{b}	73.39±0.09^{a}	1274.11±0.13^{b}
	SCRF	70.84±0.11^{b}	5.30±0.08^{a}	26.97±0.11^{a}	27.34±0.14^{a}	78.88±0.09^{c}	950.29±0.16^{a}
p-value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: Values are means ± SD; n = 3. Means with different letters (a, b, c) in the same comparing column represent a significant difference (Tukey's LSD; P <0.05). FFSF = full-fat soy flour, CFFSF = cold-water extracted full-fat soy flour, SCRF = soy curd residue flour, L* = lightness or darkness, a*= redness or greenness, b* = yellow or blue, C =chroma, h = hue angle, and BI=browning index.

Generally, the sample experienced slight darkening with time. The reduction in lightness may be attributed to pigment degradation, Maillard reaction, caramelization, or oxidation of polyphenols, as suggested by **[49]**. This is consistent with reports by **[16]**, who reported a time-dependent darkening of full-fat soy under storage. The overall colour change was more pronounced in FFSF **(**Figure 5**)**. The browning index increased sharply in all the samples; however, it was more noticeable in CFFSF. Overall, the increasing browning index of the sample may be attributed to the degradation of pigments, caramelization, or oxidation of polyphenols **[49]**. L* value is anticipated to decrease as browning is observed **[50]**. Total color change denotes a value that represents the change of color over time. The highest colour change was evident in FFSF, followed by SCRF and CFFSF.

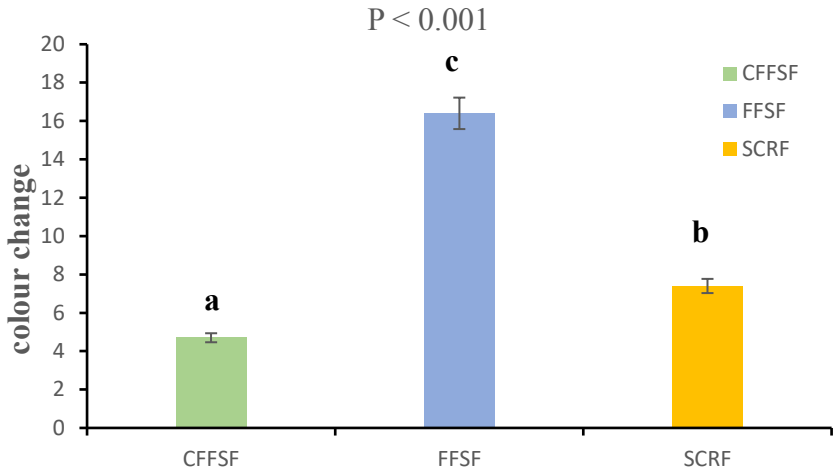


Figure 5 Total colour change in flour samples.

Note: Values are means ± SD; n = 3. Means with different letters (a, b, c) represent a significant difference (Tukey's LSD; P <0.05). FFSF = full-fat soy flour, CFFSF = cold-water extracted full-fat soy flour, SCRF = soy curd residue

Microbiological analysis

All samples observed no mold and yeast growth, except CFFSF (Table 4), which recorded 1.12 x 104 cfu/g, which is below the recommended limit of 10^5 cfu/g for flours according to **[51]**. The molds and yeast growth in CFFSF (Table 4) may be ascribed to post-production contamination from sample handling. There was no growth regarding the total plate count and total coliforms in any of the samples (Table 4). This could be due to the cleanliness of sample preparation and low water activity, which might have inhibited microbial growth. The results indicate that the samples are safe for use as food ingredients.

Table 4 Microbial growth analysis.

Flour	Microbial load (CFU/ g)		
	Total viable counts	Total coliforms	Yeast and molds
FFSF	<10 cfu/g	<10 cfu/g	<10 cfu/g
CFFSF	<10 cfu/g	<10 cfu/g	1.12E+04
SCRF	<10 cfu/g	<10 cfu/g	<10 cfu/g

Note: Values are means (n = 3). FFSF = full-fat soy flour, CFFSF = cold-water extracted full-fat soy flour, SCRF = soy curd residue flour.

CONCLUSION

The study demonstrated that processing treatment and storage time significantly impact the quality metrics under investigation. SCRF was the most oxidatively stable among the samples. However, it was notable that CFFSF was more stable than FFSF. At the end of the oxidative stability study, all samples recorded peroxide and acid values below the acceptable limit of 10 mEq/Kg and 0.6 mg/KOH/g, respectively. This work has shown a higher protein (41.22g – 53.33g / 100g) and energy yield, coupled with better storage stability in full-fat soy flour extracted with cold water (CFFSF) and soy curd residue flour (SCRF), respectively. This may offer an opportunity for protein-rich and shelf-stable product development when these ingredients are utilized in food product development. The lower bulk density alongside desirable functional properties of the samples characterized in this study may indicate their suitability for complementary food development. However, it is vital to recognize the limitations of this study. Certain constraints, such as methodological and sample size, might have influenced the results. These limitations may provide potential opportunities for future research to build upon our work and address the remaining gaps. Overall, this study contributes to the current knowledge base by comprehensively analyzing soy curd residue and full-fat soy flour and their suitability as ingredients for food formulations.

REFERENCES

1. Haidar, J., Muroki, N., Omwega, A., & Ayana, G. (2004). Malnutrition and iron deficiency anaemia in lactating women in urban slum communities from Addis Ababa, Ethiopia. In East African Medical Journal (Vol. 80, Issue 4). African Journals Online (AJOL). https://doi.org/10.4314/eamj.v80i4.8640
2. GoG. National Nutrition Policy 2014-2017. vol. 000. 2013.
3. Aryeetey, R., Atuobi-Yeboah, A., Billings, L., Nisbett, N., van den Bold, M., & Toure, M. (2021). Stories of Change in Nutrition in Ghana: a focus on stunting and anemia among children under five years (2009 – 2018). In Food Security (Vol. 14, Issue 2, pp. 355–379). Springer Science and Business Media LLC. https://doi.org/10.1007/s12571-021-01232-1
4. Forsido, S. F., Rupasinghe, H. P. V., & Astatkie, T. (2013). Antioxidant capacity, total phenolics, and nutritional content in selected Ethiopian staple food ingredients. In International Journal of Food Sciences and Nutrition (Vol. 64, Issue 8, pp. 915–920). Informa UK Limited. https://doi.org/10.3109/09637486.2013.806448
5. Goyal, R., Sharma, S., & Gill, B. S. (2012). Variability in the nutrients, antinutrients, and other bioactive compounds in soybean [Glycine max (L.) Merrill] genotypes. J. Food Legum (Vol. 25, Issue 3, pp 314–320).
6. Singh, P., Kumar, R., Sabapathy, S. N., & Bawa, A. S. (2008). Functional and Edible Uses of Soy Protein Products. In Comprehensive Reviews in Food Science and Food Safety (Vol. 7, Issue 1, pp. 14–28). Wiley. https://doi.org/10.1111/j.1541-4337.2007.00025.x
7. Dzogbefia, V. P., Arthur, P. L., & Zakpaa, H. D. (2007). Value addition to locally produced soybean in Ghana: production of soy sauce using starter culture fermentation. In Journal of Science and Technology (Ghana) (Vol. 27, Issue 2). African Journals Online (AJOL). https://doi.org/10.4314/just.v27i2.33036
8. Kamble, D. B., Singh, R., Rani, S., & Pratap, D. (2019). Physicochemical properties, in vitro digestibility and structural attributes of okara-enriched functional pasta. In Journal of Food Processing and Preservation (Vol. 43, Issue 12). Hindawi Limited. https://doi.org/10.1111/jfpp.14232
9. Guimarães, R. M., Silva, T. E., Lemes, A. C., Boldrin, M. C. F., da Silva, M. A. P., Silva, F. G., & Egea, M. B. (2018). Okara: A soybean by-product as an alternative to enrich vegetable paste. In LWT (Vol. 92, pp. 593–599). Elsevier BV. https://doi.org/10.1016/j.lwt.2018.02.058
10. Rinaldi, V. E. A., Ng, P. K. W., & Bennink, M. R. (2000). Effects of Extrusion on Dietary Fiber and Isoflavone Contents of Wheat Extrudates Enriched with Wet Okara. In Cereal Chemistry (Vol. 77, Issue 2, pp. 237–240). Wiley. https://doi.org/10.1094/cchem.2000.77.2.237
11. Atuna, R. A., Amagloh, F. C., Denwar, N. N., Asase, V. R., Faisal, S., Baako, E., Koomson, G., Gulkirpik, E., Toc, M., Donnelly, A., Amagloh, F. K., & Andrade Laborde, J. E. (2022). Filling the Protein Gap in Ghana: The Role of Soy. In Frontiers in Sustainable Food Systems (Vol. 5). Frontiers Media SA. https://doi.org/10.3389/fsufs.2021.781571
12. Cheng, Y., Shimizu, N., & Kimura, T. (2005). The viscoelastic properties of soybean curd (tofu) as affected by soymilk concentration and type of coagulant. In International Journal of Food Science & Technology (Vol. 40, Issue 4, pp. 385–390). Wiley. https://doi.org/10.1111/j.1365-2621.2004.00935.x
13. Wali, A. (2021). Microbial fermentation and quality preservation of agro-industrial wet by-products. Okayama University. https://doi.org/10.18926/62174
14. Madhujith, T., & Sivakanthan, S. (2019). Oxidative Stability of Edible Plant Oils. In Bioactive Molecules in Food (pp. 529–551). Springer International Publishing. https://doi.org/10.1007/978-3-319-78030-6_94
15. Park, S.-K., Prabakaran, M., An, Y., Kwon, C., Kim, S., Yang, Y., Kim, S.-H., & Chung, I.-M. (2018).

Impact of Storage Stability on Soybean (Glycine max L.) Flour Stored in Different Conditions and Package Materials. Korean Journal of Crop Science, 63(4), 338–359. https://doi.org/10.7740/KJCS.2018.63.4.338

16. Gulkirpik, E., Toc, M., Atuna, R. A., Amagloh, F. K., & Andrade Laborde, J. E. (2021). Evaluation of Oxidative Stability of Full Fat Soybean Flour in Storage and Sensory Quality of Tuo Zaafi-Enriched with Soy Flour as Influenced by Traditional Processing Methods. In Foods (Vol. 10, Issue 9, p. 2192). MDPI AG. https://doi.org/10.3390/foods10092192
17. AOAC, A. (2000). AOAC official methods of analysis of AOAC international. (17th Ed.).
18. Ikya, I. (2013). Proximate composition, nutritive and sensory properties of fermented maize, and full-fat soy flour blends for agidi production. In African Journal of Food Science (Vol. 7, Issue 12, pp. 446–450). Academic Journals. https://doi.org/10.5897/ajfs09.224
19. Appiah, F., & Oduro, I. (2011). Functional properties of Artocarpus altilis pulp flour as affected by fermentation. In Agriculture and Biology Journal of North America (Vol. 2, Issue 5, pp. 773–779). Science Hub. https://doi.org/10.5251/abjna.2011.2.5.773.779
20. Okaka, J. C., & Potter, N. N. (1977). Functional and storage properties of cowpea powder-wheat flour blends in breadmaking. In Journal of Food Science (Vol. 42, Issue 3, pp. 828–833). Wiley. https://doi.org/10.1111/j.1365-2621.1977.tb12614.x
21. Narayana, K., & Narasinga Rao, M. S. (1982). Functional Properties of Raw and Heat Processed Winged Bean (Psophocarpus tetragonolobus) Flour. In Journal of Food Science (Vol. 47, Issue 5, pp. 1534–1538). Wiley. https://doi.org/10.1111/j.1365-2621.1982.tb04976.x
22. Yue, X., Xu, Z., Prinyawiwatkul, W., Losso, J. N., King, J. M., & Godber, J. S. (2007). Comparison of Soybean Oils, Gum, and Defatted Soy Flour Extract in Stabilizing Menhaden Oil during Heating. In Journal of Food Science (Vol. 73, Issue 1). Wiley. https://doi.org/10.1111/j.1750-3841.2008.00586.x
23. AOCS. (2004). 1. AOCS (2004) Official Methods and Recommended Practices. American Oil Chemists' Society, Champaign, IL.
24. Chew, S.-C., Tan, C.-P., Long, K., & Nyam, K.-L. (2016). Effect of chemical refining on the quality of kenaf (hibiscus cannabinus) seed oil. In Industrial Crops and Products (Vol. 89, pp. 59–65). Elsevier BV. https://doi.org/10.1016/j.indcrop.2016.05.002
25. Mohapatra, D., Bira, Z. M., Kerry, J. P., Frías, J. M., & Rodrigues, F. A. (2010). Postharvest Hardness and Color Evolution of White Button Mushrooms (Agaricus bisporus). In Journal of Food Science (Vol. 75, Issue 3). Wiley. https://doi.org/10.1111/j.1750-3841.2010.01518.x
26. Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2012). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. In Food and Bioprocess Technology (Vol. 6, Issue 1, pp. 36–60). Springer Science and Business Media LLC. https://doi.org/10.1007/s11947-012-0867-9
27. Al-Nasiry, B. S. A.-N. (2020). Detection of bacterial contamination in filled and dried biscuit products of young children. In Annals of Tropical Medicine and Public Health (Vol. 23, Issue 16). Africa Health Research Organization. https://doi.org/10.36295/asro.2020.231602
28. Batool, S., Tahir, S., Rauf, N., & Kalsoom, R. (2013). Microbiological analysis of pasteurized and fresh fruit juice sold in Rawalpindi of Pakistan. In Bangladesh Journal of Scientific and Industrial Research (Vol. 48, Issue 3, pp. 185–192). Bangladesh Journals Online (JOL). https://doi.org/10.3329/bjsir.v48i3.17329
29. Porwal, V. B., Bharath Kumar, S., Madhumathi, R., & Prabhasankar, P. (2014). Influence of health-based ingredient and its hydrocolloid blends on noodle processing. In Journal of Food Measurement and Characterization (Vol. 8, Issue 4, pp. 283–295). Springer Science and Business Media LLC. https://doi.org/10.1007/s11694-014-9187-2
30. Gandhi, A. P. (1990). Development of HACCP procedure for the production of full-fat soy flour. In Int. Food Res. J. (Vol. 15, Issue 2, pp. 141–154).
31. Etiosa, O., Chika, N., & Benedicta, A. (2018). Mineral and Proximate Composition of Soya Bean. In Asian Journal of Physical and Chemical Sciences (Vol. 4, Issue 3, pp. 1–6). Science domain International. https://doi.org/10.9734/ajopacs/2017/38530
32. Bencini, M. C. (1986). Functional Properties of Drum-Dried Chickpea (Cicer arietinum L.) Flours. In Journal of Food Science (Vol. 51, Issue 6, pp. 1518–1521). Wiley. https://doi.org/10.1111/j.1365-2621.1986.tb13849.x
33. Guermani L, Villaume C, Bau HW, Chandrasiri V, Nicolas JP. Composition et valeur nutritionelle de l'Okara fermenté par Rhizopus oligosporus. Sci Aliments 1992;12:441–51.
34. Godswill, A. C. (2019). Proximate composition and functional properties of different grain flour composites for Industrial applications. In International Journal of Food Sciences (Vol. 2, Issue 1, pp. 43–64). IPR Journals and Books (International Peer Reviewed Journals and Books). https://doi.org/10.47604/ijf.1010
35. Tenagashaw, M. W., Kenji, G. M., Melaku, E. T., Huyskens-Keil, S., & Kinyuru, J. N. (2017). Teff-Based

Complementary Foods Fortified with Soybean and Orange-Fleshed Sweet Potato. In Journal of Food Research (Vol. 6, Issue 1, p. 112). Canadian Center of Science and Education. https://doi.org/10.5539/jfr.v6n1p112

36. Inyang, U. E., & Ekanem, J. O. (1996). Effect of dehulling methods and desolventizing temperatures on proximate composition and some functional properties of sesame (Sesamum indicum L.) seed flour. In Journal of the American Oil Chemists' Society (Vol. 73, Issue 9, pp. 1133–1136). Wiley. https://doi.org/10.1007/bf02523374
37. CODEX STAN. Codex Standard for Processed Cereal-Based Foods for Infants and Young Children. Codex Aliment Comm CODEX STAN 1994;4:74–194.
38. Twinomuhwezi, H. Awuchi, C.G., & Rachael, M. (2020). Comparative Study of the Proximate Composition and Functional Properties of Composite Flours of Amaranth, Rice, Millet, and Soybeanf. In American Journal of Food Science and Nutrition (Vol. 6, Issue 1, p 6–19) American Association for Science and Technology.
39. Akpata, M. I., & Akubor, P. I. (1999). In Plant Foods for Human Nutrition (Vol. 54, Issue 4, pp. 353–362). Springer Science and Business Media LLC. https://doi.org/10.1023/a:1008153228280
40. Li, B., Qiao, M., & Lu, F. (2012). Composition, Nutrition, and Utilization of Okara (Soybean Residue). In Food Reviews International (Vol. 28, Issue 3, pp. 231–252). Informa UK Limited. https://doi.org/10.1080/87559129.2011.595023
41. El-Adawy, T. A., Rahma, E. H., El-Bedawey, A. A., & Gafar, A. F. (2001). Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates. In Food Chemistry (Vol. 74, Issue 4, pp. 455–462). Elsevier BV. https://doi.org/10.1016/s0308-8146(01)00163-7
42. Alimentarius C. Codex standard for named vegetable oils. Codex Stan 1999;210:1–13.
43. O'Brien, R. D. (2008). Fats and Oils. CRC Press. https://doi.org/10.1201/9781420061673
44. Schaich, K. M., Shahidi, F., Zhong, Y., & Eskin, N. A. M. (2013). Lipid Oxidation. In Biochemistry of Foods (pp. 419–478). Elsevier. https://doi.org/10.1016/b978-0-08-091809-9.00011-x
45. Cucu, T., Devreese, B., Kerkaert, B., Mestdagh, F., Sucic, M., Van De Perre, I., & De Meulenaer, B. (2013). A comparative study of lipid and hypochlorous acid-induced oxidation of soybean proteins. In LWT - Food Science and Technology (Vol. 50, Issue 2, pp. 451–458). Elsevier BV. https://doi.org/10.1016/j.lwt.2012.08.027
46. Kumar, V., Rani, A., & Chauhan, G. S. (2006). Influence of germination temperature on oil content and fatty acid composition of soy sprouts. In J. Food Sci. Technol. (Vol. 43, Issue 3. pp 325–326). Association of Food Scientists and Technologists (India)
47. Dunford NT. Edible oil quality. Oklahoma Cooperative Extension Service; 2016. Available at: https://shareok.org/bitstream/handle/11244/50148/oksa_FAPC-0197_2016-01.pdf?sequence=1&isAllowed=y
48. Cong, S., Dong, W., Zhao, J., Hu, R., Long, Y., & Chi, X. (2020). Characterization of the Lipid Oxidation Process of Robusta Green Coffee Beans and Shelf Life Prediction during Accelerated Storage. In Molecules (Vol. 25, Issue 5, p. 1157). MDPI AG. https://doi.org/10.3390/molecules25051157
49. Bagheri, H., Kashaninejad, M., Ziaiifar, A. M., & Aalami, M. (2019). Textural, color, and sensory attributes of peanut kernels as affected by infrared roasting method. In Information Processing in Agriculture (Vol. 6, Issue 2, pp. 255–264). Elsevier BV. https://doi.org/10.1016/j.inpa.2018.11.001
50. Niamnuy, C., Devahastin, S., Soponronnarit, S., & Vijaya Raghavan, G. S. (2008). Kinetics of astaxanthin degradation and color changes of dried shrimp during storage. In Journal of Food Engineering (Vol. 87, Issue 4, pp. 591–600). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2008.01.013
51. Food safety and quality. (2012). Available at: https://www.wfp.org/food-safety-and-quality

Funds:
No external funding was received for this research.

Conflict of Interest:
The authors hereby declare no potential conflict of interest.

Ethical Statement:
This article does not contain any studies that would require an ethical statement.

Contact Address:

***Emmanuel Duah Osei**, Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Kamýcká 129, Suchdol, Prague 16500, Czech Republic,
E-mail: yduahosei@uniag.sk
ORCID: https://orcid.org/0000-0003-2289-0908

Abigail Ataa Pokuah, University for Development Studies, Faculty of Agriculture, Food and Consumer Sciences, Department of Food Science and Technology, Nyankpala Campus, TL 1882, Tamale, Ghana,
E-mail: abigailat178@uds.edu.gh
ORCID: https://orcid.org/0009-0002-0084-0790

Richard Atuna Atinpoore, University for Development Studies, Faculty of Agriculture, Food and Consumer Sciences, Department of Food Science and Technology, Nyankpala Campus, TL 1882, Tamale, Ghana,
E-mail: ratuna@uds.edu.gh
ORCID: https://orcid.org/0000-0002-4888-8710

Eudes Sam Faisal, University for Development Studies, Faculty of Agriculture, Food and Consumer Sciences, Department of Food Science and Technology, Nyankpala Campus, TL 1882, Tamale, Ghana,
E-mail: sameudes0@gmail.com
ORCID: https://orcid.org/0000-0003-0708-3251

Anthony Amotoe-Bondzie, Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Kamýcká 129, Suchdol, Prague 16500, Czech Republic,
E-mail: yamotoebondz@uniag.sk
ORCID: https://orcid.org/0000-0002-2529-2877

Abdul-Mateni Yussif, University for Development Studies, Faculty of Agriculture, Food and Consumer Sciences, Department of Food Science and Technology, Nyankpala Campus, TL 1882, Tamale, Ghana,
E-mail: abdulmatenibak@gmail.com
ORCID: https://orcid.org/0009-0005-7762-9434

Fortune Akabanda, University for Development Studies, Faculty of Agriculture, Food and Consumer Sciences, Department of Food Science and Technology, Nyankpala Campus, TL 1882, Tamale, Ghana,
E-mail: fakabanda@uds.edu.gh
ORCID: https://orcid.org/0000-0002-3118-7619

Francis Kweku Amagloh, University for Development Studies, Faculty of Agriculture, Food and Consumer Sciences, Department of Food Science and Technology, Nyankpala Campus, TL 1882, Tamale, Ghana,
E-mail: fkamagloh@uds.edu.gh
ORCID: https://orcid.org/0000-0001-7243-0972

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 19.11.2023
Revised: 21.12.2023
Accepted: 2.1.2024
Published: 9.1.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 50-65
https://doi.org/10.5219/1938
ISSN: 1337-0960 online
www.potravinarstvo.com

Effects of laying hens housing system on eggs microbial contamination

Ján Petrovič, Martin Mellen, Natália Čmiková, Marianna Schwarzová, Miroslava Kačániová

ABSTRACT

Microorganisms can contaminate eggs at many stages of production, handling, preparation, and consumption. The aim of our study was the microbiological quality of the internal contents of eggs from different layer housing systems. Total bacteria, coliforms, and *Salmonella* spp. were isolated and identified by mass spectrometry. Total bacterial counts were isolated on Plate count agar for 48 hours at 30 °C, coliforms on Violet red bile lactose agar for 24 hours at 37 °C and *Salmonella* spp. on Xylose lysine deoxycholate agar for 24 hours at 37 °C. The lowest total bacterial counts were found in the cage-rearing system and the highest in the aviary-rearing system for hens housing. The number of microorganisms was evaluated on days 0. and 21. Twenty species, eighteen genera, and sixteen families were isolated from enriched cages in 0 days, while three families, three genera, and five species were isolated in 21 days, according to egg content samples. Thirteen families, sixteen genera, and twenty species were isolated from egg contents samples in the deep litter on day zero and day twenty-one, respectively, by third families, fourth genera, and seventh species. Nine families, twenty genera, and fifteen species were identified in aviaries using egg content samples on day 0, and three families, three genera, and five species on day 21. *Ralstonia pickettii* was the most isolated species among all samples.

Keywords: total count of bacteria, coliform bacteria, *Salmonella* spp., mass spectrometry, microbiota, eggs

INTRODUCTION

Food eggs should have good nutritional content as well as microbiological safety. To be profitable and accepted by consumers, eggs must be of high quality **[1]**. Consumers are now strongly interested in animal products, especially those produced in a welfare-conscious manner **[2]**, which has led to a significant increase in the proportion of hens reared in alternative farming systems to cages. There are concerns about the higher potential for bacterial contamination of table eggs due to the increase in egg production in non-cage systems. Most of the eggs produced are used as major ingredients in food products and consumed as fresh table eggs, which can pose a serious threat to food safety **[3]**, **[4]**, **[5]**. Compared to eggs from cage systems, the surface of eggs from floor and free-range systems typically contain more embryos **[6]**, **[7]**. In addition, Tomczyk et al. **[8]** demonstrated that hens housed in indoor and free-range systems produced eggshells with the highest diversity and number of microorganisms. Microbiological contamination can compromise the safety, shelf life, and quality of eggs **[9]**, **[10]**. Pathogenic microbes may be present in the eggshell microbiota, as well as microbes that cause egg spoilage. Egg consumption has been shown to contribute significantly to the incidence of occasional cases of salmonellosis among many risk factors **[11]**, **[12]**. Eggs can be contaminated with microorganisms vertically or horizontally. Vertical contamination occurs when eggs develop in the ovary or oviduct of the hen **[13]**. After egg laying, horizontal contamination occurs when bacteria break through the shell **[14]**, **[15]**. Bacteria can enter the

eggs despite having defenses such as the cuticle-covered shell, the shell membrane, and antimicrobial proteins in the white. In addition, the high pH and viscosity of albumin prevent bacteria from multiplying **[16]**. A critical factor affecting table eggs' safety is the time and circumstances during storage. Changes during storage can create an ideal environment for microbial contamination of eggs. For example, the glycolytic activity of certain bacteria can cause the breakdown of the cuticle, the essential protective layer, when the relative humidity of the eggshell surface increases.

The functional (technological) properties and antibacterial properties of egg whites are affected by various physical and chemical changes caused by storage conditions and length of storage **[17]**, **[18]**, **[19]**. The migration of water between the yolk and the white and the loss of water and carbon dioxide through the eggshell pores are the main causes of changes in egg content. The height of the white decreases with prolonged storage; nevertheless, pH and foaminess increase **[20]**. In addition, the strength of the vitreous membrane that envelops the yolk is reduced **[17]**, which promotes nutrient transfer between the white and yolk **[21]**. In addition, egg white degrades due to storage conditions and time **[18]**, **[21]**, which further reduces its antibacterial properties **[18]**. The microbiological quality, sensory properties and physicochemical properties of eggs can be significantly affected by storage conditions and length of storage.

The aim of the research was to determine the effect of different laying hen-rearing strategies on the microbiological quality of egg content and the identification of bacteria by mass spectrometry.

Scientific Hypothesis

The hypothesis of the research was to investigate the effect of different laying hen-rearing strategies on the microbiological quality of egg content and the identification of bacteria by mass spectrometry. We expected that rearing conditions would also influence microbiological abundance on the day first and after storage for 21 days.

MATERIAL AND METHODOLOGY

Samples

The laying eggs under investigation were from the Bovans Brown hybrid line, and at the start of the trial, they were grown in three different systems: 30,892 pieces in enriched cages, 11,130 pieces in deep litter, and 27,958 pieces in aviaries.

The farm uses 5ON04R cage breeding equipment—the six batteries house four-story cages. There are devices to gather eggs on the front side of the hall and a gadget to remove droppings on the back side. The cages have medicine dispensers and a central power supply with a water control gauge on each floor.

The center of the hall's slatted floor is where nipple waterers, feeders, and nest-laying nests are positioned. For bedding, dry sand that is three centimeters thick is utilized. There are nine laying hens per square meter of the hall's floor. After the chickens are taken out after the laying cycle, the droppings from the litter area and the entire hall are cleared. To prevent the collected droppings from rising above the grid after the laying cycle when the hens are removed, the grid bottom is positioned 500 mm above the floor. The laying nests are near the breast feeders. When laying chickens enter the nest, they wipe their runners on the slatted portion of the floor.

This breeding strategy can increase the number of laying hens per m2. The hall is lined with three-story aviary buildings. There is sand in the spaces beneath the structure's rows and the aisles between them. In addition to being utilized as a dust bath, the litter is used to rake laying chickens.

Chemicals

Unless otherwise noted, all chemical reagents were of analytical grade and were used exactly as given, without additional purification. All other chemicals and agars indicated were obtained from Aloquence s.r.o., Vráble, Slovakia, and were used without additional purification unless otherwise specified. All solutions, except as specified, were made using metal-free ultrapure water (also known as Milli-Q water; 18.2 MΩ cm) from a Millipore Milli-Q system located in Bedford, Massachusetts.

Animals, Plants, and Biological Materials

The study was carried out on eggs from a hybrid line of production laying hens, Bovans Brown, at the chicken farm Babičkin dvor a.s., Veľký Krtíš with number 2SK VK6-33.

Instruments

For the identification of bacteria, we used MALDI TOF-MS Biotyper (Brucker Daltonics, Bremen, Germany).

Laboratory Methods

ISO 4833-2:2013 **[22]**. Microbiology of the Food Chain. Horizontal Method for the Enumeration of Microorganisms: Colony Count at 30 °C by the Pour Plate Technique.

ISO 4832:2006 **[23]**. Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Enumeration of Coliforms. Colony-Count Technique.

ISO 6579-1:2017 **[24]**. Microbiology of the food chain: Horizontal method for detecting, enumeration and serotyping Salmonella Part 1: Detection of *Salmonella* spp.

The identification was then completed using the MALDI TOF-MS Biotyper, according to Kačániová et al. **[25]**.

Description of the Experiment

Sample preparation: In all, 243 eggs from eight tests conducted in three separate locations (A, B, and C) were assessed between 0. and 21. days of storage in the lab. From enriched cages, on deep litter, and in aviaries, 144 pieces of eggs were used for both days of study. A constant temperature of 10 °C was maintained in the laboratory for 21 consecutive days. The number of bacteria in the egg contents was counted after egg samples were air-dried after being immersed in 75% ethanol for five minutes. After 5-10 seconds of flame exposure, the upper end of the egg was punctured using a sterile instrument.

Number of samples analyzed: 432 eggs from eight tests conducted in three locations.

Number of repeated analyses: 3.

Number of experiment replication: 3.

Design of the experiment: The entire egg's contents were mixed in a sterile polythene bag and then put on a PCA following serial dilution for an aerobic bacterial count. After the samples were serially diluted further, 100 µL of each dilution was applied to the plate count agar (PCA), Violet red bile agar with lactose (VRBL), and Xylose Lysine Deoxycholate agar (XLD) surfaces (Oxoid, Basingstoke, UK). The amount of *Salmonella* spp. (SS), coliform bacteria (CB), and total bacterial count (TBC) were all assessed. The plate diluting method was used to determine the quantitative CFU (Colony Forming Units) counts of the corresponding groups of bacteria in egg content. Plate count agar for the enumeration of the total count of bacteria was used for 48-72 h at 30 °C. Violet red bile agar with lactose for enumeration of coliform bacteria was used for 24-48 h at 37 °C, and Xylose Lysine Deoxycholate agar was used for the CFU segregation of *Salmonella* spp. For 24-48 h at 37 °C. All incubation was in aerobic conditions. Before being detected, the microbial colonies were cultured on TSA agar (Tryptone Soya Agar, Oxoid, UK) for 18 to 24 hours at 37 °C. A colony was produced using eight different bacterial strains. The identification was then completed using the MALDI TOF-MS Biotyper, according to **[25]**. A value more excellent than two was present in 1,523 isolates in total.

Statistical Analysis

The data were statistically evaluated using the Excel application. The arithmetic mean and standard deviation of the results were used for evaluation.

RESULTS AND DISCUSSION

Egg contents microbiota on day 0

Table 1 displays the total counts of microorganisms on day 0. The total number of bacteria, coliform counts, and *Salmonella* spp. were evaluated. According to our research, the only bacteria on eggs contained a total bacterial count (TCB). TCB was < 1 log CFU/ml in all hens housing system in the first experiment from 1.00 ±0.01 in enriched cages to 2.99 ±1.45 log CFU/ml in aviaries in the second experiment; in the third, from 1.30 ±0.36 in aviaries to 1.90 ±0.54 log CFU/ml in enriched cages; in the fourth, from < 1 in all hens housing systems to 2.53 ±1.65 log CFU/ml in enriched cages; in the fifth experiment, from < 1 in all housing systems to 1.30 ±0.78 log CFU/ml in enriched cages; and in the sixth experiment, from < 1 to 1.00 ±0.12 log CFU/ml in enriched cages; in a seventh experiment from < 1 in enriched cages to 1.30 ±1.05 log CFU/ml in same system; in the eighth experiment from < 1 in all systems to 1.48 ±0.67 log CFU/ml in deep litter.

Table 1 The total count of bacteria in the eggs in 0. days in log CFU/ml.

Day	Sample	1.	2.	3.	4.	5.	6.	7.	8.
0	ECA	< 1	2.98 ±0.04	1.70 ±1.23	2.11 ±1.45	1.30 ±0.78	< 1	1.00 ±0.03	< 1
0	ECB	< 1	1.48 ±0.12	1.90 ±0.54	2.53 ±1.65	< 1	1.00 ±0.12	1.30 ±1.05	< 1
0	ECC	< 1	1.00 ±0.01	1.70 ±0.34	< 1	< 1	< 1	< 1	< 1
0	DLA	< 1	1.30 ±0.17	1.95 ±0.45	< 1	< 1	1.00 ±0.07	1.00 ±0.05	< 1
0	DLB	< 1	1.70 ±1.23	1.48 ±1.07	1.00±0.07	1.00 ±0.05	1.00 ±0.09	1.30 ±0.78	< 1
0	DLC	< 1	1.00 ±0.09	1.70 ±1.03	< 1	< 1	< 1	1.00 ±0.05	1. 48 ±0.67
0	AA	< 1	2.90 ±1.23	1.30 ±0.36	< 1	< 1	< 1	1.30 ±0.45	< 1
0	AB	< 1	1.30 ±0.07	1.70 ±0.87	1.00 ±0.04	< 1	< 1	1.00 ±0.06	< 1
0	AC	< 1	2.99 ±1.45	1.48 ±1.23	1.00 ±0.08	1.00 ±0.07	< 1	1.00 ±0.07	< 1

Note: EC – enriched cages, DL – deep litter, A – aviaries.

From egg contents in enriched cages, a total of 135 isolates were found within a single day (Table 2). Egg content samples isolated 20 species, 18 genera, and 16 families. *Ralstonia picketii* is 20% of the most isolated species. *Methylobacterium fujisawaense* (7%) and *Enterobacter cloacae* (6%) were the other most isolated bacterial species.

Table 2 Isolated family, genera, and species of microorganisms of egg contents from enriched cages 0. day.

Family	Genera	Species	Number of isolates
Acidaminococcaceae	*Acidaminococcus*	*Acidaminococcus fermentans*	6
Microbacteriaceae	*Agromyces*	*Agromyces lapidis*	7
Microbacteriaceae	*Arthrobacter*	*Arthrobacter citreus*	4
Bacillaceae	*Bacillus*	*Bacillus subtilis*	5
Bacillaceae	*Bacillus*	*Bacillus subtilis* subsp. *subtilis*	6
Cryptococcaceae	*Cryptococcus*	*Cryptococcus neoformans*	6
Enterobacteriaceae	*Enterobacter*	*Enterobacter cloacae*	8
Micrococcaceae	*Glutamicibacter*	*Glutamicibacter arilaitensis*	4
Lactobacillaceae	*Lactobacillus*	*Lactobacillus delbrueckii* subsp. *delbrueckii*	6
Methylobacteriaceae	*Methylobacterium*	*Methylobacterium fujisawaense*	9
Methylobacteriaceae	*Methylobacterium*	*Methylobacterium* spp.	7
Moraxellaceae	*Moraxella*	*Moraxella catarrhalis*	7
Neisseriaceae	*Neisseria*	*Neisseria flavescens*	3
Sphingomonadaceae	*Novosphingobium*	*Novosphingobium resinovorum*	3
Pseudomonadaceae	*Pseudomonas*	*Pseudomonas mosselii*	5
Burkholderiaceae	*Ralstonia*	*Ralstonia pickettii*	27
Lactobacillaceae	*Schleiferilactobacillus*	*Schleiferilactobacillus harbinensis*	5
Sphingobacteriaceae	*Sphingobacterium*	*Sphingobacterium mizutaii*	4
Xanthomonadaceae	*Stenotrophomonas*	*Stenotrophomonas nitritireducens*	6
Zoogloeaceae	*Thauera*	*Thauera aromatica*	7
Total			**135**

Figure 1 Krona chart: Isolated species of microorganisms of egg contents from enriched cages 0. day.

In the deep litter 0. day egg contents, 148 strains in all were discovered (Table 3). A total of 13 families, 16 genera, and 20 species were separated from egg contents samples. Figure 1 shows 14% of the most isolated species was *Ralstonia pickettii*. Conversely, the other most isolated bacterial species was *Ralstonia insidiosa* (11%).

Table 3 Isolated family, genera, and species of microorganisms of egg contents from deep letter 0. day.

Family	Genera	Species	Number of isolates
Moraxellaceae	*Acinetobacter*	*Acinetobacter radioresistens*	6
Rhodocyclaceae	*Aromatoleum*	*Aromatoleum buckelii*	4
Enterococcaceae	*Enterococcus*	*Enterococcus faecium*	5
Micrococcaceae	*Glutamicibacter*	*Glutamicibacter arilaitensis*	5
Lactobacillaceae	*Liquorilactobacillus*	*Liquorilactobacillus nagelii*	7
Lactobacillaceae	*Lacticaseibacillus*	*Lacticaseibacillus paracasei* subsp. *paracasei*	8
Lactobacillaceae	*Latilactobacillus*	*Latilactobacillus sakei*	9
Methylobacteriaceae	*Methylobacterium*	*Methylobacterium fujisawaense*	5
Methylobacteriaceae	*Methylobacterium*	*Methylobacterium* spp.	3
Moraxellaceae	*Moraxella*	*Moraxella catarrhalis*	4
Sphingomonadaceae	*Novosphingobium*	*Novosphingobium resinovorum*	5
Pseudomonadaceae	*Pseudomonas*	*Pseudomonas balearica*	6
Burkholderiaceae	*Ralstonia*	*Ralstonia insidiosa*	15
Burkholderiaceae	*Ralstonia*	*Ralstonia pickettii*	22
Enterobacteriaceae	*Raoultella*	*Raoultella ornithinolytica*	8
Micrococcaceae	*Rothia*	*Rothia amarae*	5
Micrococcaceae	*Rothia*	*Rothia terrae*	6
Staphylococcaceae	*Staphylococcus*	*Staphylococcus haemolyticus*	7
Staphylococcaceae	*Staphylococcus*	*Staphylococcus lentus*	4
Zoogloeaceae	*Thauera*	*Thauera aromatica*	8
Total			**142**

Table 4 Isolated family, genera, and species of microorganisms of egg contents from aviaries 0. day.

Family	Genera	Species	Number of isolates
Moraxellaceae	*Acinetobacter*	*Acinetobacter calcoaceticus*	8
Moraxellaceae	*Acinetobacter*	*Acinetobacter pittii*	9
Microbacteriaceae	*Agromyces*	*Agromyces italicus*	7
Micrococcaceae	*Arthrobacter*	*Arthrobacter pyridinolis*	5
Bacillaceae	*Bacillus*	*Bacillus amyloliquefaciens* subsp. *plantarum*	6
Bacillaceae	*Bacillus*	*Bacillus cereus*	7
Bacillaceae	*Bacillus*	*Bacillus subtilis* subsp. *subtilis*	8
Debaryomycetaceae	*Candida*	*Candida krusei*	7
Debaryomycetaceae	*Candida*	*Candida utilis*	5
Enterobacteriaceae	*Enterobacter*	*Enterobacter cloacae*	7
Lactobacillaceae	*Lacticaseibacillus*	*Lacticaseibacillus paracasei* subsp. *paracasei*	8
Lactobacillaceae	*Lactobacillus*	*Lactobacillus amylovorus*	6
Lactobacillaceae	*Lactobacillus*	*Lactobacillus delbrueckii* subsp. *lactis*	6
Lactobacillaceae	*Latilactobacillus*	*Latilactobacillus sakei*	7
Lactobacillaceae	*Levilactobacillus*	*Levilactobacillus brevis*	5
Lactobacillaceae	*Ligilactobacillus*	*Ligilactobacillus agilis*	9
Bacillaceae	*Lysinibacillus*	*Lysinibacillus pakistanensis*	6
Bacillaceae	*Lysinibacillus*	*Lysinibacillus* spp.	8
Micrococcaceae	*Paeniglutamicibacter*	*Paeniglutamicibacter sulfureus*	8
Burkholderiaceae	*Paraburkholderia*	*Paraburkholderia fungorum*	8
Pseudomonadaceae	*Pseudomonas*	*Pseudomonas boreopolis*	5
Burkholderiaceae	*Ralstonia*	*Ralstonia pickettii*	9
Total			**154**

Figure 2 Krona chart: Isolated species of microorganisms of eggs content from deep litter 0. day.

A total of 154 isolates were found in the aviaries 0. day egg contents (Table 4). From egg content samples, a total of nine families, twenty genera, and fifteen species were identified. The three most isolated species were *Acinetobacter pittii, Ligilactobacillus agilis* and *Ralstonia pickettii*, with 6% of the total. However, *Acinetobacter calcoaceticus* and *Bacillus subtilis* subsp. *subtilis*, *Lacticaseibacillus paracasei* subsp. *paracasei, Lysinibacillus spp., Paeniglutamicibacter sulfureus, Paraburkholderia fungorum* and other (5%) were the other most isolated bacterial species (Figure 2).

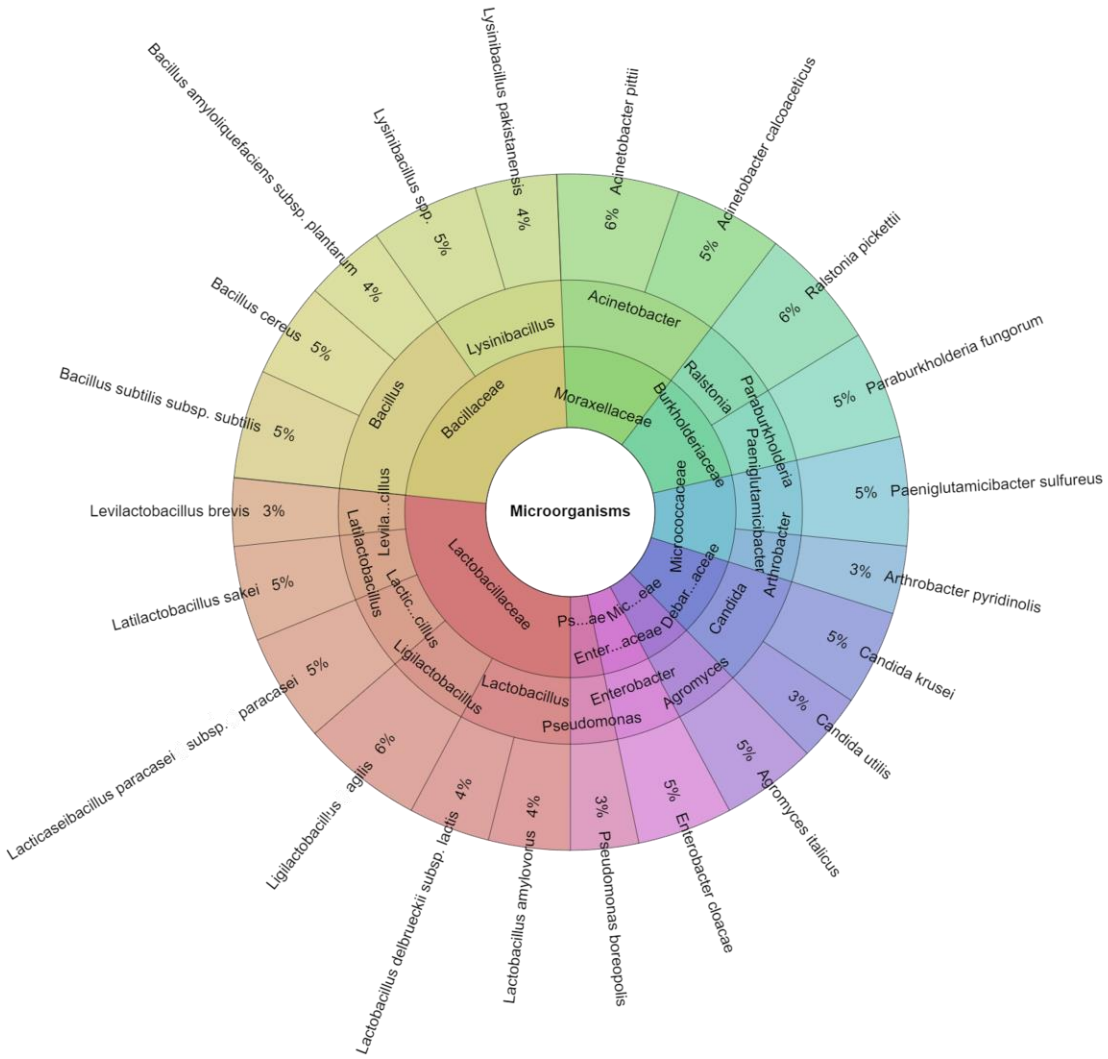


Figure 3 Krona chart: Isolated species of microorganisms of egg contents from aviaries 0. day.

Eggs content microbiota in day 21

The total number of microorganisms counted on day 21 is displayed in Table 5. The total number of bacteria, coliform bacteria, and *Salmonella* spp. were all counted in our investigation. According to our research, there were only total counts of bacteria (TCB) in eggs content. TCB in first experiment ranged from < 1 in deep litter to 2.08 ±1.34 log CFU/ml in deep litter; in second experiment from < 1 in enriched cages to 1.70 ±0.65 log CFU/ml in aviaries; in third from < 1 in enriched cages and deep litter to 2.40 ±1.23 log CFU/ml in aviaries; in fourth from < 1 in enriched cages and deep litter to 1.30 ±0.74 log CFU/ml in deep litter; in fifth from < 1 in all systems to 1.00 ±0.18 log CFU/ml in deep litter; in sixth from < 1 in deep litter to 1.60 ±0.77 log CFU/ml in aviaries; in seventh from < 1 in all systems to 1.30 ±0.77 log CFU/ml in aviaries and eight experiments were in all systems < 1.

Table 5 The total count of bacteria in egg contents in 21. day in log CFU/ml.

Day	Sample	1.	2.	3.	4.	5.	6.	7.	8.
0	ECA	1.00 ±0.07	< 1	1.30 ±0.45	1.00 ±0.06	< 1	1.30 ±0.23	1.00 ±0.06	< 1
0	ECB	1.70 ±1.12	1.70 ±0.67	1.00 ±0.15	< 1	< 1	1.00 ±0.67	< 1	< 1
0	ECC	1.00 ±0.21	1.48 ±0.45	< 1	< 1	< 1	1.00 ±0.04	1.00 ±0.08	< 1
0	DLA	< 1	1.70 ±0.49	1.00 ±0.05	< 1	1.00 ±0.18	1.60 ±0.08	< 1	< 1
0	DLB	< 1	1.70 ±0.43	1.00 ±0.09	< 1	< 1	< 1	< 1	< 1
0	DLC	< 1	1.78 ±0.56	< 1	1.30 ±0.74	< 1	< 1	< 1	< 1
0	AA	2.08 ±1.34	1.00 ±0.06	2.40 ±1.23	1.00 ±0.05	< 1	1.00 ±0.12	1.30 ±0.77	< 1
0	AB	1.60 ±0.78	1.60 ±0.45	2.18 ±1.52	1.00 ±0.07	< 1	1.60 ±0.77	< 1	< 1
0	AC	1.00 ±0.98	1.70 ±0.65	1.00 ±0.07	1.00 ±0.12	< 1	1.00 ±0.08	< 1	< 1

Note: EC – enriched cages, DL – deep litter, A – aviaries.

After 21 days, 62 isolates from egg contents in enriched cages were found (Table 6). From egg content samples, 3 families, 3 genera, and 5 species were isolated. *Ralstonia picketii* was the most isolated species 40% (Figure 4). The remaining most frequently isolated bacterial species were *Lactobacillus delbrueckii* subsp. *lactis*, and *Ralstonia mannitolilytica* (16%).

Table 6 Isolated family, genera, and species of microorganisms of egg contents from enriched cages 21. day.

Family	Genera		Number of isolates
Lactobacillaceae	*Lactobacillus*	*Lactobacillus delbrueckii* subsp. *lactis*	10
Micrococcaceae	*Ralstonia*	*Ralstonia mannitolilytica*	10
Micrococcaceae	*Ralstonia*	*Ralstonia pickettii*	25
Staphylococcaceae	*Staphylococcus*	*Staphylococcus equorum* subsp. *equorum*	8
Staphylococcaceae	*Staphylococcus*	*Staphylococcus lugdunensis*	9
Total			**62**

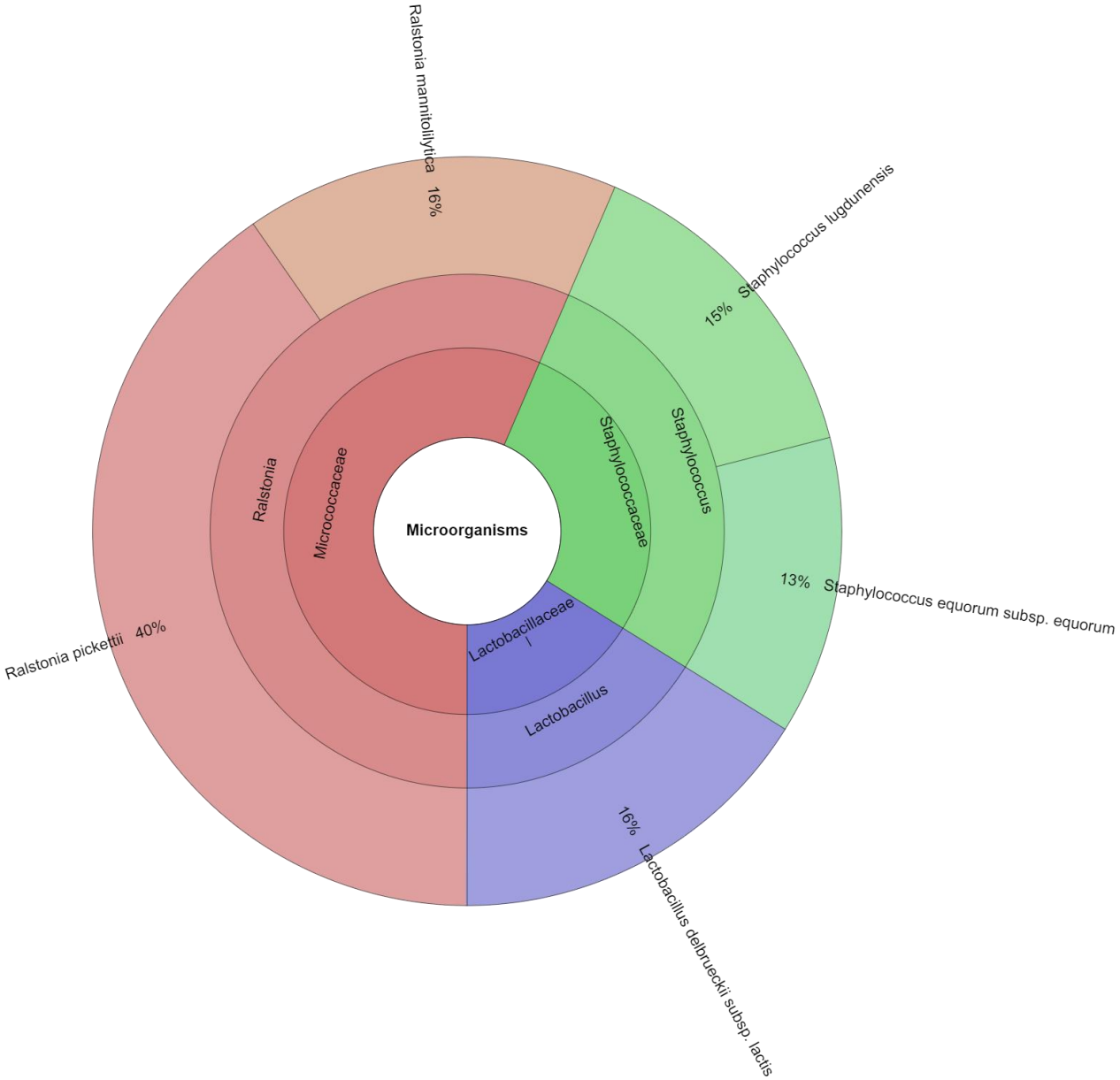


Figure 4 Krona chart: Isolated species of microorganisms of egg contents from enriched cages 21. day.

In the deep litter 21. day old egg contents, 81 isolates in total were discovered (Table 7). Third families, fourth genera, and seventh species were separated from eggs content samples. Among the most isolated species, 38% were *Ralstonia picketii* (Figure 5). Conversely, the other most isolated bacterial species were *Ralstonia mannitolilytica* (19%) and *Lactobacillus amylovorus* (12%).

Table 7 Isolated family, genera, and species of microorganisms of egg contents from deep litter 21. day.

Family	Genera	Species	Number of isolates
Debaryomycetaceae	*Candida*	*Candida glabrata*	5
Lactobacillaceae	*Lactobacillus*	*Lactobacillus amylovorus*	10
Micrococcaceae	*Ralstonia*	*Ralstonia mannitolilytica*	15
Micrococcaceae	*Ralstonia*	*Ralstonia pickettii*	31
Staphylococcaceae	*Staphylococcus*	*Staphylococcus arlettae*	5
Staphylococcaceae	*Staphylococcus*	*Staphylococcus equorum subsp. equorum*	8
Staphylococcaceae	*Staphylococcus*	*Staphylococcus xylosus*	7
Total			**81**

Figure 5 Krona chart: Isolated species of microorganisms of egg contents from deep litter 21. day.

A total of 82 isolates were found in the aviaries 21-day egg contents (Table 8). From egg content samples, a total of 3 families, 3 genera, and 5 species were identified. Ralstonia pickettii, *Ralstonia mannitolilytica*, and *Aromatoleum buckelii* accounted for 38%, 26%, and 16% of the most isolated species.

Table 8 Isolated family, genera, and species of microorganisms of egg from aviaries 21. day.

Family	Genera	Species	Number of isolates
Rhodocyclaceae	*Aromatoleum*	*Aromatoleum buckelii*	13
Micrococcaceae	*Ralstonia*	*Ralstonia insidiosa*	12
Micrococcaceae	*Ralstonia*	*Ralstonia mannitolilytica*	21
Micrococcaceae	*Ralstonia*	*Ralstonia pickettii*	31
Sphingobacteriaceae	*Sphingobacterium*	*Sphingobacterium faecium*	5
Total			**82**

Figure 6 Krona chart: Isolated species of microorganisms of egg contents from aviaries 21. day.

There are various ways that pathogenic bacteria might contaminate eggs, and there are a number of foodborne pathogens that can enter the egg and remain there for the duration of its shelf life. Considerable investigation has been conducted on the topic of bacterial contamination of table eggs **[15]**. Total viable count (TVC) on eggshells plays an important role in egg safety and product shelf life, which makes it a topic of great interest. Regulating bodies in some but not all countries suggest acceptable TVC limits for eggs or egg products **[26]**. One excellent illustration of the significant adjustments made to the agriculture industry in response to shifting societal expectations is the production of eggs. Consumer demand for the consumption of high-quality, healthful animal products that consider sustainability and animal welfare is currently quite strong. Numerous instances of significant alterations to the production process of eggs in response to societal needs may be traced back to the consideration of ethical dimensions in this industry. The primary modifications to the production system include the gradual removal of cage-housing arrangements, the requirement that no male chicks be killed, and an extension of the production period **[2]**. Our study aimed to find out how different laying hen-raising practices affected the egg contents' microbiological quality and the ability to identify germs using mass spectrometry. The laying hen strain and the housing environment may impact the microbial growth on the eggshell because of the dynamic nature of microorganisms **[27]**. This is relevant to food safety in the egg industry. Eggs from conventional cages have a lower eggshell bacterial load than eggs from alternative housing systems like aviaries, litter, free-range, or organic systems **[24]**, **[25]**, **[26]**, **[27]**, **[28]** and similar results were found in our study. There was a noticeably greater bacterial load in caged eggs, according to the few and early observations on the hygienic

quality of eggs produced in the initial types of cages **[29]**, **[30]**, **[31]**, **[32]**. Eggs from backyard and free-range hens were shown to have a significant level of microbial contamination in tests conducted 30-40 years ago **[36]**. There are not many differences between cage eggs and experimental research (assuming that the eggs placed on the ground are excluded) **[33]**. However, they are often smaller at the commercial level, ranging from 0.5 to 1 log units, and tend to be larger in aviaries. Coliform numbers can occasionally be marginally lower than in conventional cages (CC). Thirteen laying houses from three EU nations were examined by De Reu et al. **[34]** with furnished cages and 7 with access to range). The amount of total aerobic bacteria found in the shells of non-cage eggs was just 0.2 log units higher than that of furnished cages (FC) eggs, which is a negligible difference in hygiene. Most of the time, the Enterobacteriaceae counts were not statistically different and almost at the detection limit (< 10 CFU). The egg contents count was relatively low (≈ 2%) in all housing systems. Still, a great deal of variation has been noted among farms that use the same technology **[38]**, **[39]**. The distinctions between NCS and cage eggs are becoming less noticeable in egg grading and packaging **[40]**. Additionally, during storage, the bacterial burden on eggshells decreases. Various elements can impact the housing system's consequences **[33]**, including layer facilities design and farming practices **[35]**. According to Rossi et al. **[37]**, it was not anticipated that the rise in non-cage systems (NCS) in the EU would substantially impact the sanitary quality of eggs. The most recent published research, which is not from the EU, corroborates the previously mentioned tendencies. When Jones et al. **[27]** studied the eggshells of hens kept in conventional cages (CC), barns, and free-range (FR) in the United States, they found that the hens in FR had greater levels of *Enterobacteriaceae*, but only in certain strains of the bird, due to their varying propensity to lay eggs on the floor. According to Parisi et al. **[38]**, there were, on average, 1.0 log CFU more *Enterobacteriaceae* in FR eggs than in CC hen eggs. According to **[31]**, FR production was the primary source of the majority of coliform isolates (62%), with *E. coli* being the predominant strain (55%), particularly in FR nest boxes (44%). Of these isolates, CC production accounted for only 15%. Samiullah et al. **[7]** compared eggs from one FR and one cage commercial farm in Australia. Although the overall bacterial burden was low in both systems, they discovered a considerably lower total microbial load in cage eggs. It is possible to counteract the tendency of increased sale contamination in NCS eggs by practicing appropriate husbandry and hygiene, collecting eggs more frequently, and, most importantly, avoiding floor lay. Additional risk factors for NCS include nest contamination, placing next boxes directly on the litter, and the buildup of eggs in egg belts **[41]**, **[42]**. However, the disparities between facilities with CC or FC might vary greatly, ranging from 5 to 15 or even 100 times larger **[38]**. The total count of bacteria (TCB) in the internal egg content in cardboard boxes was 0.00 to 1.92 log CFU/ml, while in plastic boxes, the range was 0.00 to 2.49 log CFU/ml **[43]**. In our study, the total count of bacteria ranged from < 1 to 2.99 ±1.45 CFU/ml id day 0 and from < 1 to 2.40 ±1.23 log CFU/ml. The egg white was initially thought to be infertile. On the other hand, microbes were found inside the egg white in a recent study **[44]**. Egg yolks have also been found to contain bacteria **[45]**. Past studies **[46]**, **[47]** verified the existence of bacteria in one-day-old chicks' digestive and reproductive systems. Numerous investigations have demonstrated the connection between hen stomach, fecal bacteria, and egg production and fertility **[48]**, **[49]**. In our study, the most identified bacteria were Ralstonia pickettii in all egg variants. In different study *Acinetobacter, Bacillus, Carnobacterium, Enterobacter, Herbaspirillum, Kocuria, Pseudomonas, Staphylococcus, Stenotrophomonas* genera were isolated from egg content **[43]**. After the eggshells are laid, a variety of control techniques are used to reduce the amount of bacteria present, including as cleaning and cold storage **[50]**. There are two reasons why it's critical to keep eggs refrigerated after laying. The eggs' shelf life is extended by cold storage, which also inhibits the growth of mesophilic microbes **[51]**. The average shelf life of an egg is 21-35 days, depending on the nation of origin. Egg quality and shelf life are related, and environmental factors like temperature and relative humidity can have an impact on both **[52]**. Over a 21-day period, albumin quality and egg weight dropped at storage temperatures between 20 and 22 °C but remained steady when eggs were held at 4 °C **[53]**.

CONCLUSION

Caution should be exercised when comparing the effects of housing systems, as several production factors can influence the different components of commercial egg quality. Our study aimed to determine microbial contamination of the internal contents of eggs at day 0 and during day 21 of storage at laboratory temperature. Our results showed good microbiological quality of egg contents, and the most isolated bacteria was *Ralstonia picketti*. Our results did not show the presence of *Salmonella* spp. and coliforms. The level of bacterial contamination of the egg contents depends on the housing system and is related to temperature and days of storage. According to our findings, there were more contaminated eggs at the beginning of the experiment. At the beginning of the experiment, isolated species of microorganisms also represented a larger proportion of all species. Complex processes are involved in the changes in egg microbiological quality parameters that occur during storage in various housing systems. The current study's findings highlight the significance of

microbiological quality in various housing systems for variations in egg quality during storage. Eggs placed in aviaries and cage-housing systems were found to be of a greater quality than eggs put on litter.The bacterial content of egg shells varies more in various housing systems than in cages.Richer cages and aviaries are a good alternative to traditional cages when it comes to laying hen performance and egg quality.

REFERENCES

1. Pires, P. G. da S., Bavaresco, C., Prato, B. S., Wirth, M. L., & Moraes, P. de O. (2021). The relationship between egg quality and hen housing systems - A systematic review. In Livestock Science (Vol. 250, p. 104597). Elsevier BV. https://doi.org/10.1016/j.livsci.2021.104597
2. Gautron, J., Réhault-Godbert, S., Van de Braak, T. G. H., & Dunn, I. C. (2021). Review: What are the challenges facing the table egg industry in the next decades and what can be done to address them? In Animal (Vol. 15, p. 100282). Elsevier BV. https://doi.org/10.1016/j.animal.2021.100282
3. Park, J. H., Kang, M. S., Park, K. M., Lee, H. Y., Ok, G. S., Koo, M. S., Hong, S. I., & Kim, H. J. (2020). A dynamic predictive model for the growth of Salmonella spp. and Staphylococcus aureus in fresh egg yolk and scenario-based risk estimation. In Food Control (Vol. 118, p. 107421). Elsevier BV. https://doi.org/10.1016/j.foodcont.2020.107421
4. Tian, L., Hu, S., Jia, J., Tan, W., Yang, L., Zhang, Q., Liu, X., & Duan, X. (2021). Effects of short-term fermentation with lactic acid bacteria on the characterization, rheological and emulsifying properties of egg yolk. In Food Chemistry (Vol. 341, p. 128163). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.128163
5. Wilson, P. B. (2017). Recent advances in avian egg science: A review. In Poultry Science (Vol. 96, Issue 10, pp. 3747–3754). Elsevier BV. https://doi.org/10.3382/ps/pex187
6. Englmaierová, M., Tůmová, E., Charvátová, V., & Skřivan, M. (2014). Effects of laying hens housing system on laying performance, egg quality characteristics, and egg microbial contamination. In Czech Journal of Animal Science (Vol. 59, Issue 8, pp. 345–352). Czech Academy of Agricultural Sciences. https://doi.org/10.17221/7585-cjas
7. Samiullah, Roberts, J. R., & Chousalkar, K. K. (2014). Effect of production system and flock age on egg quality and total bacterial load in commercial laying hens. In Journal of Applied Poultry Research (Vol. 23, Issue 1, pp. 59–70). Elsevier BV. https://doi.org/10.3382/japr.2013-00805
8. Tomczyk, Ł., Stępień, Ł., Urbaniak, M., Szablewski, T., Cegielska-Radziejewska, R., & Stuper-Szablewska, K. (2018). Characterisation of the Mycobiota on the Shell Surface of Table Eggs Acquired from Different Egg-Laying Hen Breeding Systems. In Toxins (Vol. 10, Issue 7, p. 293). MDPI AG. https://doi.org/10.3390/toxins10070293
9. Tomczyk, Ł., Szablewski, T., Stuper-Szablewska, K., Nowaczewski, S., & Cegielska-Radziejewska, R. (2019). The influence of the conditions of acquisition and storage of table eggs on changes in their quality and the presence of mycobiota and Fusarium mycotoxins. In Poultry Science (Vol. 98, Issue 7, pp. 2964–2971). Elsevier BV. https://doi.org/10.3382/ps/pez156
10. Rodríguez-Navarro, A. B., Domínguez-Gasca, N., Muñoz, A., & Ortega-Huertas, M. (2013). Change in the chicken eggshell cuticle with hen age and egg freshness. In Poultry Science (Vol. 92, Issue 11, pp. 3026–3035). Elsevier BV. https://doi.org/10.3382/ps.2013-03230
11. Guillier, L., Thébault, A., Fravalo, P., Mughini-Gras, L., Jourdan-da Silva, N., David, J., Kooh, P., Cadavez, V., & Gonzales-Barron, U. (2021). Risk factors for sporadic salmonellosis: a systematic review and meta-analysis. In Microbial Risk Analysis (Vol. 17, p. 100138). Elsevier BV. https://doi.org/10.1016/j.mran.2020.100138
12. Mughini-Gras, L., Enserink, R., Friesema, I., Heck, M., van Duynhoven, Y., & van Pelt, W. (2014). Risk Factors for Human Salmonellosis Originating from Pigs, Cattle, Broiler Chickens and Egg Laying Hens: A Combined Case-Control and Source Attribution Analysis. In Y.-F. Chang (Ed.), PLoS ONE (Vol. 9, Issue 2, p. e87933). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0087933
13. Gast, R. K., Regmi, P., Guraya, R., Jones, D. R., Anderson, K. E., & Karcher, D. M. (2019). Contamination of eggs by Salmonella Enteritidis in experimentally infected laying hens of four commercial genetic lines in conventional cages and enriched colony housing. In Poultry Science (Vol. 98, Issue 10, pp. 5023–5027). Elsevier BV. https://doi.org/10.3382/ps/pez222
14. Sharaf Eddin, A., Ibrahim, S. A., & Tahergorabi, R. (2019). Egg quality and safety with an overview of edible coating application for egg preservation. In Food Chemistry (Vol. 296, pp. 29–39). Elsevier BV. https://doi.org/10.1016/j.foodchem.2019.05.182

15. Sharaf Eddin, A., Ibrahim, S. A., & Tahergorabi, R. (2019). Egg quality and safety with an overview of edible coating application for egg preservation. In Food Chemistry (Vol. 296, pp. 29–39). Elsevier BV. https://doi.org/10.1016/j.foodchem.2019.05.182
16. Rzedzicki, J., & Stępień-Pyśniak, D. (2009). Antimicrobial defence mechanisms of chicken eggs and possibilities for their use in protecting human and animal health. In Annales UMCS, Medicina Veterinaria (Vol. 64, Issue 2, pp. 1–8). Walter de Gruyter GmbH. https://doi.org/10.2478/v10082-009-0004-7
17. Berardinelli, A., Ragni, L., Giunchi, A., Gradari, P., & Guarnieri, A. (2008). Physical-Mechanical Modifications of Eggs for Food-Processing During Storage. In Poultry Science (Vol. 87, Issue 10, pp. 2117–2125). Elsevier BV. https://doi.org/10.3382/ps.2007-00216
18. Rehault-Godbert, S., Baron, F., Mignon-Grasteau, S., Labas, V., Gautier, M., Hincke, M. T., & Nys, Y. (2010). Effect of Temperature and Time of Storage on Protein Stability and Anti-Salmonella Activity of Egg White. In Journal of Food Protection (Vol. 73, Issue 9, pp. 1604–1612). Elsevier BV. https://doi.org/10.4315/0362-028x-73.9.1604
19. Samli, H. E., Agma, A., & Senkoylu, N. (2005). Effects of Storage Time and Temperature on Egg Quality in Old Laying Hens. In Journal of Applied Poultry Research (Vol. 14, Issue 3, pp. 548–553). Elsevier BV. https://doi.org/10.1093/japr/14.3.548
20. Silversides, F. G., & Budgell, K. (2004). The Relationships Among Measures of Egg Albumen Height, pH, and Whipping Volume. In Poultry Science (Vol. 83, Issue 10, pp. 1619–1623). Elsevier BV. https://doi.org/10.1093/ps/83.10.1619
21. Gao, D., Qiu, N., Liu, Y., & Ma, M. (2016). Comparative proteome analysis of egg yolk plasma proteins during storage. In Journal of the Science of Food and Agriculture (Vol. 97, Issue 8, pp. 2392–2400). Wiley. https://doi.org/10.1002/jsfa.8052
22. ISO, 4833-2:2013. (n.d.). ISO 4833-2:2013(en), Microbiology of the food chain—Horizontal method for the enumeration of microorganisms—Part 2: Colony count at 30 degrees C by the surface plating technique.
23. ISO 4832:2006. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coliforms. Colony-count technique.
24. ISO 6579-1:2017. Microbiology of the food chain. Horizontal method for the detection, enumeration and serotyping of Salmonella. Part 1: Detection of Salmonella spp.
25. Kačániová, M., Klūga, A., Kántor, A., Medo, J., Žiarovská, J., Puchalski, C., & Terentjeva, M. (2019). Comparison of MALDI-TOF MS Biotyper and 16S rDNA sequencing for the identification of Pseudomonas species isolated from fish. In Microbial Pathogenesis (Vol. 132, pp. 313–318). Elsevier BV. https://doi.org/10.1016/j.micpath.2019.04.024
26. van Veelen, H. P. J., Salles, J. F., & Tieleman, B. I. (2018). Microbiome assembly of avian eggshells and their potential as transgenerational carriers of maternal microbiota. In The ISME Journal (Vol. 12, Issue 5, pp. 1375–1388). Springer Science and Business Media LLC. https://doi.org/10.1038/s41396-018-0067-3
27. Holt, P. S., Davies, R. H., Dewulf, J., Gast, R. K., Huwe, J. K., Jones, D. R., Waltman, D., & Willian, K. R. (2011). The impact of different housing systems on egg safety and quality. In Poultry Science (Vol. 90, Issue 1, pp. 251–262). Elsevier BV. https://doi.org/10.3382/ps.2010-00794
28. Buhr, R. J., Hannah, J. F., Wilson, J. L., Cox, N. A., Richardson, L. J., Cason, J. A., & Musgrove, M. T. (2009). Eggshell bacteria levels of non-washed and washed eggs from caged and cage-free hens. In World Poultry Science Association (pp. 1–7). WPSA.
29. De Reu, K., Grijspeerdt, K., Heyndrickx, M., Zoons, J., De Baere, K., Uyttendaele, M., Debevere, J., & Herman, L. (2005). Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. In British Poultry Science (Vol. 46, Issue 2, pp. 149–155). Informa UK Limited. https://doi.org/10.1080/00071660500065359
30. Galvão, J. A., Biondo, A. W., Possebon, F. S., Spina, T. L. B., Correia, L. B. N., Zuim, C. V., Guerra Filho, J. B. P., Pantoja, J. C. F., & Pinto, J. P. D. A. N. (2018). Microbiological vulnerability of eggs and environmental conditions in conventional and free-range housing systems. In Semina: Ciências Agrárias (Vol. 39, Issue 1, p. 133). Universidade Estadual de Londrina. https://doi.org/10.5433/1679-0359.2018v39n1p133
31. Jones, D. R., Cox, N. A., Guard, J., Fedorka-Cray, P. J., Buhr, R. J., Gast, R. K., Abdo, Z., Rigsby, L. L., Plumblee, J. R., Karcher, D. M., Robison, C. I., Blatchford, R. A., & Makagon, M. M. (2015). Microbiological impact of three commercial laying hen housing systems. In Poultry Science (Vol. 94, Issue 3, pp. 544–551). Elsevier BV. https://doi.org/10.3382/ps/peu010
32. Singh, R., Cheng, K. M., & Silversides, F. G. (2009). Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. In Poultry Science (Vol. 88, Issue 2, pp. 256–264). Elsevier BV. https://doi.org/10.3382/ps.2008-00237

33. Capero, R., Yanguela, L., Lidon, M. D., & Hernandis, A. (2000). Calidad del huevo en jaulas enriquecidas. In Córdoba (pp. 61–80). University of Córdoba.

34. Capero, R., María, G., & Hernandis, A. (2001). Calidad del huevo en jaulas enriquecidas: Resultados en la fase final de puesta. In Córdoba (pp. 209–218). University of Córdoba.

35. Mallet, S., Guesdon, V., Ahmed, A. M. H., & Nys, Y. (2006). Comparison of eggshell hygiene in two housing systems: Standard and furnished cages. In British Poultry Science (Vol. 47, Issue 1, pp. 30–35). Informa UK Limited. https://doi.org/10.1080/00071660500468132

36. Sauveur, B. (1991). Mode d'élevage des poules et quatité de l'oeuf de consommation. In INRAE Productions Animales (Vol. 4, Issue 2, pp. 123–130). Universite de Bordeaux. https://doi.org/10.20870/productions-animales.1991.4.2.4324

37. De Reu, K., Messens, W., Heyndrickx, M., Rodenburg, T. B., Uyttendaele, M., & Herman, L. (2008). Bacterial contamination of table eggs and the influence of housing systems. In World's Poultry Science Journal (Vol. 64, Issue 1, pp. 5–19). Informa UK Limited. https://doi.org/10.1017/s0043933907001687

38. De Reu, K., Rodenburg, T. B., Grijspeerdt, K., Messens, W., Heyndrickx, M., Tuyttens, F. A. M., Sonck, B., Zoons, J., & Herman, L. (2009). Bacteriological contamination, dirt, and cracks of eggshells in furnished cages and noncage systems for laying hens: An international on-farm comparison. In Poultry Science (Vol. 88, Issue 11, pp. 2442–2448). Elsevier BV. https://doi.org/10.3382/ps.2009-00097

39. Huneau-Salaün, A., Guinebretière, M., & Michel, V. (2014). Effect of substrate provision on performance and behaviour of laying hens in the pecking and scratching area of furnished cages. In British Poultry Science (Vol. 55, Issue 4, pp. 409–418). Informa UK Limited. https://doi.org/10.1080/00071668.2014.92508

40. De Reu, K., Grijspeerdt, K., Messens, W., Heyndrickx, M., Uyttendaele, M., Debevere, J., & Herman, L. (2006). Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including Salmonella enteritidis. In International Journal of Food Microbiology (Vol. 112, Issue 3, pp. 253–260). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2006.04.011

41. Rossi, M., De Reu, K., & Ferrante, V. (2010). Will the move from conventional cage to alternative production systems for laying hens to impact egg quality and hen welfare? Proc. XIIIth Eur. Poultry Conf, Tours.

42. Rodenburg, T. B., Tuyttens, F. A. M., Sonck, B., De Reu, K., Herman, L., & Zoons, J. (2005). Welfare, Health, and Hygiene of Laying Hens Housed in Furnished Cages and in Alternative Housing Systems. In Journal of Applied Animal Welfare Science (Vol. 8, Issue 3, pp. 211–226). Informa UK Limited. https://doi.org/10.1207/s15327604jaws0803_5

43. Sokołowicz, Z., Kačániová, M., Dykiel, M., Augustyńska-Prejsnar, A., & Topczewska, J. (2023). Influence of Storage Packaging Type on the Microbiological and Sensory Quality of Free-Range Table Eggs. In Animals (Vol. 13, Issue 12, p. 1899). MDPI AG. https://doi.org/10.3390/ani13121899

44. Lee, S., La, T.-M., Lee, H.-J., Choi, I.-S., Song, C.-S., Park, S.-Y., Lee, J.-B., & Lee, S.-W. (2019). Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. In Scientific Reports (Vol. 9, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-019-43280-w

45. Ding, J., Dai, R., Yang, L., He, C., Xu, K., Liu, S., Zhao, W., Xiao, L., Luo, L., Zhang, Y., & Meng, H. (2017). Inheritance and Establishment of Gut Microbiota in Chickens. In Frontiers in Microbiology (Vol. 8). Frontiers Media SA. https://doi.org/10.3389/fmicb.2017.01967

46. Pajurek, M., Pietron, W., Maszewski, S., Mikolajczyk, S., & Piskorska-Pliszczynska, J. (2019). Poultry eggs as a source of PCDD/Fs, PCBs, PBDEs and PBDD/Fs. In Chemosphere (Vol. 223, pp. 651–658). Elsevier BV. https://doi.org/10.1016/j.chemosphere.2019.02.023

47. Zhou, Q., Lan, F., Li, X., Yan, W., Sun, C., Li, J., Yang, N., & Wen, C. (2021). The Spatial and Temporal Characterization of Gut Microbiota in Broilers. In Frontiers in Veterinary Science (Vol. 8). Frontiers Media SA. https://doi.org/10.3389/fvets.2021.712226

48. Elokil, A. A., Abouelezz, K., Adetula, A. A., Ahmad, H. I., Mo, C., Sun, C., & Li, S. (2020). Investigation of the impact of gut microbiotas on fertility of stored sperm by types of hens. In Poultry Science (Vol. 99, Issue 2, pp. 1174–1184). Elsevier BV. https://doi.org/10.1016/j.psj.2019.10.048

49. Elokil, A. A., Magdy, M., Melak, S., Ishfaq, H., Bhuiyan, A., Cui, L., Jamil, M., Zhao, S., & Li, S. (2020). Faecal microbiome sequences in relation to the egg-laying performance of hens using amplicon-based metagenomic association analysis. In Animal (Vol. 14, Issue 4, pp. 706–715). Elsevier BV. https://doi.org/10.1017/s1751731119002428

50. Fikiin, K., Akterian, S., & Stankov, B. (2020). Do raw eggs need to be refrigerated along the food chain? In Trends in Food Science & Technology (Vol. 100, pp. 359–362). Elsevier BV. https://doi.org/10.1016/j.tifs.2020.04.003

51. Saleh, G., El Darra, N., Kharroubi, S., & Farran, M. T. (2020). Influence of storage conditions on quality and safety of eggs collected from Lebanese farms. In Food Control (Vol. 111, p. 107058). Elsevier BV. https://doi.org/10.1016/j.foodcont.2019.10705

52. Vlčková, J., Tůmová, E., Míková, K., Englmaierová, M., Okrouhlá, M., & Chodová, D. (2019). Changes in the quality of eggs during storage depending on the housing system and the age of hens. In Poultry Science (Vol. 98, Issue 11, pp. 6187–6193). Elsevier BV. https://doi.org/10.3382/ps/pez401

53. Jones, D. R., Ward, G. E., Regmi, P., & Karcher, D. M. (2018). Impact of egg handling and conditions during extended storage on egg quality. In Poultry Science (Vol. 97, Issue 2, pp. 716–723). Elsevier BV. https://doi.org/10.3382/ps/pex351

Funds:

This research received no external funding.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Ján Petrovič, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Animal Husbandry, Tr. A. Hlinku 2, 949 76, Nitra, Slovakia,
E-mail: xpetrovicj@uniag.sk
ORCID: https://orcid.org/0000-0002-3659-0087

Martin Mellen, Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Animal Husbandry, Tr. A. Hlinku 2, 949 76, Nitra, Slovakia,
E-mail: martin.mellen@gmail.com
ORCID: https://orcid.org/0009-0003-9503-9265

Natália Čmiková, Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Institute of Horticulture, Tr. A. Hlinku 2, 949 76, Nitra, Slovakia,
E-mail: xcmikova@uniag.sk
ORCID: https://orcid.org/0000-0003-4419-0590

Marianna Schwarzová, Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Institute of Horticulture, Tr. A. Hlinku 2, 949 76, Nitra, Slovakia,
Tel.: +421 37 641 4352
E-mail: marianna.schwarzova@uniag.sk
ORCID: https://orcid.org/0000-0002-0694-952X

***Miroslava Kačániová**, Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Institute of Horticulture, Tr. A. Hlinku 2, 949 76, Nitra, Slovakia,
University of Economics and Human Sciences in Warsaw, School of Medical and Health Sciences, Okopowa 59, Warszawa, 01 043, Poland,
Tel.: +421 37 641 4715
E-mail: miroslava.kacaniova@uniag.sk
ORCID: https://orcid.org/0000-0002-4460-0222

potravinárstvo®
OPEN ACCESS
Received: 29.11.2023
Revised: 12.12.2023
Accepted: 29.12.2023
Published: 9.1.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 66-76
https://doi.org/10.5219/1941
ISSN: 1337-0960 online
www.potravinarstvo.com

Development of wheat composite bread using barley β-glucan rich flour

Boris Kovac, Andreja Bregar

ABSTRACT

The study highlights the potential for enhancing the nutritional value of wheat bread by incorporating β-glucan-enriched flour derived from barley. Initially, the study explores the air separation of barley flour into fractions rich in β-glucans. The procedure significantly increases the β-glucan concentration from 4.5% in barley to 8.0-10.5% in the separated coarse fractions, with yields varying from 33% to 77%. The optimal fraction of β-glucan-rich flour (β-GRF), balancing β-glucan concentration and yield, was chosen for subsequent evaluations. The impact of different levels of β-GRF supplementation (ranging from 0% to 30%) on the dough texture and sensory characteristics of the mixed wheat bread was tested. The addition of β-GRF has a noticeable influence on the rheological properties of the dough, resulting in longer development times and decreased stability compared to control samples. As the concentration of β-GRF increases to 10%, the specific volume generally rises, reaching 3.5 cm³/g, compared to the control bread with a specific volume of 3.2 cm³/g. However, beyond the 10% β-GRF level, the specific volume starts to decrease. Furthermore, β-GRF addition affects sensory and texture aspects, including bread volume, crumb, and crust characteristics. Despite these alterations, the bread remains within acceptable sensory parameters, and the final product, with 3g of β-glucan per 100 g of bread, meets the criteria for a health claim related to cholesterol reduction. This research underscores the potential to create healthier bread options by harnessing the nutritional benefits of dry concentrated β-glucans from barley, offering a promising avenue for improving the nutritional profile of bread products.

Keywords: β-glucan, barley, supplementation, mixed bread, health claim

INTRODUCTION

There is a growing interest in foods with added nutritional value that come with approved health claims. With its valuable nutrients, including β-glucans, barley has become an attractive option for functional food preparation due to its nutritional and technological benefits **[1]**. On the other hand, white wheat bread, consumed in large quantities in many European countries, is lacking in dietary fiber. Hence, public demand and research interest is in enriching bread with non-digestible prebiotic ingredients with health-promoting effects **[2]**. According to Hu et al. **[3]**, β-glucan reduces the glycemic index of bread and α-amylase activity by interacting with starch to form a more stable gel network structure, reducing the contact area between amylase and starch. The European Food Safety Authority (EFSA), through Commission Regulation **[4]** and the US Food and Drug Administration, has approved health claims for β-glucans. β-glucans are a key component for reducing the glycemic index of various processed foods **[5]**. EFSA's health claim depends on the source of β-glucans and the amount of β-glucan in one portion of the meal. Consumption of β-glucans from oats or barley as part of a meal reduces blood glucose levels after that meal. The claim may be used only for food that contains at least 4 g of β-glucans from oats or barley for every 30 g of available carbohydrates in a quantified portion as part of the meal. β-glucans contribute to maintaining normal blood cholesterol levels, and the claim may be used only for food containing at least 1 g of

β-glucans from cereals or mixtures of these sources per quantified portion. The health claims related to β-glucans have led to increased interest in β-glucan-rich cultivars of barley and oats and concentrated β-glucan flours. Garcia-Gimenez et al. **[6]** reported that barley has a considerably high β-glucan concentration, ranging from 2.5% to 8% w/w, compared to other cereals such as wheat or rice, where the concentration does not exceed 1%. Various extraction and purification methods are available to produce cereal β-glucans **[7]**. The nature of the extraction procedure has a profound effect on the structure and molecular weight of β-glucan. Water and alkali extractions are preferred as the yield and recovery of extracted β-glucan concentration exceed 50% **[8]**. Pure β-glucan was commercially produced through high-temperature extraction with concentrated sodium hydroxide. However, using pure or highly concentrated β-glucan fractions to enrich cereal products is unsustainable and unnecessary. Practitioners in industrial-scale applications are constantly seeking the most cost-effective and environmentally sustainable procedures for obtaining highly concentrated β-glucan meal. Dry processing methods, such as grinding, screening, and/or air classification, use less energy and are environmentally friendly. The concentration of the final product can vary and is closely connected to the separation technique used and the initial content in the cereal. β-Glucans enriched flour by air classification could be a good ingredient for functional food preparation. However, the concentration of β-glucan will likely remain relatively low compared to wet extraction procedures. Ferrari et al. **[9]** concentrated β-glucan using a combination of micronization and air classification processes, resulting in a product ideal for integration into bread recipes. By combining grinding and screening **[10]**, fraction yields ranged from 30-70%, and the content of β-glucans ranged from 9.31% to 18.19%. While sieving allows the separation of fractions based solely on particle size and volume, the advantage of air classification is that fractions in the air stream are also separated based on specific gravity. The process of dry air separation is both environmentally and financially superior. Wheat flours are optimal for bread-making quality and can be blended with barley β-glucan. However, integrating purified β-glucan into a dough recipe can affect its rheological properties. Several authors **[11]** and **[12]** have reported that the overall effect may not necessarily be positive when integrating purified β-glucan into a dough recipe, affecting bread texture quality. The successful integration of β-glucan flours, rather than purified β-glucan, with minimal impact on the final bread quality, still presents a challenge.

Scientific Hypothesis

There is growing interest in foods with added nutritional value and labeled with approved health claims. Preparing β-glucan-enriched functional products, such as mixed wheat bread, using pure or highly concentrated β-glucan fractions may pose technological and economic challenges. This concern has prompted our study, where we explored two hypotheses:

a. The β-glucan-rich fraction (β-GRF) obtained from barley through the dry separation method is sufficiently concentrated to support health claims related to lowering cholesterol levels.

b. Integrating β-GRF into mixed wheat bread yields acceptable dough characteristics and final bread quality.

These hypotheses aim to address the feasibility and effectiveness of incorporating β-glucan into mixed wheat bread, both in terms of health benefits and overall product quality.

MATERIAL AND METHODOLOGY

Samples

Commercially known as Beta Gerstel, Barley was used to produce 21 samples of β-GRF through the dry separation method. In the second level, dough samples were prepared by blending wheat flour with the β-GRF samples to determine the rheological characteristics of the dough. The third level involves preparing bread samples for final analyses. The same ratio of wheat flour to β-GRF, as used in the second phase, is employed, along with yeast and salt.

Chemicals

Kit for β-glucan determination: β-Glucan Assay Kit K-BGLU, Megazyme Ltd., Ireland. All chemicals were of analytical grade quality.

Animals, Plants, and Biological Materials

Wheat (*Triticum aestivum*) flour (12.5% protein, 0.50% ash, 1.3% crude fat, 14.7% moisture) produced by Mlinotest d.d., Slovenia.

Barley (*Hordeum vulgare*), Beta gerste, Germany (10.5% protein, 2.50% ash, 4,5% β-glucan,14.0% moist.

Instruments

Mill, hammermill, microniser: Sangatti, Italy.

Farinograph: Brabender, Germany.

Spiral mixer: IP10F Fimar, Italy.

Baking oven: Dibas, Germany.
Spectrophotometer: Mettler Toledo, USA.

Laboratory Methods

The amount of β-glucan was determined using the Megazym kit, following the AAAC Method 32-23 modified according to McCleary and Codd **[13]**. This method relies on the enzymatic degradation of polysaccharides by β-glucan hydrolases (specific for mixed-linkage [(1-3)(1-4)]-β-D-glucan), followed by spectrophotometric measurement of the generated D-glucose.

Dough rheology was determined following the AACC Farinograph Method 54-29 **[14]**. The farinograph is an instrument used to measure and record the rheological properties of dough during mixing. It is used to evaluate flour's water absorption and to determine dough development time, stability, and other dough mixing characteristics.

The seed displacement method determined the bread volume and specific volume according to AACC Method 10-05.01 **[15]**. It can be used to accurately measure the volume of oddly shaped objects by measuring the volume of displaced material when an object is submerged in a volumetric tube. This method relies on the principle that the bread volume displaces an equivalent volume of the rapeseed.

A sensory profile that included the attributes related to texture was completed according to ISO 13299 **[16]**. A sensory panel of eight trained experts (five females, three males; age range, 28-58 years) was used to evaluate the bread samples. All of the experts had experience in descriptive evaluation of bread. The initial panel discussion and training session were compressed to two 2-h sessions. In the first phase of the test, the panel developed 9 descriptors for the sensory attributes of the control bread and the bread with β-GRF addition. The panellists created a 10-point unstructured line scale with descriptor labels at either end. Attribute ranges (weak = 0 vs. intense = 10): Crust thickness: thick = 0 vs. thin = 10, crumb colour: white = 0 vs. brown = 10, crumb pore homogeneity: inhomogeneous = 0 vs. homogeneous = 10, crumb pore size: small = 0 vs. big = 10. Texture cohesiveness: less cohesive = 0, very cohesive = 10, springiness: springless = 0 vs. springy = 10, toughness: weak = 0 vs. intense = 10, hardness: smooth = 0 vs. hard = 10, adhesivity: not stick = 0 vs. sticky = 10. The control bread was chosen as the reference sample, to reduce the variation among the panellists. The panellists defined the intensity value of the reference sample concerning each sensory attribute. Based on the questionnaires, the panelists rated the samples (one whole loaf of bread, two slices/per person) individually, using a balanced test design in which the serving order was randomized for each panellist.

The β-GRF was prepared following the procedure outlined in reference **[9]**. Barley underwent dehusking, with 12% of the peripheral parts being separated. The grains were then hammer-milled and subjected to two rounds of micronization using a high-speed rotor micronizer operating at 10,000 rotations per minute. Micronized fractions were separated in a cyclone under an airflow. An aspirating pump, positioned at the system's end, propelled the airflow, regulated by an inlet valve. Before the pump, a cyclone and a filter were placed to eliminate excessively fine powder. The apparatus sorted the flour into two portions: a coarse fraction and a fine fraction. Particle size control and separation were achieved by modulating the airflow using an inlet valve.

Description of the Experiment

Sample preparation: Preparation of β-GRF samples from barley:

Micronized flours were separated into coarse, medium, and fine fractions in cyclone by a controlled radial airflow. The yield and β-glucan concentration of fractions were determined. β-GRF coarse fraction was adjusted to 10% of β-glucan and used in dough preparation and baking trials.

Farinograph dough samples: The wheat flour was blended with β-GRF at 5%, 10%, 15%, 20%, 25%, and 30% (w/w). A control sample was prepared without β-GRF. The water addition was adjusted based on the farinograph result.

Bread samples: The dough was prepared from wheat flour blended with β-GRF at levels of 0%, 5%, 10%, 15%, 20%, 25%, and 30% (w/w), 2% yeast, 2% salt and 56.5% to 60% water (all w/w). The water addition was adjusted based on the previous farinograph results. Subsequently, the mixed dough was divided into 450 g pieces, shaped manually, and placed into baking pans. After fermenting at 30 °C and 85% relative humidity for 60 minutes, the loaves were baked in a convection oven at 230 °C for 30 minutes.

Number of samples analyzed: 21 samples of β-GRF, 7 samples of dough, 7 samples of bread.

Number of repeated analyses: 3.

Number of experiment replications: The sensory evaluation involved 16 replications, while other tests were conducted with 6 replications.

Design of the experiment:

1. Preparation of β-GRF from barley by milling, micronization, and dry separation; samples were used to determine β-glucan content and yield.

2. Dough preparation and determination of rheological characteristics by farinograph. Water absorption development time, stability, degree of softening, and quality number were determined.
3. Baking trials and final bread characteristics determination; moisture content, loaf height, volume, and specific volume were determined.
4. Sensory evaluation; crust thickness and colour, crumb homogeneity and size, texture cohesiveness, springiness, toughness, hardness, and adhesivity were determined.
In the final phase, the obtained results were processed, subjected to statistical analysis, and verified the validity of our hypotheses.

Statistical Analysis

The SPSS software version 26 (IBM, Armonk, NY, USA) was used to confirm the statistical significance of differences between each experimental set of wells. Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by *post-hoc* tests to determine any differences between group means. Differences with $p < 0.05$ were considered to be statistically significant. The data are presented as means ± standard deviation for 6 replicates, and 16 replicates were conducted for sensory evaluation.

RESULTS AND DISCUSSION

Separation of barley flour into β-glucan enriched fractions

The primary aim was to separate micronized barley flour into fractions with as high a concentration of β-glucans as possible using an air cyclone. The cyclone could classify flour particles into coarse, medium, and fine fractions based on airflow settings. The results, presented in Table 1, show the yield and concentration of β-glucans in g/100g dry matter for all three fractions were obtained through classification at various airflow settings. As the intensity of air flow increases by airflow variation (valve settings from 1 to 7) the β-glucan content in the coarse fraction tends to concentrate. The β-glucan content in the medium fraction generally decreases with higher airflow intensities. As the airflow intensity increases, the yield of the coarse fraction decreases steadily. In contrast, the yield of the medium fraction increases as the airflow intensity increases. The separation phenomenon is consistent with the results of **[10]**. This suggests that β-glucan particles are redistributed from the medium to the coarse fraction at higher air flow intensities. The trends of the yield and β-glucan curves are similar to those described by Ferrari **[9]**. The yield of the fine fraction remains relatively low compared to the coarse and medium fractions across all airflow intensities. Overall, the results indicate that the choice of airflow intensity in cyclone separation can significantly impact both yield and β-glucan content in different fractions. The study found that at valve setting 6, the maximal β-glucan content in the coarse fraction was 2.1 times higher than that of the initial material. The initial material had a β-glucan content of 4.5 g/100 g dry matter, which is lower than that reported in barley tested by another source **[10]**. The efficiency of β-glucan concentration in the coarse fraction is comparable to the findings of Ferrari et al. **[9]**. They started with 8.7% β-glucan content in barley flour and, after two cycles of air classification, with a relatively low yield (29.8%), achieved 15.6%, β-glucan content respectively. The classic extraction of β-glucans from cereals typically encompasses a minimum of four comprehensive stages **[17]**: (a) wet milling of raw cereal and deactivation of β-glucanase to preserve the native high molecular weight of β-glucan, (b) extraction of β-glucans, (c) a purification and isolation step, and (d) drying of the final product.

Table 1 The yields and β-glucan content (g/100g) and SD in coarse, medium, and fine fractions as a function of airflow regulated by separator valve settings.

Airflow settings	Fraction yield (yield ±SD)			β-glucan content (g.100g^{-1} ±SD)		
	coarse fraction	medium fraction	fine fraction	coarse	medium	fine fraction
1	0.75 ±0.0^{def}	0.22 ±0.01^{a}	0.03 ±0.01^{a}	8.01 ±0.02^{a}	6.15 ±0.02^{d}	5.91 ±0.28^{f}
2	0.70 ±0.07^{cf}	0.28 ±0.02^{b}	0.03 ±0.01^{a}	8.33 ±0.02^{a}	5.23 ±0.03^{c}	4.78 ±0.20^{d}
3	0.66 ±0.05^{be}	0.31 ±0.01^{bc}	0.04 ±0.01^{a}	8.69 ±0.05^{ab}	5.25 ±0.04^{c}	3.75n ±0.12^{d}
4	0.65 ±0.03^{bd}	0.34 ±0.02^{cd}	0.03 ±0.01^{a}	9.13 ±0.03^{b}	4.71 ±0.04^{bc}	1.22 ±0.10^{b}
5	0.61 ±0.03^{ac}	0.35 ±0.0^{d}	0.03 ±0.01^{a}	10.18 ±0.03^{c}	4.13 ±0.03^{ab}	1.3 ±0.13^{b}
6	0.59 ±0.04^{ab}	0.38 ±0.02^{d}	0.02 ±0.01^{a}	10.51 ±0.04^{c}	3.99 ±0.03^{a}	1.84 ±0.21^{c}
7	0.53 ±0.07^{a}	0.45 ±0.01^{e}	0.02 ±0.01^{a}	10.01 ±0.04^{c}	3.9 ±0.02^{a}	0.28 ±0.04^{a}

Note: Data are medians. Data with different superscript letters in the same column are significantly different ($p < 0.05$).

In contrast, our procedure involves only the milling and mechanical separation phase, making the process more sustainable and cost-effective compared to the traditional wet process with its multiple stages. Cajzek et al. **[18]** also asserted that a two-step process involving milling and sieving is effective in preparing flours rich in beta-glucan and bioactive compounds, offering potential enhancements in both functional and health-related properties for bakery products. However, it is important to note that our approach as well as the previously mentioned process **[18]** results in a lower final concentration of beta-glucan. This drawback could potentially be mitigated by selecting higher-quality raw materials. We can conclude that the dry process offers great potential for environmentally and economically friendly β-glucan concentration; however, we have to stress the importance of barley cultivars with higher initial β-glucan content for large-scale production. Industrial production would require scale-up with further process optimization. Based on the yield and β-glucan concentration, we identified coarse fractions that are suitable for our upcoming tests in dough preparation and baking trials. The β-GRF fraction was adjusted to contain 10% β-glucan.

Dough characteristics

Table 2 shows notable effects on all determined Farinograph parameters when β-GRF is added to wheat flour dough. β-glucan supplementation leads to an increase in dough hydration. This observation is consistent with findings from a study by Zeng et al. **[19]**, which reported that hydrophilic colloids, like β-glucan, commonly increase dough viscosity, requiring slightly more water for dough preparation. β-glucan impacts hydration, development times, dough stability, and quality. In general, the trend is that β-glucan enhances these aspects, but the specific effects depend on its concentration. Up to 2% of β-glucan supplements contribute to the formation and development of gluten networks, promoting the formation of harder, stronger, and more stable gluten networks **[20]**. However, as the concentration of β-glucan continues to rise, the gluten structure weakens to a greater extent, as indicated by the work of Lii et al. **[21]**, which is not preferred in regard to dough stability **[22]**. Previous research **[12]** demonstrated that substituting hulless barley flour for wheat flour adversely affected gluten microstructure and dough mixing behavior. This was attributed to the partial breaking of cross-links in the gluten network, resulting in shortened dough development and stability times. The current study's results align with the mentioned findings, showing a significant increase in development time when 5% β-GRF is added to the dough. This suggests that β-glucan doughs require more time for proper gluten development during mixing than control dough, potentially leading to better stability during processing and handling. The current study indicates that β-GRF supplementation higher than 10% (equivalent to 1% β-glucan) negatively correlates with development time.

Table 2 Farinograph parameters of wheat flour dough as a control (0) and fortified dough with β-GRF at a range to 30% (β-GRF).

B-GRF (%)	Farinograph dough parameters				
	Water absorption (% ±SD)	Development time (min ±SD)	Stability (min ±SD)	Degree of softening (BU* ±SD)	Quality number (number ±SD)
0	56.5 ±3.0^{a}	7.0 ±0.8^{ab}	12.14 ±1.63^{ab}	40 ±1.63^{a}	160 ±14^{bcd}
5	57.5 ±0.6^{a}	9.1 ±0.3^{df}	15.3 ±0.9^{c}	48.0 ±1.8^{bc}	160 ±15^{cd}
10	57.5 ±0.7^{a}	8.35 ±0.3^{cd}	13.40 ±1.2^{bc}	56 ±1.5^{cd}	144 ±6^{ac}
15	58.0 ±0.8^{a}	8.15 ±0.4^{cf}	10.1 ±0.5^{a}	61 ±1.8^{d}	134 ±6^{ab}
20	58.5 ±0.2^{a}	7.5 ±0.6^{bc}	10.2 ±1.2^{a}	51 ±2.9^{bc}	146 ±5^{ad}
25	59.5 ±2.4^{a}	6.1 ±0.2^{a}	10.1 ±0.8^{a}	50.0 ±4.1^{b}	129 ±10^{a}
30	60.0 ±1.8^{a}	6.15 ±0.3^{a}	10.0 ±0.8^{a}	49 ±1.5^{b}	124 ±6^{a}

Note: *BU Brabender farinograph units. Data are medians. Data with different superscript letters in the same column are significantly different (p <0.05).

This aligns with the observations of a previous study **[23]**, which noted that at concentrations over 1%, β-glucan molecules organize into a dense, pseudoplastic complex. The trend in dough stability mirrors that of development time, increasing at low β-GRF additions but decreasing when more than 10% β-GRF is added. The current study's findings are consistent with previous research **[11]**, **[24]**, where increased water absorption and β-glucan concentration resulted in delayed dough development time. However, it contrasts with the results of Mohebbi et al. **[24]**, who found a negative effect on stability and development time even with a 1% β-glucan addition.

Bread moisture content, volume, and specific volume

The moisture content of the final bread samples presented in Table 3 appears to remain relatively stable across different levels of β-glucan content, with slight variations ranging from 43.7% to 46%. This suggests that changes in β-glucan content significantly impact the moisture content of the bread. Increased water absorption and, consequently, higher bread moisture content have been reported in other studies where β-glucan from different sources was used to fortify wheat flour **[11]**. The literature describes a statistically significant correlation between dough characteristics and bread quality. That means that the results of the farinograph dough testing reliably predict the final characteristics of the enriched bread **[25]**.

Table 3 Bread moisture content, volume, and specific volume and loaf height for control (0) and fortified dough with β-GRF at range to 30%.

β-GRF (%)	Moisture content (% ±SD)	Loaf hight (cm ±SD)	Volume (cm^3 ±SD)	Specific volume ($cm^3.g^{-1}$ ±SD)
0	38.7 ±0.8^{a}	8.1 ±0.1^{b}	1200 ±8.1^{c}	3.2 ±0.1^{bc}
5	39.2 ±1.6^{a}	8.2 ±0.1^{b}	1240 ±8.7^{d}	3.3 ±0.1^{c}
10	40.0 ±1.6^{ab}	8.3 ±0.1^{b}	1250 ±8.7^{d}	3.5 ±0.2^{c}
15	40.5 ±1.3^{ab}	7.9 ±0.2^{ab}	1015 ±6.4^{b}	3.1 ±0.1^{b}
20	41.5 ±0.3^{ab}	7.9 ±0.2^{ab}	990 ±9.1^{a}	2.9 ±0.1^{ab}
25	42.2 ±0.8^{ab}	7.8 ±0,1^{a}	980 ±9.1^{a}	2.8 ±0.1^{a}
30	42.4 ±1.6^{b}	7.7 ±0.3^{a}	977 ±9.2^{a}	2.8 ±0.1^{a}

Note: Data are medians. Data with different superscript letters in the same column are significantly different (p <0.05).

The data indicates a significant impact of β-GRF addition on bread properties, particularly a noticeable decrease in loaf height when the β-glucan content exceeds 10%. The decrease in loaf height (Figure 1) is attributed to the higher levels of β-glucan, which intensively affect the bread's volume, leading to denser or less risen bread. Škrbic et al. **[26]** noted a similar effect when barley flour was added to wheat flour, causing a decrease in bread volume due to gluten dilution and less retention of CO_2 gas. The study observes that supplemented bread volume generally increases up to 10% β-GRF, after which it starts to decrease. Xu et al. **[3]** also reported a similar trend when adding different doses of oat β-glucan. Specific volume, which measures the volume per gram of bread, follows the same trend as bread volume, becoming denser and less voluminous as more β-glucan is added. The findings align with a previous report **[12]** for added barley β-glucan, and similar studies **[27]** describe a significant reduction in specific bread volume and an increase in firmness when enriched with fiber-rich substrates. Depending on the desired characteristics of the bread, such as loaf height and specific volume, one can use this data to determine the optimal level of β-glucan content. For instance, if a lighter and more voluminous bread, popular in some European regions, is preferred, a lower β-glucan supplement may be the choice.

Figure 1 Influence of β-glucan on the appearance of the baked product. The concentration increases from left to right. Far left is the control with 0%, followed by samples with 5, 10, 15, 20, 25, and 30% β-glucan.

Crust and crumb attributes and texture profile of the bread

There is a clear trend of decreasing crust thickness as the percentage of β-GRF increases, indicating that higher β-GRF content leads to thinner crusts. The crust colour darkens with higher levels of β-GRF supplementation, likely due to the Maillard reaction during baking, resulting in crust browning. The data indicates a decrease in crumb pore homogeneity with β-GRF supplementation, suggesting that β-GRF may lead to an inconsistent and non-uniform crumb structure throughout the bread. Crumb pore size increases with β-GRF supplementation up to 15%, associated with a more open and airy texture, preferred for certain types of bread, such as artisanal loaves. Beyond 15% supplementation, crumb size reduces, leading to a decrease in bread volume. Similar behaviour was

observed in a study **[28]** where wheat flour was partially substituted with an oat flour fraction rich in β-glucans. Springiness represents the ability of bread to return to its original shape after compression. Current work indicates that the springiness of the bread samples increases with higher β-GRF supplementation levels, suggesting improved elasticity. This aligns with findings from Kurek et al. **[29]**, who observed increased springiness with low levels of β-glucan supplementation. The toughness and hardness of bread samples in current research decrease as β-GRF supplementation increases. This is consistent with the study by Skendi et al. **[11]**, where an increasing level of pure β-glucan supplementation resulted in a coarser structure and decreased breadcrumb firmness. The cohesiveness of tested bread samples increases, indicating a decreasing tendency of the bread to fracture and crumble with higher β-GRF levels. This contrasts with the findings of **[28]**, where oat beta-glucans led to decreasing cohesiveness, although not significantly different between samples. Adhesivity of bread samples increases with higher β-GRF supplementation, in line with other works showing that bread containing β-glucans exhibits lower moisture loss during storage **[11]**. Abdel-Gawad **[30]** reported that β-glucan enrichment could prolong bread shelf life, and the reduction in moisture loss is evident with an increasing concentration of β-glucans. The results confirm that β-glucan can impact bread quality parameters, potentially causing some damage, as observed by other authors **[31]**. The results conclude that further research and testing may be necessary to optimize β-GRF levels for achieving the desired bread texture and optimal nutritional properties. In summary, the results provide comprehensive insights into how β-glucan supplementation influences various textural properties of bread. The observed effects may have implications for product quality and nutritional considerations, highlighting the need for careful optimization based on intended use and consumer preferences.

Table 4 Crust and crumb attributes, texture profile of the bread prepared from the wheat flour dough for control (0) and fortified dough with β-GRF at range to 30%.

β-GRF (%)		0	5	10	15	20	25	30
Crust	Thickness (score ±SD)	6.3 ±1.0^{de}	6.1 ±0.9^{bcde}	6.0 ±0.5^{ae}	5.5 ±0.7^{ad}	5.3 ±0.7^{ac}	5.0 ±0.8^{ab}	5.0 ±0.9^{a}
	Colour (score ±SD)	2.0 ±0.8^{ae}	2.5 ±0.5^{abf}	3.0 ±0.8^{ac}	3.0 ±0.7^{ad}	3.5 ±1.0^{bcde}	4.0 ±0.8^{cdf}	4.0 ±1.0^{cd}
Crum pore	Homogeneity (score ±SD)	8.0 ±1.1^{e}	7.0 ±0.7^{bcde}	6.3 ±0.7^{ad}	6.3 ±0.7^{ac}	6.0 ±0.8^{ab}	5.8 ±0.8^{a}	5.8 ±0.8^{a}
	Size (score ±SD)	5.0 ±0.8^{a}	5.3 ±0.9^{a}	6.0 ±0.7^{a}	6.0 ±0.7^{a}	5.5 ±0.9^{a}	5.0 ±0.8^{a}	4.9 ±0.8^{a}
Texture	Cohesiveness (score ±SD)	5.9 ±0.1^{a}	6.0 ±0.5^{a}	6.1 ±0.7^{a}	6.0 ±0.7^{a}	6.0 ±0.7^{a}	7.0 ±0.7^{b}	7.0 ±0.8^{b}
	Springiness (score ±SD)	5.6 ±0.8^{ac}	6.0 ±0.8^{ab}	6.3 ±0.5^{bc}	7.8 ±0.9^{d}	8.0 ±0.7^{d}	8.0 ±0.7^{d}	9.6 ±0.5^{e}
	Toughness (score ±SD)	8.0 ±0.6^{cde}	7.0 ±0.9^{be}	7.0 ±0.9^{bd}	7.0 ±0.9^{bc}	6.3 ±0.7^{ab}	6.0 ±0.8^{ab}	5.8 ±1.0^{a}
	Hardness (score ±SD)	6.0 ±1.6^{cdef}	5.3 ±0.6^{bcde}	5.0 ±0.6^{ae}	5.1 ±0.9^{ad}	5.0 ±0.7^{ac}	4.8 ±0.6^{abf}	4.0 ±0.7^{a}
	Adhesivity (score ±SD)	3.0 ±0.5^{a}	4.0 ±0.7^{a}	3.9 ±0.7^{a}	5.5 ±0.6^{b}	7.3 ±0.6^{c}	8.0 ±1.1^{c}	8.1 ±1.1^{c}

Note: Attribute ranges; crust thickness: thick = 0 vs. thin = 10, crumb colour: white = 0 vs. brown = 10, crumb pore homogeneity: inhomogeneous = 0 vs. homogeneous = 10, crumb pore size: small = 0 vs. big = 10. Texture cohesiveness: less cohesive = 0, very cohesive = 10, springiness: springless = 0 vs. springy = 10, toughness: weak = 0 vs. intense = 10, hardness: smooth = 0 vs. hard = 10, adhesivity: not stick = 0 vs. sticky = 10. Data are medians. Data with different superscript letters in the same column differ significantly (p <0.05).

Nutritional value concerning health claims

The data in Table 5 reveals that β-GRF supplementation significantly impacts the nutritional composition of bread, particularly concerning β-glucan content. The results of many previous studies confirmed that β-glucans contribute to maintaining normal blood cholesterol levels, mainly when the food portion contains at least 1 g of β-glucans from sources like oats, oat bran, barley, barley bran, or mixtures of these per quantified portion **[32]**. To claim that the food has a beneficial effect on maintaining normal blood cholesterol levels, the consumer should be informed that this effect is obtained with a daily intake of 3 g of β-glucans from oats, oat bran, barley, barley bran, or mixtures of these β-glucans **[4]**.

At 5% β-GRF supplementation, the β-glucan content is low, requiring a larger amount of bread (600 g) to meet the daily recommended β-glucans for a health claim related to cholesterol reduction. However, at 30% β-GRF supplementation, only 100 g of bread is needed to meet the daily recommended β-glucans, making it more feasible without significant changes to bread consumption habits. The consumption of β-glucans helps reduce the post-meal rise in blood glucose **[4]**, a capability not shared by other polysaccharides present in bread. A health claim related to cholesterol reduction can be used for food containing at least 4 g of β-glucans from oats or barley for every 30 g of available carbohydrates in a quantified portion as part of the meal. The best ratio of 1.91 g β-glucan per 30 g carbohydrates was obtained at 30% β-GRF supplementation. However, it falls short of reaching the minimal criteria for the health claim related to blood glucose reduction. While our study demonstrates promising results in enhancing the nutritional value of wheat bread by incorporating β-glucan-enriched flour from barley, it's essential to acknowledge potential limitations. The study identifies a lower final concentration of beta-glucan compared to classic extraction methods. Further optimization of the milling and mechanical separation phase might be necessary to enhance the final concentration without compromising other desirable characteristics. While the bread remains within acceptable sensory parameters, individual preferences can vary. Conducting broader sensory studies with diverse consumer groups might provide more comprehensive insights into the acceptability of the β-GRF-enriched bread across different populations. Although the bread meets the criteria for a health claim related to cholesterol reduction, a broader nutritional assessment, considering factors such as overall caloric content, and other nutrients, would provide a more comprehensive understanding of the product's nutritional profile.

Table 5 Quantity of β-glucan-enriched bread per day, determined through calculation to meet the recommended amount for the health claim regarding lowering.

% of β-GRF supplement	β-glucan/100g bread ($g.100g^{-1}$)	Quantity of bread (g)
0	0	-
5	0.5	600
10	1	300
15	1.5	200
20	2	150
25	2.5	120
30	3	100

CONCLUSION

The procedure of air separation significantly increases β-glucan from 4.5% in barley to 8.0-10.5% in the separated coarse fractions, with yields varying from 33% to 77%. Because our process involves only milling and mechanical sieving, we can infer that it is more sustainable and cost-effective compared to the wet process, which typically includes at least four stages. The supplementation of wheat flour by β-GRF fraction, containing 10% β-glucan has a noticeable influence on the rheological properties of the dough, resulting in longer development times and decreased stability when compared to control samples. The specific volume generally increases up to a 10% β-GRF supplement, reaching 3.5 cm^3/g, in comparison to the control bread with a specific volume of 3.2 cm^3/g. However, beyond the 10% concentration, the specific volume begins to decrease. Furthermore, β-GRF addition affects various sensory aspects of bread including crumb, crust, and texture characteristics. Despite these alterations, the bread compared to the control remains within acceptable sensory parameters and the final 3g β-glucan/100g bread meets the criteria for a health claim related to cholesterol reduction. Overall, these findings can contribute to the development of functional foods enriched with β-glucan and promote human health.

REFERENCES

1. Aoe, S., Morita, T., & Ohno, N. (Eds.). (2022). Beta-Glucan in Foods and Health Benefits. MDPI. https://doi.org/10.3390/books978-3-0365-5108-1
2. Mohebbi, Z., Homayouni, A., Azizi, M. H., & Hosseini, S. J. (2017). Effects of beta-glucan and resistant starch on wheat dough and prebiotic bread properties. In Journal of Food Science and Technology (Vol. 55, Issue 1, pp. 101–110). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-017-2836-9
3. Xu, S., Gong, Y., Rafique, H., He, T., & Hu, X. (2021). Effect of oat β-glucan addition on the staling properties of wheat-oat blended flour Chinese steamed bread. In Bioactive Carbohydrates and Dietary Fibre (Vol. 26, p. 100285). Elsevier BV. https://doi.org/10.1016/j.bcdf.2021.100285
4. Regulation (EC) No 1924/2006 of the European Parliament and of the council of 20 December 2006 on nutrition and health claims made on foods.
5. Bozbulut, R., & Sanlier, N. (2019). Promising effects of β-glucans on glyceamic control in diabetes. In Trends in Food Science & Technology (Vol. 83, pp. 159–166). Elsevier BV. https://doi.org/10.1016/j.tifs.2018.11.018
6. Garcia-Gimenez, G., Russell, J., Aubert, M. K., Fincher, G. B., Burton, R. A., Waugh, R., Tucker, M. R., & Houston, K. (2019). Barley grain (1,3;1,4)-β-glucan content: effects of transcript and sequence variation in genes encoding the corresponding synthase and endohydrolase enzymes. In Scientific Reports (Vol. 9, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-019-53798-8
7. Zhu, F., Du, B., & Xu, B. (2016). A critical review on production and industrial applications of beta-glucans. In Food Hydrocolloids (Vol. 52, pp. 275–288). Elsevier BV. https://doi.org/10.1016/j.foodhyd.2015.07.003
8. Maheshwari, G., Sowrirajan, S., & Joseph, B. (2017). Extraction and Isolation of β-Glucan from Grain Sources—A Review. In Journal of Food Science (Vol. 82, Issue 7, pp. 1535–1545). Wiley. https://doi.org/10.1111/1750-3841.13765
9. Ferrari, B., Finocchiaro, F., Stanca, A. M., & Gianinetti, A. (2009). Optimization of air classification for the production of β-glucan-enriched barley flours. In Journal of Cereal Science (Vol. 50, Issue 2, pp. 152–158). Elsevier BV. https://doi.org/10.1016/j.jcs.2009.04.007
10. Messia, M. C., De Arcangelis, E., Candigliota, T., Trivisonno, M. C., & Marconi, E. (2020). Production of ß-glucan enriched flour from waxy barley. In Journal of Cereal Science (Vol. 93, p. 102989). Elsevier BV. https://doi.org/10.1016/j.jcs.2020.102989
11. Skendi, A., Papageorgiou, M., & Biliaderis, C. G. (2010). Influence of water and barley β-glucan addition on wheat dough viscoelasticity. In Food Research International (Vol. 43, Issue 1, pp. 57–65). Elsevier BV. https://doi.org/10.1016/j.foodres.2009.08.012
12. Yu, L., Ma, Y., Zhao, Y., Pan, Y., Tian, R., Yao, X., Yao, Y., Cao, X., Geng, L., Wang, Z., Wu, K., & Gao, X. (2021). Effect of Hulless Barley Flours on Dough Rheological Properties, Baking Quality, and Starch Digestibility of Wheat Bread. In Frontiers in Nutrition (Vol. 8). Frontiers Media SA. https://doi.org/10.3389/fnut.2021.785847
13. McCleary, B. V., & Codd, R. (1991). Measurement of (1 → 3),(1 → 4)-β-<scp>D</scp>-glucan in barley and oats: A streamlined enzymic procedure. In Journal of the Science of Food and Agriculture (Vol. 55, Issue 2, pp. 303–312). Wiley. https://doi.org/10.1002/jsfa.2740550215
14. AACC International. Approved Methods of Analysis, 11th Ed. Method 54-21. Farinograph Method for Flour. Cereals & Grains Association, St. Paul, MN, U.S.A. Retrived from https://www.cerealsgrains.org/resources/Methods/tools/Documents/54-21-01.pdf.
15. AACC International. Approved Methods of Analysis, 11th Ed. Method 10-05.01. Guidelines for Measurement of Volume by Rapeseed Displacement. St. Paul, MN, U.S.A. Retrived from https://www.cerealsgrains.org/resources/Methods/Pages/10BakingQuality.aspx.
16. ISO 13299:2016 Sensory analysis – Methodology – General guidance for establishing a sensory profile Retrived from: https://www.iso.org/obp/ui/en/#iso:std:iso:13299:ed-2:v1:en
17. Benito-Román, Ó., Alonso, E., Palacio, L., Prádanos, P., & Cocero, M. J. (2014). Purification and isolation of β-glucans from barley: Downstream process intensification. In Chemical Engineering and Processing: Process Intensification (Vol. 84, pp. 90–97). Elsevier BV. https://doi.org/10.1016/j.cep.2013.12.006
18. Cajzek, F., Bertoncelj, J., Kreft, I., Poklar Ulrih, N., Polak, T., Požrl, T., Pravst, I., Polišenská, I., Vaculová, K., & Cigić, B. (2019). Preparation of β-glucan and antioxidant-rich fractions by stone milling of hull-less barley. In International Journal of Food Science & Technology (Vol. 55, Issue 2, pp. 681–689). Wiley. https://doi.org/10.1111/ijfs.14322

19. Huang, Z., Wang, J. J., Chen, Y., Wei, N., Hou, Y., Bai, W., & Hu, S.-Q. (2020). Effect of water-soluble dietary fiber resistant dextrin on flour and bread qualities. In Food Chemistry (Vol. 317, p. 126452). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.126452
20. Zeng, F., Hu, Z., Yang, Y., Jin, Z., & Jiao, A. (2023). Regulation of baking quality and starch digestibility in whole wheat bread based on β-glucans and protein addition strategy: Significance of protein-starch-water interaction in dough. In International Journal of Biological Macromolecules (p. 128021). Elsevier BV. https://doi.org/10.1016/j.ijbiomac.2023.128021
21. Li, Z., Gao, W., Liang, J., Fan, H., Yang, Y., Suo, B., & Ai, Z. (2023). Mechanism underlying the weakening effect of β-glucan on the gluten system. In Food Chemistry (Vol. 420, p. 136002). Elsevier BV. https://doi.org/10.1016/j.foodchem.2023.136002
22. Selaković, A., Nikolić, I., Dokić, L., Šoronja-Simović, D., Šimurina, O., Zahorec, J., & Šereš, Z. (2021). Enhancing rheological performance of laminated dough with whole wheat flour by vital gluten addition. In LWT (Vol. 138, p. 110604). Elsevier BV. https://doi.org/10.1016/j.lwt.2020.110604
23. Lante, A., Canazza, E., & Tessari, P. (2023). Beta-Glucans of Cereals: Functional and Technological Properties. In Nutrients (Vol. 15, Issue 9, p. 2124). MDPI AG. https://doi.org/10.3390/nu15092124
24. Mohebbi, Z., Homayouni, A., Azizi, M. H., & Hosseini, S. J. (2017). Effects of beta-glucan and resistant starch on wheat dough and prebiotic bread properties. In Journal of Food Science and Technology (Vol. 55, Issue 1, pp. 101–110). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-017-2836-9
25. Torbica, A., Mocko Blažek, K., Belović, M., & Janić Hajnal, E. (2019). Quality prediction of bread made from composite flours using different parameters of empirical rheology. In Journal of Cereal Science (Vol. 89, p. 102812). Elsevier BV. https://doi.org/10.1016/j.jcs.2019.102812
26. Škrbić, B., Milovac, S., Dodig, D., & Filipčev, B. (2009). Effects of hull-less barley flour and flakes on bread nutritional composition and sensory properties. In Food Chemistry (Vol. 115, Issue 3, pp. 982–988). Elsevier BV. https://doi.org/10.1016/j.foodchem.2009.01.028
27. Andrzej, K. M., Małgorzata, M., Sabina, K., Horbańczuk, O. K., & Rodak, E. (2019). Application of rich in β-glucan flours and preparations in bread baked from frozen dough. In Food Science and Technology International (Vol. 26, Issue 1, pp. 53–64). SAGE Publications. https://doi.org/10.1177/1082013219865379
28. Ortiz de Erive, M., He, F., Wang, T., & Chen, G. (2020). Development of β-glucan enriched wheat bread using soluble oat fiber. In Journal of Cereal Science (Vol. 95, p. 103051). Elsevier BV. https://doi.org/10.1016/j.jcs.2020.103051
29. Kurek, M. A., Wyrwisz, J., Brzeska, M., Moczkowska, M., Karp, S., & Wierzbicka, A. (2018). Effect of different beta-glucan preparation pretreatments on fortified bread quality. In Food Science and Technology (Vol. 38, Issue 4, pp. 606–611). FapUNIFESP (SciELO). https://doi.org/10.1590/fst.06917
30. Abdel-Gawad, A., Youssef, M., Abou-Elhawa, S., & Abdel-Rahman, A. (2018). Different Moisture Contents of Tempered Hull Barley and Hull-Less Barley Grains Prior to Milling 2. Effect on Physical and Sensory Properties of Bread Baked from these Barley Flours. In Journal of Food and Dairy Sciences (Vol. 2018, Issue 0, pp. 77–90). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/jfds.2018.77757
31. Gill, S., Vasanthan, T., Ooraikul, B., & Rossnagel, B. (2002). Wheat Bread Quality as Influenced by the Substitution of Waxy and Regular Barley Flours in Their Native and Extruded Forms. In Journal of Cereal Science (Vol. 36, Issue 2, pp. 219–237). Elsevier BV. https://doi.org/10.1006/jcrs.2001.0458
32. Cicero, A. F. G., Fogacci, F., Veronesi, M., Strocchi, E., Grandi, E., Rizzoli, E., Poli, A., Marangoni, F., & Borghi, C. (2020). A Randomized Placebo-Controlled Clinical Trial to Evaluate the Medium-Term Effects of Oat Fibers on Human Health: The Beta-Glucan Effects on Lipid Profile, Glycemia and inTestinal Health (BELT) Study. In Nutrients (Vol. 12, Issue 3, p. 686). MDPI AG. https://doi.org/10.3390/nu12030686

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you Tina Stokelj providing beta-glucan analyses.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

*Boris Kovac, University of Primorska, Faculty of Health Sciences, Department of dietetics, Polje, 42, 6310 Izola, Slovenia,
Tel.: 00386 56626465
E-mail: boris.kovac@fvz.upr.si
ORCID: https://orcid.org/0009-0008-0033-3450

Andreja Bregar, Mlinotest d.d., Quality department, Tovarniška, 14., 5270, Ajdovščina, Slovenia,
Tel.:00386 53644500
E-mail: andreja.bregar@mlinotest.si
ORCID: https://orcid.org/0009-0001-6896-5517

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 24.12.2023
Revised: 21.1.2024
Accepted: 10.2.2024
Published: 12.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 77-96
https://doi.org/10.5219/1947
ISSN: 1337-0960 online
www.potravinarstvo.com

The importance of camel milk and its dairy products – a review

Aikerim Zhumabay, Assiya Serikbayeva, Sabira Kozykan, Saule Sarimbekova, Gaukhar Kossaliyeva, Aitbay Alimov

ABSTRACT

Camel milk and dairy products based on camel milk are consumed by people in various countries, particularly Asia and Africa. Traditionally, products based on camel milk products have been an essential source of protein for people living in arid countries of the world. Here, we have discussed the chemical composition of camel milk and the technological features and limitations in the production of dairy products from camel milk. Moreover, different species or strains of LAB such as *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus casei, Enterococcus faecium, Streptococcus thermophilus, Weissella confusa* were detected as the prevalent bacteria in camel milk and camel milk products. Although camel milk has been subjected to numerous studies, technical analyses on an industrial scale remain scarce, especially for processed camel milk products. Further comprehensive research is needed to improve the quality of camel milk dairy products so that they can compete with milk from other livestock.

Keywords: camel milk composition, dairy products, shubat, LAB, review

INTRODUCTION

For thousands of years, the camel has been used by humans as a means of transportation and goods. However, it is unfair to think this way about this animal because camels greatly support people in arid areas, giving their milk to satisfy hunger and thirst **[1]**. Camel breeding is developed in the desert and semi-desert zones in African, Arab, and Central Asian countries and Mongolia **[2]**. Camels are markedly superior to other farm animal species in resilience to extreme desert conditions **[3]**. Camel breeding in Kazakhstan is also a traditional branch of livestock breeding. Currently, Kazakhstan is experiencing an increase in the number of camels (both one-humped and two-humped camels and hybrids). Moreover, camel breeding in Kazakhstan is developing in Mangyshlak, Kyzylorda, and South Kazakhstan regions **[4]**. The genus Camelus includes three species of camels, of which there are two (*C. dromedarius* and *C. bactrianus*) in Kazakhstan. *Dromedaries* have one, and Bactrians have two humps, and they are resistant to harsh climatic conditions of deserts and semi-desert zones **[5]**.

In the global community, cow's milk produces fermented milk products. However, it contains casein, which clogs blood vessels. Daily consumption of cow's milk in some people due to intolerance to this product destroys pancreatic cells and worsens carbohydrate metabolism, which leads to diabetes mellitus. Camel milk (CM) is very nutritious and completely secure for consumption **[6]**, **[7]**. Recently, diabetes mellitus has been increasing all over the world **[8]**. Camel milk is albuminous in its physical and chemical parameters and is considered the closest to mother's milk **[9]**. Although the demand for dairy products made from camel milk is growing every day, the camel milk dairy industry needs to pay more attention to its resources. This article discusses the general composition of camel milk, in particular, an overview of isolated LAB strains in camel milk and camel milk products, and the possibility of using camel milk to produce food products.

Figure 1 Fresh camel milking. Camels are being bred for the first time in the Bukhar-Zhyrau district of the Karagandy Region and now local residents produce their own shubat, a fermented camel milk beverage. Even though the main camel breeding regions in Kazakhstan are usually in the south and west parts of the country, the Karagandy Region is located in the central part of Kazakhstan. Kazakh Bactrian and single-humped dromedary camels produce milk with 4 percent fat or more. (Source: **[137]**).

Camel milk composition

Camel milk has a white, sweet, or sweet-salty taste, thick consistency, and foams strongly when poured. Camel milk varies considerably from cow's milk in physical and chemical parameters. A meta-analysis conducted on camel milk reported a fat content of 3.82%, total protein of 3.35%, lactose of 4.46%, dry matter

of 12.47%, and ash of 0.79% **[10]**. The caloric value of CM was found to be about 61 Kcal/100 mL, while *C. bactrianus* showed a much higher value of 82.70 Kcal/100 mL **[11]**. The protein content in camel milk varies between 2.9% to 4.9%, consisting mainly of two types: caseins and whey proteins. The fat content ranges from 1.2% to 6.4%, characterized by a higher proportion of long-chain fatty acids and a notable amount of linoleic acid and unsaturated fatty acids, essential for nutrition. Camel milk is also relatively rich in lactose, with an average of 4.4%. One of the distinguishing features of camel milk is its high vitamin content, including fat-soluble vitamins (A, E, D) and water-soluble vitamins (B, and particularly vitamin C). This makes it an excellent source of essential nutrients. Additionally, camel milk is rich in minerals, particularly calcium and potassium, vital for various bodily functions. Overall, the average composition of camel milk includes 3.1% protein, 3.5% fat, 4.4% lactose, 0.79% ash, and 11.9% total solids. The milk's low content of short-chain fatty acids and its rich profile of vitamins and minerals make it a highly nutritious option, suitable for various dietary needs **[136]**.

Proteins

Milk proteins are high molecular weight elements comprising amino acids linked by peptide bonds. According to the literature, the total protein content per 100 g in raw camel milk ranges between 2.15-4.9%, with an average of 3%, while the amount of casein varies between 50-87%, and whey is 20-25% of the total protein **[12]**, **[13]**, **[14]**. Proteins in milk are divided into caseins and whey proteins. Casein belongs to complex proteins and is found in milk as granules, formed with the participation of calcium ions, phosphorus ions, and others **[15]**. Casein contains four main fractions: α_{S1}-, α_{S2}-, β-, and k-casein. Scientists have found that the main protein allergens of cow's milk proteins are considered to be the presence of β-lactoglobulin (as well as in the milk of other ruminants) **[16]**. Under the action of acids, enzymes, and acid salts, casein coagulates and precipitates, which is used in cheese production, etc. **[17]**. After casein removal, soluble whey proteins remain in milk whey, the main ones being λ-lactalbumin, β-lactoglobulin, lactoferrin, lysozyme, immunoglobulins (IgG), which belong to blood plasma proteins **[18]**. The protein concentrations in camel milk ranges as follows: α_S-casein (21%), β-casein (65%), and κ-casein (3.5%) of the total casein **[19]**. According to several authors, the fraction of α_{S1}-casein fraction is much lower than in other livestock's milk, and β-lactoglobulin is almost absent **[20]**, **[21]**, which is of interest in studying the allergenicity of camel milk and dairy products **[22]**. Protein of camel milk is dominated by immunoglobulin and lactoferrin, which have therapeutic, antioxidant, and immunostimulant properties that protect the human body **[23]**, **[24]**. Konuspayeva et al. detected the mean values of IgG of 0.718 ±0.330 mg/mL in Kazakhstan camel milk. The authors also indicated seasonal variations in IgG concentrations, with higher values observed in winter **[25]**. IgG changes were also detected at different milking times (11.8-211.1 mg/mL) **[26]**. Lactoferrin in camel milk samples varies from 0.02-7.2 mg/mL **[27]**, **[28]**, and it was found to be higher in colostrum **[29]**. It also said that camel milk contains more lactoferrin than bovine milk **[30]**, **[31]**. The biological and nutritional value of proteins is determined by the amino acid composition, especially the essential amino acids. It has been studied that camel milk proteins are richer than other animals' milk proteins in essential amino acids such as lysine, phenylalanine, threonine, etc. **[32]**. According to studies, around 17-18 amino acids were detected in camel milk **[4]**, **[33]**. They are essential for vital processes such as building proteins and synthesizing hormones and neurotransmitters.

Vitamins and minerals

Vitamins are low molecular weight organic compounds, which, present in food in small amounts, are essential components that regulate metabolism. Studies showed camel milk contains more vitamin C and niacin (PP) **[34]**, **[35]**, **[36]**. The amount of vitamin C in camel milk samples from different areas of Xinjiang province ranges between 12.10-41.25 μg/mL **[37]**. It is believed that the amount of vitamin C in camel milk depends on the diet and individual characteristics of the animal. Notably, Konuspaeva et al. reported variability in vitamin C content by region and season **[38]**. The relatively high amount of vitamin C in camel milk results from eating important nutrients in an arid area where fruits and vegetables are deficient in vitamin C. However, compared to cow's milk, camel milk has a small amount of A, E, B1, B2-, B9, and B5 vitamins **[39]**. Minerals are metal ions and inorganic and organic acid salts of milk. Camel milk and shubat are superior to cow's milk in micronutrient content, especially iron and copper **[40]**, **[41]**. Faye and Beugoumi reported variations in camel milk copper (30-800 μg/100 mL) and calcium (0.27-2.57 μg/100 mL) **[42]**.

Lipids

The nutritive importance of food products especially milk is assessed by the amount and composition of milk fat lipids, the content of unsaturated fatty acids, which play an important role in metabolism. Camel milk has much less cholesterol and fat and is a great source of unsaturated fatty acids **[43]**, **[44]**. The fat content of camel milk is only 1.2-4.5% **[45]**. However, a study of camel milk in different regions of Xinjiang (China) shows that

the fat content of camel milk can be as high as 7.02% **[37]**. According to the research of Rafiq et al. **[33]** and Konuspayeva et al. **[43]** a high content of saturated fatty acids was found in Mongolian and Kazakh camel milk samples, respectively. In a previous study, camel milk contained higher levels of n-3 PUFAs compared to other livestock and was considered a functional food for the human diet **[46]**. In addition, the difference in lipid composition can be explained mainly by the different feeding conditions and camel breeds **[47]**. It has been demonstrated that camel milk from East Africa contained more fat than samples from Western Asia **[10]**. Also, seasonal variations influence most alterations in the compounds of camel milk **[48]**.

Camel farming

Camel breeding is a branch of animal husbandry engaged in the breeding and utilization of camels. It is developed in the zone of deserts, semi-deserts, and dry steppes. In Africa, the Middle East, Uzbekistan, Tajikistan, India, Pakistan, and Afghanistan breed *Dromedaries*; Kazakhstan, Kyrgyzstan, Mongolia, and China breed *Bactrians*. In every country where camels are raised, camel milk is consumed. As a dairy animal, the camel has the advantage of a long lactation period and the ability to be used for many years. It is difficult to determine the milk production of camels, as they are milked with a camel calf that has time to suck most of the milk. Dairy productivity also depends on the age of the animal **[49]**. For instance, low milk yields were observed in the first and ninth parties **[50]**. Interestingly, camel calving from November to February had higher peak milk yield and longer lactation periods **[51]**. According to Faye, camel milk production ranges from 1,000 to 12,000 liters per lactation, and the lactation period varies between 9-18 months **[52]**. Moreover, the mean daily milk yield was 8.17 L in Pakistan **[53]**. Overall, it was found that the daily milk yield of the camelid ranges from 3.5 to 40 litres **[54]**. Figure 1 shows the process of camel milking in the Karaganda region of Kazakhstan.

Raw camel milk and microbiological safety

Camel milk and dairy products based on camel milk are consumed by people in various countries, particularly, in Asia, and Africa. However, the consumption of unpasteurized raw camel milk from family farms still continues in some places worldwide. The most important characteristics of dairy products are their safety and microbiological stability. Raw camel milk may be contaminated with various pathogenic bacteria from many sources (udder, equipment, environment, etc.). Somatic cells are cells of various tissues and organs that are part of the tissues of the milk passages involved in milk secretion and milk excretion. In the udder, there is a constant renewal of epithelial tissue cells. The presence of a certain level of somatic cells in milk is natural. Still, an elevated level of somatic cells indicates that there are issues, primarily mastitis, in the dairy herd, the symptoms of which will manifest significantly later. In camel milk, somatic cell count (SCC) levels are not yet determined. It was recommended that 150 x 10^3 SCC cells/ml is a threshold value for healthy camel milk **[55]**. Furthermore, *Staphylococcus aureus* and *Salmonella* were detected in raw milk samples in Saudi Arabia **[56]**, **[57]**. Notably, Garcell et al. reported the Brucellosis outbreak related to camel milk consumption **[58]**. In addition, tetracycline and oxytetracycline residues were found in some cow and camel milk samples **[59]**. Overall, camel health, milking procedures, environment storage, and transport conditions can affect the microbial contamination of camel raw milk. Therefore, it is important to test raw camel milk and its processed product samples for microbiological quality before use **[60]**.

Pasteurizated camel milk

Pasteurization is one of the mandatory steps in the industrial production of milk and other products. The pasteurization of camel milk is complicated by the fact that it is impossible to maintain the temperature. Konuspayeva et al. reported that no standards for pasteurization conditions have been developed for camel milk **[61]**. In addition, alkaline phosphatase (ALP), the main marker of pasteurisation, is immediately negative after heat treatment. However, the amount and activity of ALP varies between animal species and cannot be applied to non-bovine milk. Moreover, it is well-known that pasteurization influences the quality of milk and its products. For instance, Elyas revealed that low fat and protein levels in fermented camel milk after pasteurization compared to un-pasteurized one **[62]**. Another study showed that low-temperature long-time pasteurization did not influence casein micelles size in camel milk. At the same time, high-temperature short-time, ultra-high temperature, and high-pressure treatment (HPP) caused decreases in particle size. Besides, HPP treatment kept a higher amount of bacteria **[63]**. Therefore, there is a need to develop standardized pasteurization conditions that can be applied to camel milk.

Fermented camel milk

It has been proven that camel milk and fermented products based on it have a high nutritional value and biomedical properties, which makes them a significant foodstuff **[64]**. Lactic acid bacteria (LAB) are widely used in many biotechnological and food industries – in the production of fermented milk products, enrichment of various foods, and creation of probiotics and dietary supplements **[65]**. Food products enriched with probiotic cultures have also been popular in the last decade. Scholars from different countries have noted that bacteria of the genera *Lactobacillus*, *Enterococcus, and Bifidobacterium* are the most commonly used bacteria in the food and biotechnology industries as probiotic cultures **[66]**. Raw camel milk and spontaneously fermented dairy products may be considered as sources of potential probiotics. Sourdough cultures specifically designed for camel milk fermentation could be an important step toward better utilization of camel milk. The Table 1 shows the different species, such as *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Enterococcus faecium*, etc., which were identified as the predominant bacteria in camel milk and camel milk products.

Table 2 LAB species isolated from camel milk and products across the globe.

Product type	*Species/strains*	Countries	Sources
Raw camel milk	*Enterococcus faecium* *Lactococcus lactis* *Pediococcus pentosaceus* *Pediococcus acidilactici* *Weissella confusa* *Leuconostoc pseudomesenteroides* *Lactobacillus reuteri*	Kuwait	**[67]**
	Lactococcus lactis *Enterococcus lactis* *Lactobacillus plantarum*	India	**[68]**
	Lactobacillus fermentum *Pediococcus acidilactici* *Pediococcus pentosaceus* *Weissella confusa* *Lactobacillus rhamnosus* *Lactobacillus plantarum* *Lactobacillus brevis*	Kazakhstan	**[69]**
Fermented milk	*Lactobacillus oryzae* *Lactobacillus casei* *Lactobacillus paracasei* *Lactobacillus curizae* *Lactobacillus plantarum* *Enterococcus lactis* *Pediococcus pentosaceu* *Pediococcus acidilactici* *Enterococcus faecium* *Weissella confusa*	Kazakhstan	**[69]**
Raw camel milk	*Lactococcus lactis* subsp. *lactis* *Lactobacillus helveticus* *Streptococcus salivarius* subsp. *thermophilus* *Lactobacillus casei* subsp. *casei* *Lactobacillus plantarum*	Morocco	**[70]**
	Streptococcus thermophilus *Leuconostoc mesenteroides.* *Lactobacillus helveticus,* *Lactococcus lactis*	Inner Mongolia	**[71]**
Raw camel milk	*Enterococcus faecium BagHom4* *Streptococcus equinus Omer9*	Saudi Arabia	**[72]**
Stirred yogurt (Laban) made from camel milk	*Streptococcus equinus Anwr4* *Streptococcus equinus Zaki1* *Streptococcus equinus Salam7*	Saudi Arabia	**[72]**

Table 2 Cont.

Product type	*Species/strains*	Countries	Sources
Raw camel milk	*Enterococcus bulliens sp. nov.* *Enterococcus sulfureus ATCC 49903(T)* *Enterococcus italicus DSM 15952(T)* *E. sulfureus LMG 13084(T)*	Morocco	**[73]**
Shubat	*Lactobacillus helveticus* *Lactobacillus kefiranofacien* *Streptococcus salivarius*	Mongolia, Inner Mongolia	**[74]**
Raw camel milk	*Leuconostoc mesenteroides* *Lactobacillus casei* *Weissella cibaria* *Pediococcus pentosaceus* *Enterococcus durans* *Enterococcus faecium* *Enterococcus lactis*	Iran	**[75]**
	Lactococcus lactiis *Lactobacillus plantarum* *Lactobacillus paracasei* *Enterococcus spp.* *Lactobacillus spp.* *Lactococcus spp.* *Lactobacillus brevis*	Morocco	**[76]**
	Leuconostoc mesenteroides *Lactobacillus plantarum* *Weissella paramesenteroides* *Weissella confuse*	Iran	**[77]**
	Lactococcus garvieae C47	United Arab Emirates	**[78]**
Raw camel milk	*Lactococcus* *Enterococcus* *Streptococcus* *Leuconostoc*	Morocco	**[79]**
Raw and fermented camel milk	*Lactobacillus*	Saudi Arabia (Makkah area) Egypt (Fayoum	**[80]**
Shubat	*Lactobacillus plantarum* *Lactobacillus casei* *Lactobacillus helveticus* *Lactococcus lactis* *Lactobacillus paracasei spp. paracasei*	Kazakhstan	**[81]**
Tarag	*L. delbrueckii ssp. bulgaricus* *L. helveticus* *L. delbrueckii ssp. lactis* *L. fermentum* *Lactococcus lactis ssp. lactis.*	Mongolia	**[82]**
Fermented camel milk	*Lactobacillus paracasei subsp. paracasei*	Mongolia	**[83]**
Raw camel milk	*Weissella confusa* *Weissella cibaria* *Enterococcus durans*	Morocco	**[84]**
Ethiopian traditional fermented camel milk	*Streptococcus* *Lactococcus* *Weissella*	Ethiopia	**[85]**

Table 2 Cont.

Product type	*SPECIES/STRAINS*	Countries	Sources
Raw camel milk	*Limosilactobacillus reuteri strain 2892*	Iran	**[86]**
	Enterococcus faecium	Iran	**[87]**
	L. mesenteroides	Algeria	**[88]**
	L. brevis	Algeria	**[89]**
	Lactiplantibacillus plantarum LC38	TUNISIA	**[90]**
	Lactococcus lactis KX881768 *Lactobacillus plantarum KX881772* *Lactococcus lactis KX881782* *Lactobacillus plantarum KX881779*	Abu Dhabi, UAE	**[91]**
	Lactococcus lactis SCC133 and SLch14	Tunisia	**[92]**
	Lactobacillu *Lactococcus* *Streptococcus* *Enterococcus*	Ethiopia	**[93]**
Spontaneously fermented camel milk	*Lactococcus lactis* *Lactobacillus plantarum* *Pediococcus acidilactici*	Ethiopia	**[94]**
Chal	*L. plantarum* *L. paraplantarum* *L. kefiri* *L. gasseri* *L. paracasei,* *Leuconostoc (Leu.) lactis* *Weissella (W.) cibaria* *Enterococcus (E.) faecium*	Iran	**[95]**
Raw and fermented camel milk (Suusac)	*S. thermophilus* *Lc. lactis subsp. lactis* *S. infantarius subsp. infantarius*	Kenya	**[96]**
Gariss	*Lactobacillus plantarum* *Lactococcus raffinolactis*	Sudan	**[97]**
Raw camel milk	*Leuconostoc mesenteroides subsp. dextranicum* *Lactococcus lactis subsp. lactis* *Lb. plantarum* *Lactobacillus brevis*	Algeria	**[98]**
"Shmen", a traditional butter made from camel milk	*Lactobacillus plantarum* *Lactobacillus delbrueckii ssp. bulgaricus* *Lactococcus lactis ssp. lactis biovar diacetylacti* *Lactococcus lactis ssp. cremoris* *Leuconostoc pseudomesenteroides* *Lactobacillus paracasei ssp. paracasei* *Leuconostoc gelidum* *Enterococcus faecium*	Algeria	**[99]**

Sour milk products have been known since antiquity. Depending on climate and lifestyle, each region has its national product. They have in common that they are obtained by fermenting milk from different animals. *Shubat* is a lactic acid beverage produced from natural camel milk due to lactic acid and alcoholic fermentation. It is also called as "*Khoormog*" in Mongolia or "*Chal*" in Iran **[100]**. It has quiet specific taste and smell. There is a distinction between ready Shubat by aging: one-day aging – young, two-day aging – medium strength, and three-day aging – strong. The best is a drink of 2-3 days of aging. During fermentation, *Shubat* density, dry residue, and milk sugar content decrease, lactic acid level, and acidity increase, resulting in the synthesis of ethyl alcohol and carbon dioxide. The the high content of vitamin C is preserved **[101]**. The previous study showed that some PUFAs were detected only in Shubat. Besides, the concentration of some amino acids, including threonine,

aspargine, alanine increased compared to raw milk and milk powder **[4]**. The pH values in *Shubat* were between 3.7-4.1 **[102]**. Manaer et al. reported the effect of shut in type-2 diabetic rats. Furthermore, the authors speculated that its probiotic function may increase the function of β-cells **[103]**. Shubat has also been successfully used to treat tuberculosis **[104]**.

In addition, spontaneous fermentation has created many traditional products such as *Gariss* in Sudan, Laben in Arab countries, and *Suusac* in Kenya. Sulieman et al. (2022) reported that *Gariss* contains 3.4-3.85% protein, 2.2-2.9% fat, 1.3-1.4% lactose, 0.75-0.8% ash, 1.35-1.4% ethanol, 1-1.8% acidity **[105]**. Furthermore, forty-five strains of LAB were detected in *Suusac*, with *L. mesenteroides* and *L. plantarum* predominating **[106]**. A comparative study of *Gariss, Suusac,* and *Shubat* revealed that they had 2.8-5%, 4%, and 4.3% fat; *Gariss* and *Suusac* had 2.3-3.4% and 3% protein; *Gariss* and *Shubat* had 0.51-1.3% and 0.75% ash, respectively **[107]**. However, very few studies have been published on the chemical composition and medicinal properties of shubat or other traditionally fermented camel milk products.

Camel milk cheese

Today, an important direction is the creation of new types of products with functional properties, high quality, and biological value based on the use of local sources of raw materials with a specific chemical composition. Coagulation of milk proteins is an important part of the technological production process of most dairy products. In industrial production conditions, controlling and regulating technological parameters during milk coagulation remains important. Camel milk is rarely used to make cheese because of the thickening problem **[108]**. The basic principle of cheese making is to thicken the milk to form curds and whey. The difficulty in obtaining cheese from camel milk alone is because casein, which plays a determining role in the rennet curdling of milk, has a different primary structure than casein in cow milk. According to current theory, the enzyme chymosin in casein cleaves the peptide bond between phenylalanine-105-methionine 106, broken 200 times faster than other peptide bonds. Camel milk does not curdle well, forming a loose and delicate clot that escapes the whey **[109]**.

Current cheese-making methods speed up this process, thanks to the involvement of rennet (bacteria that produce lactic acid). Ramet studied that camel milk can be coagulated by adding calcium phosphate and vegetable rennet **[110]**. Al-Zoreky et al. used recombinant camel chymosin to produce soft cheese from camel milk **[111]**. Furthermore, soft white cheese was obtained by using STI-12, RST-743, and R-707 starter cultures **[112]**, **[113]**. A previous study showed that the calcium concentration of camel and cow milk blend cheese (449.50 mg/100g) was higher than that of camel milk cheese (325.50 mg/100g) [113]. Cheese made using starter culture showed higher cheese yield (13.22), total dry matter (44.36), fat (19.0), and protein (21.3) than cheese made by direct acidification **[114]**. However, research on camel milk cheese's chemical and other properties is still being determined.

Nowadays, camel's milk is transformed into many types of cheese, such as camembert, ricotta, halloumi, feta, etc. The Caravane cheese (also known as Camelbert) is the first camel cheese produced in Mauritania, it's one of the few commercially produced camel milk cheeses. Caravane is soft and creamy. Also, it is a very healthy cheese as it contains low lactose and fat content. Camel milk feta is made with camel milk is also creamy and has a delicate flavour. Camel milk halloumi is a variation of the traditional Cypriot cheese made from camel milk. Camel milk ricotta is a soft, fresh cheese often used in desserts or as a spread. Shubat cheese is derived from fermented camel milk with a unique tangy and rich flavor. Inayat et al. observed that fat (3.62 to 2.96), ash (11.79 to 7.30), and chloride (3.18 to 2.29) decreased in soft unripened cheese made from skimmed camel milk, but protein (44.72 to 78.88) and casein (21.17 to 59.56) increased significantly **[115]**. Although it is possible to thicken camel milk, using different cheesemaking technologies is still challenging.

Milk or fermented milk powder

Among the methods of milk preservation, drying occupies a special place, which is one of the most widespread and rational ways of creating reserves of dairy products, allowing them to reduce the cost of transporting and increase their shelf life. Food product drying methods differ in how heat is supplied to the product being dried. Several drying methods are used in the food industry, including spray drying, freeze drying, thermal radiation drying, etc.

Dried products are made by evaporating the water from the milk. Whole milk powder is obtained by drying pasteurized whole milk. It can have a mild aftertaste and a light cream color. The research has shown sufficient prospectivity of the developed technology of freeze-drying milk. The form of camel milk is powder using modern innovative technology by evaporation at low temperatures with appropriate pasteurization. Freeze drying is when a small amount of product is frozen and placed under a vacuum. Due to the removal of moisture under vacuum at low temperatures, the product's structure remains unchanged, allowing the highest quality products to be obtained **[116]**. For instance, Zou et al. reported that direct freeze-dried camel milk powder had

minimum alterations in protein profile than direct spray-dried powder **[117]**. A comparative study between these two methods showed that freeze-dried camel milk powder displayed greater calcium preservation (15.33 g/kg) and iron (0.012 g/kg) **[118]**. Spray-dried whole camel milk powder had an average moisture content of 4%, total dry matter 96%, fat 29%, protein 31.6%, and lactose 29.6% **[110]**. High outlet temperature and pressure decreased vitamin C recovery during spray drying **[119]**. Overall, the freeze-drying method is recommended to obtain camel milk powder. Figure 2 shows some camel milk products produced in Kazakhstan.

Yogurt

Yogurt is a fermented milk product similar in consistency and flavor to nonfat sour cream. It is obtained by fermenting milk with specific yogurt bacteria – thermophilic *Streptococcus thermophilus* and *Bulgarian bacillus* **[120]**. The challenges and possibilities for processing camel milk into dairy products is given to assess the opportunities for developing functional camel milk products. The relative structure, distribution, and molecular composition of cow and camel milk elements are not similar. Consequently, producing camel milk products such as yogurt and cheese using the same technology as for cow milk dairy products may result in processing obstacles. However, scientific evidence indicates that it is possible to transform camel milk into products by optimizing processing parameters. The main problem of camel milk yogurt production is the texture characteristics **[121]**. In the meantime, several efforts have been made to solve the issues associated with the poor texture of fermented camel milk. Ali et al. recently prepared camel milk-soy yogurt with two different starter culture strains **[122]**. A slight increase in sensorial properties of camel milk yogurt was obtained by adding glutathione-treated transglutaminase enzyme **[123]**. It was observed that yogurt prepared from camel milk contained less protein (3.23%), fat (4.27%), lactose (3.43%), and a high amount of moisture (88.17%), ash (0.84%) as compared to yogurt prepared from cow milk **[124]**. Hashim et al. reported that cow's milk yogurt prepared using different formulations had a pH ranging from 4.3 to 4.5 and acidity ranging from 0.98 to 1.16%. In addition, yogurt prepared using 0.75% alginate and 0.075% calcium had similar sensory characteristics to cow milk yogurt **[125]**.

Balkaimak

Innovative advances are opening up opportunities to expand the range of camel milk products. “Balkaimak” is the Kazakh national dessert **[126]**. It translates as honey sour cream, i.e. “bal” – honey, “kaimak” – sour cream. For its preparation, fresh sour cream is cooked over low heat so that it does not turn into butter, and a small amount of honey and flour of the highest grade is added. Properly cooked Balkaimak has an orange color and is slightly stretchy.

Figure 2 Camel milk powder and shubat produced in Daulet-Beket. Daulet-Beket is an agricultural enterprise in Almaty region that produces mare and camel milk, and *Shubat.* (Sources: **[138]**, **[139]**).

Camel milk butter

Butter from camel is in high consumer demand due to its unique properties. However, camel milk butter production technology has significant differences compared to production from cow's milk. The butter made from camel milk differs from cow's milk by its white color and physical and chemical properties. The main problem is that camel milk slightly tends to form cream due to the lack of agglutinin, the fat distributed as small micelle-like globules, and the strong adhesion of fat to proteins. Despite this, pastoralists in Algeria make the traditional "Shmen" butter from camel's milk by churning. Also, another study revealed the possibility of

obtaining butter from camel milk using high churning force but a very long churning time **[127]**. It was reported that butter made from camel milk contains more long-chain fatty acids (C14-C18) and fewer short-chain fatty acids (C4:0-C12:0) **[128]**. Several authors detected 64-65% total solids and 49-59.6% fat in camel milk butter **[129]**. Another study showed that the moisture content of butter made from pure camel milk (39.2%) was higher than butter mixed with goat milk (14.27-32.97%), while fat content was much less in butter from pure camel milk (56.8%) than butter mixed with goat milk (60.57-80%) **[130]**. However, camel's milk butter requires further research to compete with cow's milk butter production.

Camel milk ice-cream

To create a new functionally enriched ice cream that meets the requirements of the diet of ordinary, average people that would meet the dietary requirements. Thus, the development of a soft ice cream made from camel's milk is a relevant solution to several problems such as satisfying the need to to eat "sweet" without harming health and figure, as well as saturating the body with protein. Soft ice cream is usually marketed at the point of production because such ice cream is obtained after a freezing process, which in turn involves a higher defrosting temperature. The technological process of soft ice cream preparation consists of the following operations: preparation of the mixture, maturation of the mixture, and its whipping. Haijian et al. obtained low-fat ice- cream with improved sensory characteristics by enriching camel milk ice cream with 2% casein hydrolysate **[131]**. It was also suggested that using additives and flavorings in camel milk ice cream improves the nutritional value and sensory attributes **[132]**. It was observed that there was no significant difference in fat (10.1-10.2%) and protein (3.4-3.6%) content in camel milk ice cream as compared to bovine milk ice cream **[133]**.

Camel milk chocolate

Consumers like the production of chocolate milk desserts with probiotic properties based on camel milk. The study showed that the survival rate of *L. casei* in desserts prepared with camel milk was higher than those prepared with bovine milk **[134]**.

Camel beauty products

Numerous nutrients in camel milk provide natural skin protection. Alpha hydroxyl acids present in camel milk help to smooth fine lines and wrinkles, while the antimicrobial properties of camel milk act as a natural detergent, making the soap an effective treatment for skin conditions, including acne and eczema **[135]**.

Challenges in camel milk production and marketing

Many studies have proven camel milk is beneficial and close to human milk. Despite the increase in camel milk production, its consumption at the global level is limited. Due to its high cost, it is less utilized for daily milk production than other livestock's milk. Processing camel milk presents different challenges compared to bovine milk. The reason camel milk products are difficult to produce is that camel milk's composition differs significantly from cow milk. Camel milk does not show a particular tendency to coagulate, primarily due to the absence of the kappa-casein and β-lactoglobulin interactions, which causes many difficulties in producing cheese, yogurt, etc. Therefore, intensive research is needed to better understand camel milk proteins' chemical composition. Although camel milk has been subjected to numerous studies, technical analyses on an industrial scale remain scarce, especially for processed camel milk products. Further comprehensive research is needed to improve the quality of camel milk dairy products to compete with other livestock.

The results of this review indicate that various camel milk products were successfully processed due to optimization and adjustment of processing steps. The development of new technologies and improved processing methods are needed to process camel milk products intended for special dietary or medical use.

CONCLUSION

The increasing population demand for functional food products makes it necessary to investigate in depth all potential understudied and poorly known food sources. It is necessary to create new types of foods with high nutritional and biological value, providing the needs of adults and children in substances affecting the harmonious development of the organism. Producing camel milk products using the same technology as cow's milk dairy products can lead to processing. Despite these technological limitations, scientific evidence indicates that it is possible to transform camel milk into products by optimizing processing parameters. Camel milk is a nutritious beverage known for its unique composition, including various beneficial components. The protein content in camel milk varies between 2.9% to 4.9%, consisting mainly of two types: caseins and whey proteins. The fat content ranges from 1.2% to 6.4%, characterized by a higher proportion of long-chain fatty acids and a notable amount of linoleic acid and unsaturated fatty acids, essential for nutrition. Camel milk is also relatively rich in lactose, with an average of 4.4%. One of the distinguishing features of camel milk is its high vitamin content, including fat-soluble vitamins (A, E, D) and water-soluble vitamins (B, and particularly vitamin C). This makes it an excellent source of essential nutrients. Additionally, camel milk is rich in minerals, particularly calcium and potassium, vital for various bodily functions. Overall, the average composition of camel milk includes 3.1% protein, 3.5% fat, 4.4% lactose, 0.79% ash, and 11.9% total solids. The milk's low content of short-chain fatty acids and its rich profile of vitamins and minerals make it a highly nutritious option, suitable for various dietary needs.

REFERENCES

1. Burger, P. A., Ciani, E., & Faye, B. (2019). Old World camels in a modern world – a balancing act between conservation and genetic improvement. In Animal Genetics (Vol. 50, Issue 6, pp. 598–612). Wiley. https://doi.org/10.1111/age.12858
2. Ho, T. M., Zou, Z., & Bansal, N. (2022). Camel milk: A review of its nutritional value, heat stability, and potential food products. In Food Research International (Vol. 153, p. 110870). Elsevier BV. https://doi.org/10.1016/j.foodres.2021.110870
3. Behrouz, S., Saadat, S., Memarzia, A., Sarir, H., Folkerts, G., & Boskabady, M. H. (2022). The Antioxidant, Anti-Inflammatory and Immunomodulatory Effects of Camel Milk. In Frontiers in Immunology (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fimmu.2022.855342
4. Zhumabay, A., Serikbayeva, A., Kozykan, S., Yusof, Y. A., & Kozhakhmetova, A. (2023). Determination of the fatty and amino acid composition of camel milk, milk powder and shubat. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 918–928). HACCP Consulting. https://doi.org/10.5219/1931
5. Akhmetsadykova, S. H., Konuspayeva, G., & Akhmetsadykov, N. (2022). Camel breeding in Kazakhstan and future perspectives. In Animal Frontiers (Vol. 12, Issue 4, pp. 71–77). Oxford University Press (OUP). https://doi.org/10.1093/af/vfac048
6. Benslama, Y., Dennouni-Medjati, N., Dali-Sahi, M., Meziane, F. Z., & Harek, Y. (2021). Childhood type 1 diabetes mellitus and risk factor of interactions between dietary cow's milk intake and HLA-DR3/DR4 genotype. In Journal of Biomolecular Structure and Dynamics (Vol. 40, Issue 21, pp. 10931–10939). Informa UK Limited. https://doi.org/10.1080/07391102.2021.1953599
7. Ejtahed, H. S., Niasari Naslaji, A., Mirmiran, P., Zraif Yeganeh, M., Hedayati, M., Azizi, F., & Moosavi Movahedi, A. (2014). Effect of Camel Milk on Blood Sugar and Lipid Profile of Patients With Type 2 Diabetes: A Pilot Clinical Trial. In International Journal of Endocrinology and Metabolism (Vol. 13, Issue 1). Briefland. https://doi.org/10.5812/ijem.21160
8. Balooch Hasankhani, M., Mirzaei, H., & Karamoozian, A. (2023). Global trend analysis of diabetes mellitus incidence, mortality, and mortality-to-incidence ratio from 1990 to 2019. In Scientific Reports (Vol. 13, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-023-49249-0
9. Zou, Z., Duley, J. A., Cowley, D. M., Reed, S., Arachchige, B. J., Koorts, P., Shaw, P. N., & Bansal, N. (2022). Digestibility of proteins in camel milk in comparison to bovine and human milk using an in vitro infant gastrointestinal digestion system. In Food Chemistry (Vol. 374, p. 131704). Elsevier BV. https://doi.org/10.1016/j.foodchem.2021.131704
10. Konuspayeva, G., Faye, B., & Loiseau, G. (2009). The composition of camel milk: A meta-analysis of the literature data. In Journal of Food Composition and Analysis (Vol. 22, Issue 2, pp. 95–101). Elsevier BV. https://doi.org/10.1016/j.jfca.2008.09.008
11. Alhaj, O. A., Ahmad, L., Alenezi, A. F., Abodoleh, G., Ghazzawi, H., Trabelsi, K., Bragazzi, N. L., Mehta, B. M., Faye, B., & Jahrami, H. A. (2023). Does total caloric count of camel milk differ by species, country, season and year of publication: A systematic review, meta-analysis and meta-regression. In International

Journal of Dairy Technology (Vol. 77, Issue 1, pp. 15–34). Wiley. https://doi.org/10.1111/1471-0307.13017

12. Swelum, A. A., El-Saadony, M. T., Abdo, M., Ombarak, R. A., Hussein, E. O. S., Suliman, G., Alhimaidi, A. R., Ammari, A. A., Ba-Awadh, H., Taha, A. E., El-Tarabily, K. A., & Abd El-Hack, M. E. (2021). Nutritional, antimicrobial and medicinal properties of Camel's milk: A review. In Saudi Journal of Biological Sciences (Vol. 28, Issue 5, pp. 3126–3136). Elsevier BV. https://doi.org/10.1016/j.sjbs.2021.02.057
13. Benmeziane – Derradji, F. (2021). Evaluation of camel milk: gross composition—a scientific overview. In Tropical Animal Health and Production (Vol. 53, Issue 2). Springer Science and Business Media LLC. https://doi.org/10.1007/s11250-021-02689-0
14. Alhaj, O. A., Lajnaf, R., Jrad, Z., Alshuniaber, M. A., Jahrami, H. A., & Serag El-Din, M. F. (2022). Comparison of Ethanol Stability and Chemical Composition of Camel Milk from Five Samples. In Animals (Vol. 12, Issue 5, p. 615). MDPI AG. https://doi.org/10.3390/ani12050615
15. Hailu, Y., Hansen, E. B., Seifu, E., Eshetu, M., Ipsen, R., & Kappeler, S. (2016). Functional and technological properties of camel milk proteins: a review. In Journal of Dairy Research (Vol. 83, Issue 4, pp. 422–429). Cambridge University Press (CUP). https://doi.org/10.1017/s0022029916000686
16. Wal, J.-M. (2002). Cow's milk proteins/allergens. In Annals of Allergy, Asthma & Immunology (Vol. 89, Issue 6, pp. 3–10). Elsevier BV. https://doi.org/10.1016/s1081-1206(10)62115-1
17. Mbye, M., Ayyash, M., Abu-Jdayil, B., & Kamal-Eldin, A. (2022). The Texture of Camel Milk Cheese: Effects of Milk Composition, Coagulants, and Processing Conditions. In Frontiers in Nutrition (Vol. 9). Frontiers Media SA. https://doi.org/10.3389/fnut.2022.868320
18. Laleye, L. C., Jobe, B., & Wasesa, A. A. H. (2008). Comparative Study on Heat Stability and Functionality of Camel and Bovine Milk Whey Proteins. In Journal of Dairy Science (Vol. 91, Issue 12, pp. 4527–4534). American Dairy Science Association. https://doi.org/10.3168/jds.2008-1446
19. Kumar, D., Verma, A. K., Chatli, M. K., Singh, R., Kumar, P., Mehta, N., & Malav, O. P. (2016). Camel milk: alternative milk for human consumption and its health benefits. In Nutrition & Food Science (Vol. 46, Issue 2, pp. 217–227). Emerald. https://doi.org/10.1108/nfs-07-2015-0085
20. Hinz, K., O'Connor, P. M., Huppertz, T., Ross, R. P., & Kelly, A. L. (2012). Comparison of the principal proteins in bovine, caprine, buffalo, equine and camel milk. In Journal of Dairy Research (Vol. 79, Issue 2, pp. 185–191). Cambridge University Press (CUP). https://doi.org/10.1017/s0022029912000015
21. Yang, Y., Bu, D., Zhao, X., Sun, P., Wang, J., & Zhou, L. (2013). Proteomic Analysis of Cow, Yak, Buffalo, Goat and Camel Milk Whey Proteins: Quantitative Differential Expression Patterns. In Journal of Proteome Research (Vol. 12, Issue 4, pp. 1660–1667). American Chemical Society (ACS). https://doi.org/10.1021/pr301001m
22. Talarico, V., Mazza, G., Rubino, M., Monti, G., Giancotti, L., Bua, A., Mohamed, A. M., & Miniero, R. (2021). Camel milk: a possible alternative for children with cow's milk allergy? In Minerva Pediatrics (Vol. 73, Issue 4). Edizioni Minerva Medica. https://doi.org/10.23736/s2724-5276.19.05632-9
23. Habib, H. M., Ibrahim, W. H., Schneider-Stock, R., & Hassan, H. M. (2013). Camel milk lactoferrin reduces the proliferation of colorectal cancer cells and exerts antioxidant and DNA damage inhibitory activities. In Food Chemistry (Vol. 141, Issue 1, pp. 148–152). Elsevier BV. https://doi.org/10.1016/j.foodchem.2013.03.039
24. Alkhulaifi, M. M., Alosaimi, M. M., Khan, M. S., Tabrez, S., Shaik, G. M., Alokail, M. S., Hassan, M. A., Awadalla, M. E., & Husain, F. M. (2023). Assessment of Broad-Spectrum Antimicrobial, Antibiofilm, and Anticancer Potential of Lactoferrin Extracted from Camel Milk. In Applied Biochemistry and Biotechnology. Springer Science and Business Media LLC. https://doi.org/10.1007/s12010-023-04579-7
25. Konuspayeva, G., Faye, B., Loiseau, G., & Levieux, D. (2007). Lactoferrin and Immunoglobulin Contents in Camel's Milk (Camelus bactrianus, Camelus dromedarius, and Hybrids) from Kazakhstan. In Journal of Dairy Science (Vol. 90, Issue 1, pp. 38–46). American Dairy Science Association. https://doi.org/10.3168/jds.s0022-0302(07)72606-1

26. El-Hatmi, H., Levieux, A., & Levieux, D. (2006). Camel (Camelus dromedarius) immunoglobulin G, α-lactalbumin, serum albumin and lactoferrin in colostrum and milk during the early post partum period. In Journal of Dairy Research (Vol. 73, Issue 3, pp. 288–293). Cambridge University Press (CUP). https://doi.org/10.1017/s0022029906001713

27. Arain, M. A., Khaskheli, G. B., Shah, A. H., Marghazani, I. B., Barham, G. S., Shah, Q. A., Khand, F. M., Buzdar, J. A., Soomro, F., & Fazlani, S. A. (2022). Nutritional significance and promising therapeutic/medicinal application of camel milk as a functional food in human and animals: a

comprehensive review. In Animal Biotechnology (Vol. 34, Issue 6, pp. 1988–2005). Informa UK Limited. https://doi.org/10.1080/10495398.2022.2059490

28. Elagamy, E. I., Ruppanner, R., Ismail, A., Champagne, C. P., & Assaf, R. (1996). Purification and characterization of lactoferrin, lactoperoxidase, lysozyme and immunoglobulins from camel's milk. In International Dairy Journal (Vol. 6, Issue 2, pp. 129–145). Elsevier BV. https://doi.org/10.1016/0958-6946(94)00055-7

29. Zhang, H., Yao, J., Zhao, D., Liu, H., Li, J., & Guo, M. (2005). Changes in Chemical Composition of Alxa Bactrian Camel Milk During Lactation. In Journal of Dairy Science (Vol. 88, Issue 10, pp. 3402–3410). American Dairy Science Association. https://doi.org/10.3168/jds.s0022-0302(05)73024-1

30. Li, X., Li, Z., Xu, E., Chen, L., Feng, H., Chen, L., Deng, L., & Guo, D. (2019). Determination of Lactoferrin in Camel Milk by Ultrahigh-Performance Liquid Chromatography-Tandem Mass Spectrometry Using an Isotope-Labeled Winged Peptide as Internal Standard. In Molecules (Vol. 24, Issue 22, p. 4199). MDPI AG. https://doi.org/10.3390/molecules24224199

31. Ipsen, R. (2017). Opportunities for Producing Dairy Products from Camel Milk: A Comparison with Bovine Milk. East African Journal of Sciences, Vol. 11 No. 2 (2017): East African Journal of Sciences. https://doi.org/10.20372/EAJS.V11I2.404

32. Konuspayeva, G., Faye, B., Loiseau, G., & Levieux, D. (2007). Lactoferrin and Immunoglobulin Contents in Camel's Milk (Camelus bactrianus, Camelus dromedarius, and Hybrids) from Kazakhstan. In Journal of Dairy Science (Vol. 90, Issue 1, pp. 38–46). American Dairy Science Association. https://doi.org/10.3168/jds.s0022-0302(07)72606-1

33. Rafiq, S., Huma, N., Pasha, I., Sameen, A., Mukhtar, O., & Khan, M. I. (2015). Chemical Composition, Nitrogen Fractions and Amino Acids Profile of Milk from Different Animal Species. In Asian-Australasian Journal of Animal Sciences (Vol. 29, Issue 7, pp. 1022–1028). Asian Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.15.0452

34. He, J., Xiao, Y., Orgoldol, K., Ming, L., Yi, L., & Ji, R. (2019). Effects of Geographic Region on the Composition of Bactrian Camel Milk in Mongolia. In Animals (Vol. 9, Issue 11, p. 890). MDPI AG. https://doi.org/10.3390/ani9110890

35. Stahl, T., Sallmann, H. P., & Duehlmeier, R. (2006). Selected vitamins and fatty acid patterns in dromedary milk and colostrum. In Journal of Camel Practice and Research (Vol. 13, Issue 1, pp. 53–57). Camel Publishing House.

36. Bouhaddaoui, S., Chabir, R., Errachidi, F., El Ghadraoui, L., El Khalfi, B., Benjelloun, M., & Soukri, A. (2019). Study of the Biochemical Biodiversity of Camel Milk. In The Scientific World Journal (Vol. 2019, pp. 1–7). Hindawi Limited. https://doi.org/10.1155/2019/2517293

37. Miao, J., Xiao, S., & Wang, J. (2023). Comparative Study of Camel Milk from Different Areas of Xinjiang Province in China. In Food Science of Animal Resources (Vol. 43, Issue 4, pp. 674–684). Korean Society for Food Science of Animal Resources. https://doi.org/10.5851/kosfa.2023.e27

38. Konuspayeva, G., Faye, B., & Loiseau, G. (2011). Variability of vitamin C content in camel milk from Kazakhstan. In Journal of Camelid Science (Vol. 4, pp. 63–69). International society of Camelid Research and Development.

39. Wang, S. Y., Liang, J. P., Shao, W., & Wen, H. (2011). Mineral, vitamin and fatty acid contents in the camel milk of dromedaries in the anxi gansu China. In Journal of Camel Practice and Research (Vol. 18, Issue 2, pp. 273–276). Camel Publishing House.

40. Mostafidi, M., Moslehishad, M., Piravivanak, Z., & Pouretedal, Z. (2016). Evaluation of mineral content and heavy metals of dromedary camel milk in Iran. In Food Science and Technology (Vol. 36, Issue 4, pp. 717–723). FapUNIFESP (SciELO). https://doi.org/10.1590/1678-457x.16116

41. Soliman, G. Z. A. (2005). Comparison Of Chemical And Mineral Content Of Milk From Human, Cow, Buffalo, Camel And Goat In Egypt. In The Egyptian Journal of Hospital Medicine (Vol. 21, Issue 1, pp. 116–130). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/ejhm.2005.18054

42. Faye, B., & Bengoumi, M. (2018). Camel clinical biochemistry and hematology. New-York: Springer Publ.; p. 346.

43. Konuspayeva, G., Lemarie, É., Faye, B., Loiseau, G., & Montet, D. (2008). Fatty acid and cholesterol composition of camel's (Camelus bactrianus, Camelus dromedariusand hybrids) milk in Kazakhstan. In Dairy Science and Technology (Vol. 88, Issue 3, pp. 327–340). Springer Science and Business Media LLC. https://doi.org/10.1051/dst:2008005

44. Faye, B., Bengoumi, M., Al-Masaud, A., & Konuspayeva, G. (2015). Comparative milk and serum cholesterol content in dairy cow and camel. In Journal of King Saud University - Science (Vol. 27, Issue 2, pp. 168–175). Elsevier BV. https://doi.org/10.1016/j.jksus.2014.11.003

45. Swelum, A. A., El-Saadony, M. T., Abdo, M., Ombarak, R. A., Hussein, E. O. S., Suliman, G., Alhimaidi, A. R., Ammari, A. A., Ba-Awadh, H., Taha, A. E., El-Tarabily, K. A., & Abd El-Hack, M. E. (2021). Nutritional, antimicrobial and medicinal properties of Camel's milk: A review. In Saudi Journal of Biological Sciences (Vol. 28, Issue 5, pp. 3126–3136). Elsevier BV. https://doi.org/10.1016/j.sjbs.2021.02.057

46. Wang, F., Chen, M., Luo, R., Huang, G., Wu, X., Zheng, N., Zhang, Y., & Wang, J. (2022). Fatty acid profiles of milk from Holstein cows, Jersey cows, buffalos, yaks, humans, goats, camels, and donkeys based on gas chromatography–mass spectrometry. In Journal of Dairy Science (Vol. 105, Issue 2, pp. 1687–1700). American Dairy Science Association. https://doi.org/10.3168/jds.2021-20750

47. Chamekh, L., Calvo, M., Khorchani, T., Castro-Gómez, P., Hammadi, M., Fontecha, J., & Yahyaoui, M. H. (2020). Impact of management system and lactation stage on fatty acid composition of camel milk. In Journal of Food Composition and Analysis (Vol. 87, p. 103418). Elsevier BV. https://doi.org/10.1016/j.jfca.2020.103418

48. Zou, Z., Duley, J. A., Cowley, D. M., Reed, S., Arachchige, B. J., Shaw, P. N., & Bansal, N. (2022). Comprehensive biochemical and proteomic characterization of seasonal Australian camel milk. In Food Chemistry (Vol. 381, p. 132297). Elsevier BV. https://doi.org/10.1016/j.foodchem.2022.132297

49. Swelum, A. A.-A., Saadeldin, I. M., Abdelnour, S. A., Ba-Awadh, H., Abd El-Hack, M. E., & Sheiha, A. M. (2019). Relationship between concentrations of macro and trace elements in serum and follicular, oviductal, and uterine fluids of the dromedary camel (Camelus dromedarius). In Tropical Animal Health and Production (Vol. 52, Issue 3, pp. 1315–1324). Springer Science and Business Media LLC. https://doi.org/10.1007/s11250-019-02137-0

50. Raziq, A., Younas, M. T., Khan, M. S., & Iqbal, A. (2010). Milk production potential as affected by parity and age in the Kohi dromedary camel. In Journal of Camel Practice and Research (Vol. 17, Issue 2, pp. 195–198). Camel Publishing House.

51. Musaad, A., Faye, B., & Nikhela, A. A. (2012). Lactation curves of dairy camels in an intensive system. In Tropical Animal Health and Production (Vol. 45, Issue 4, pp. 1039–1046). Springer Science and Business Media LLC. https://doi.org/10.1007/s11250-012-0331-x

52. Faye, B. (2008). Dairy productivity potential of camels. In: Cardellion, R., Rosati A., and Mosconi C., editors. Current status of genetic resources, recording and production systems in African, Asian and American Camelids. ICAR, Rome, Italy: ICAR technical series No. 11, Proceedings of the ICAR/FAO Seminar; p. 93–104.

53. Ahmad, S., Yaqoob, M., Bilal, M. Q., Khan, M. K., Muhammad, G., Yang, L.-G., & Tariq, M. (2012). Factors affecting yield and composition of camel milk kept under desert conditions of central Punjab, Pakistan. In Tropical Animal Health and Production (Vol. 44, Issue 7, pp. 1403–1410). Springer Science and Business Media LLC. https://doi.org/10.1007/s11250-012-0079-3

54. Khan, B. B., & Iqbal, A. (2001). Production and composition of camel milk - review. In Pakistan Journal of Agricultural Sciences (Vol. 38, pp. 64–68). Pakistan Association of Advancement in Agricultural Sciences.

55. Kaskous, S., Al-Momani, A. Q., Al-Yacoub, A. N., & Al-Najjar, K. A. (2021). Physiological Perspective of Milk Somatic Cell Count in Lactating Camels. In Journal of Camel Practice and Research (Vol. 28, Issue 3, pp. 319–325). Diva Enterprises Private Limited. https://doi.org/10.5958/2277-8934.2021.00049.7

56. El -Ziney, M. G. (2007). Microbiological quality and safety assessment of camel milk (camelus dromedaries) in saudi arabia (qassim region). In Applied Ecology and Environmental Research (Vol. 5, Issue 2, pp. 115–122). ALOKI Ltd. https://doi.org/10.15666/aeer/0502_115122

57. Alaoui Ismaili, M., Saidi, B., Zahar, M., Hamama, A., & Ezzaier, R. (2019). Composition and microbial quality of raw camel milk produced in Morocco. In Journal of the Saudi Society of Agricultural Sciences (Vol. 18, Issue 1, pp. 17–21). Elsevier BV. https://doi.org/10.1016/j.jssas.2016.12.001

58. Garcell, H. G., Garcia, E. G., Pueyo, P. V., Martín, I. R., Arias, A. V., & Alfonso Serrano, R. N. (2016). Outbreaks of brucellosis related to the consumption of unpasteurized camel milk. In Journal of Infection and Public Health (Vol. 9, Issue 4, pp. 523–527). Elsevier BV. https://doi.org/10.1016/j.jiph.2015.12.006

59. Alnassrallah, M. N., Alzoman, N. Z., & Almomen, A. (2022). Qualitative immunoassay for the determination of tetracycline antibiotic residues in milk samples followed by a quantitative improved HPLC-DAD method. In Scientific Reports (Vol. 12, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-022-18886-2

60. Zhumabay, A. N., Serikbayeva, A. D., Myrzabek, K. A., & Musulmanova, M. M. (2023). Microbiological analysis of raw camel milk, dry camel milk and shubat from a farm in Almaty region. In Journal of Microbiology and Virology (Vol. 2, Issue 41, pp. 167–172). Research and Production Center for Microbiology and Virology. https://doi.org/10.53729/MV-AS.2023.02.10

61. Konuspayeva, G., & Faye, B. (2021). Recent Advances in Camel Milk Processing. In Animals (Vol. 11, Issue 4, p. 1045). MDPI AG. https://doi.org/10.3390/ani11041045

62. Hassan, R. A., El Zubeir, I. E. M., & Babiker, S. A. (2007). Effect of Pasteurization of Raw Camel Milk and Storage Temperature on the Chemical Composition of Fermented Camel Milk. In International Journal of Dairy Science (Vol. 2, Issue 2, pp. 166–171). Science Alert. https://doi.org/10.3923/ijds.2007.166.171

63. Ayyash, M., Abdalla, A., Abu-Jdayil, B., Huppertz, T., Bhaskaracharya, R., Al-Mardeai, S., Mairpady, A., Ranasinghe, A., & Al-Nabulsi, A. (2022). Rheological properties of fermented milk from heated and high pressure-treated camel milk and bovine milk. In LWT (Vol. 156, p. 113029). Elsevier BV. https://doi.org/10.1016/j.lwt.2021.113029

64. Alharbi, Y. M., Sakr, S. S., Albarrak, S. M., Almundarij, T. I., Barakat, H., & Hassan, M. F. Y. (2022). Antioxidative, Antidiabetic, and Hypolipidemic Properties of Probiotic-Enriched Fermented Camel Milk Combined with Salvia officinalis Leaves Hydroalcoholic Extract in Streptozotocin-Induced Diabetes in Rats. In Antioxidants (Vol. 11, Issue 4, p. 668). MDPI AG. https://doi.org/10.3390/antiox11040668

65. De Filippis, F., Pasolli, E., & Ercolini, D. (2020). The food-gut axis: lactic acid bacteria and their link to food, the gut microbiome and human health. In FEMS Microbiology Reviews (Vol. 44, Issue 4, pp. 454–489). Oxford University Press (OUP). https://doi.org/10.1093/femsre/fuaa015

66. Gupta, V., & Garg, R. (2009). PROBIOTICS. In Indian Journal of Medical Microbiology (Vol. 27, Issue 3, pp. 202–209). Elsevier BV. https://doi.org/10.4103/0255-0857.53201

67. Rahmeh, R., Akbar, A., Kishk, M., Al-Onaizi, T., Al-Azmi, A., Al-Shatti, A., Shajan, A., Al-Mutairi, S., & Akbar, B. (2019). Distribution and antimicrobial activity of lactic acid bacteria from raw camel milk. In New Microbes and New Infections (Vol. 30, p. 100560). Elsevier BV. https://doi.org/10.1016/j.nmni.2019.100560

68. Sharma, A., Lavania, M., Singh, R., & Lal, B. (2021). Identification and probiotic potential of lactic acid bacteria from camel milk. In Saudi Journal of Biological Sciences (Vol. 28, Issue 3, pp. 1622–1632). Elsevier BV. https://doi.org/10.1016/j.sjbs.2020.11.062

69. Nagyzbekkyzy, E., Sembayeva, D., Sarsenova, A., Mansurov, N., Moldabayeva, A., & Moldagulova, N. (2020). Data on the diversity of lactic acid bacteria isolated from raw and fermented camel milk. In Data in Brief (Vol. 31, p. 105956). Elsevier BV. https://doi.org/10.1016/j.dib.2020.105956

70. Khedid, K., Faid, M., Mokhtari, A., Soulaymani, A., & Zinedine, A. (2009). Characterization of lactic acid bacteria isolated from the one humped camel milk produced in Morocco. In Microbiological Research (Vol. 164, Issue 1, pp. 81–91). Elsevier BV. https://doi.org/10.1016/j.micres.2006.10.008

71. Zhao, J., Fan, H., Kwok, L.-Y., Guo, F., Ji, R., Ya, M., & Chen, Y. (2020). Analyses of physicochemical properties, bacterial microbiota, and lactic acid bacteria of fresh camel milk collected in Inner Mongolia. In Journal of Dairy Science (Vol. 103, Issue 1, pp. 106–116). American Dairy Science Association. https://doi.org/10.3168/jds.2019-17023

72. Bin Masalam, M. S., Bahieldin, A., Alharbi, M. G., Al-Masaudi, S., Al-Jaouni, S. K., Harakeh, S. M., & Al-Hindi, R. R. (2018). Isolation, Molecular Characterization and Probiotic Potential of Lactic Acid Bacteria in Saudi Raw and Fermented Milk. In Evidence-Based Complementary and Alternative Medicine (Vol. 2018, pp. 1–12). Hindawi Limited. https://doi.org/10.1155/2018/7970463

73. Kadri, Z., Spitaels, F., Cnockaert, M., Praet, J., El Farricha, O., Swings, J., & Vandamme, P. (2015). Enterococcus bulliens sp. nov., a novel lactic acid bacterium isolated from camel milk. In Antonie van Leeuwenhoek (Vol. 108, Issue 5, pp. 1257–1265). Springer Science and Business Media LLC. https://doi.org/10.1007/s10482-015-0579-z

74. Yu, Z., Peng, C., Kwok, L., & Zhang, H. (2021). The Bacterial Diversity of Spontaneously Fermented Dairy Products Collected in Northeast Asia. In Foods (Vol. 10, Issue 10, p. 2321). MDPI AG. https://doi.org/10.3390/foods10102321

75. Davati, N., Yazdi, F. T., Zibaee, S., Shahidi, F., & Edalatian, M. R. (2015). Study of Lactic Acid Bacteria Community From Raw Milk of Iranian One Humped Camel and Evaluation of Their Probiotic Properties. In Jundishapur Journal of Microbiology (Vol. 8, Issue 5). Briefland. https://doi.org/10.5812/jjm.8(5)2015.16750

76. Moussaid, S., Bouhlal, O., Benali, A., Kabbour, M. R., Ounine, K., & El Maadoudi, E. H. (2021). Technological characterization of indigenous lactic acid bacteria from Moroccan camel milk for their

potential use as starter or adjunct culture. In Folia Microbiologica (Vol. 66, Issue 5, pp. 761–774). Springer Science and Business Media LLC. https://doi.org/10.1007/s12223-021-00885-x

77. Edalati, E., Saneei, B., Alizadeh, M., Hosseini, S. S., Zahedi Bialvaei, A., & Taheri, K. (2019). Isolation of probiotic bacteria from raw camel's milk and their antagonistic effects on two bacteria causing food poisoning. In New Microbes and New Infections (Vol. 27, pp. 64–68). Elsevier BV. https://doi.org/10.1016/j.nmni.2018.11.008
78. Baig, M. A., Turner, M. S., Liu, S.-Q., Shah, N. N., & Ayyash, M. M. (2022). Heat, cold, acid, and bile salt induced differential proteomic responses of a novel potential probiotic Lactococcus garvieae C47 isolated from camel milk. In Food Chemistry (Vol. 397, p. 133774). Elsevier BV. https://doi.org/10.1016/j.foodchem.2022.133774
79. Kadri, Z., Spitaels, F., Cnockaert, M., Amar, M., Joossens, M., & Vandamme, P. (2021). The bacterial diversity of raw Moroccon camel milk. In International Journal of Food Microbiology (Vol. 341, p. 109050). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2021.109050
80. Elbanna, K., El Hadad, S., Assaeedi, A., Aldahlawi, A., Khider, M., & Alhebshi, A. (2018). In vitro and in vivo evidences for innate immune stimulators lactic acid bacterial starters isolated from fermented camel dairy products. In Scientific Reports (Vol. 8, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-018-31006-3
81. Ishii, S., Nurtazin, S. (2014). Properties of camel milk liquor ("shubat") in the Republic of Kazakhstan. In Milk Science (Vol. 63, Issue 2, pp. 55–62). Japanese Dairy Science Association. https://doi.org/10.11465/milk.63.55
82. Takeda, S., Yamasaki, K., Takeshita, M., Kikuchi, Y., Tsend-Ayush, C., Dashnyam, B., Ahhmed, A. M., Kawahara, S., & Muguruma, M. (2011). The investigation of probiotic potential of lactic acid bacteria isolated from traditional Mongolian dairy products. In Animal Science Journal (Vol. 82, Issue 4, pp. 571–579). Wiley. https://doi.org/10.1111/j.1740-0929.2011.00874.x
83. Xu, R. H., Xiu, L., Zhang, Y. L., Du, R. P., & Wang, X. (2019). Probiotic and hepatoprotective activity of lactobacillus isolated from Mongolian camel milk products. In Beneficial Microbes (Vol. 10, Issue 6, pp. 699–710). Wageningen Academic Publishers. https://doi.org/10.3920/bm2018.0131
84. Mercha, I., Lakram, N., Kabbour, M. R., Bouksaim, M., Zkhiri, F., & El Maadoudi, E. H. (2020). Probiotic and technological features of Enterococcus and Weissella isolates from camel milk characterised by an Argane feeding regimen. In Archives of Microbiology (Vol. 202, Issue 8, pp. 2207–2219). Springer Science and Business Media LLC. https://doi.org/10.1007/s00203-020-01944-6
85. Berhe, T., Ipsen, R., Seifu, E., Kurtu, M. Y., Fugl, A., & Hansen, E. B. (2019). Metagenomic analysis of bacterial community composition in Dhanaan: Ethiopian traditional fermented camel milk. In FEMS Microbiology Letters (Vol. 366, Issue 11). Oxford University Press (OUP). https://doi.org/10.1093/femsle/fnz128
86. Forooghi Nia, F., Rahmati, A., Ariamanesh, M., Saeidi, J., Ghasemi, A., & Mohtashami, M. (2023). The Anti-Helicobacter pylori effects of Limosilactobacillus reuteri strain 2892 isolated from Camel milk in C57BL/6 mice. In World Journal of Microbiology and Biotechnology (Vol. 39, Issue 5). Springer Science and Business Media LLC. https://doi.org/10.1007/s11274-023-03555-x
87. Kordesedehi, R., Taheri-Kafrani, A., Rabbani-Khorasgani, M., Kazemi, R., Mutangadura, D., & Haertle, T. (2018). Modification of IgE binding to αS1-casein by proteolytic activity of Enterococcus faecium isolated from Iranian camel milk samples. In Journal of Biotechnology (Vols. 276–277, pp. 10–14). Elsevier BV. https://doi.org/10.1016/j.jbiotec.2018.04.005
88. Benmechernene, Z., Fernández-No, I., Quintela-Baluja, M., Böhme, K., Kihal, M., Calo-Mata, P., & Barros-Velázquez, J. (2014). Genomic and Proteomic Characterization of Bacteriocin-ProducingLeuconostoc mesenteroidesStrains Isolated from Raw Camel Milk in Two Southwest Algerian Arid Zones. In BioMed Research International (Vol. 2014, pp. 1–10). Hindawi Limited. https://doi.org/10.1155/2014/853238
89. Derriche, I., Nogacka, A. M., Salazar, N., Ruas-Madiedo, P., Gueimonde, M., Bensalah, F., & de los Reyes-Gavilán, C. G. (2020). Effect of inulin-type fructans and galactooligosaccharides on cultures of Lactobacillus strains isolated in Algeria from camel's milk and human colostrum. In Food Science and Technology International (Vol. 27, Issue 3, pp. 223–233). SAGE Publications. https://doi.org/10.1177/1082013220944661
90. Chouikhi, A., Ktari, N., Bardaa, S., Hzami, A., Ben Slima, S., Trabelsi, I., Asehraou, A., & Ben Salah, R. (2021). A novel probiotic strain, Lactiplantibacillus plantarum LC38, isolated from Tunisian camel milk promoting wound healing in Wistar diabetic rats. In Archives of Microbiology (Vol. 204, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1007/s00203-021-02634-7

91. Abushelaibi, A., Al-Mahadin, S., El-Tarabily, K., Shah, N. P., & Ayyash, M. (2017). Characterization of potential probiotic lactic acid bacteria isolated from camel milk. In LWT - Food Science and Technology (Vol. 79, pp. 316–325). Elsevier BV. https://doi.org/10.1016/j.lwt.2017.01.041

92. Fguiri, I., Ziadi, M., Atigui, M., Ayeb, N., Arroum, S., Assadi, M., & Khorchani, T. (2015). Isolation and characterisation of lactic acid bacteria strains from raw camel milk for potential use in the production of fermented Tunisian dairy products. In International Journal of Dairy Technology (Vol. 69, Issue 1, pp. 103–113). Wiley. https://doi.org/10.1111/1471-0307.12226

93. Hawaz, E., Guesh, T., Kebede, A., & Menkir, S. (2016). Characterization of Lactic Acid Bacteria from Camel Milk and their Technological Properties to Use as a Starter Culture. East African Journal of Sciences, Vol. 10 No. 1 (2016): East African Journal of Sciences. https://doi.org/10.20372/EAJS.V10I1.320

94. Fugl, A., Berhe, T., Kiran, A., Hussain, S., Laursen, M. F., Bahl, M. I., Hailu, Y., Sørensen, K. I., Guya, M. E., Ipsen, R., & Hansen, E. B. (2017). Characterisation of lactic acid bacteria in spontaneously fermented camel milk and selection of strains for fermentation of camel milk. In International Dairy Journal (Vol. 73, pp. 19–24). Elsevier BV. https://doi.org/10.1016/j.idairyj.2017.04.007

95. Soleymanzadeh, N., Mirdamadi, S., & Kianirad, M. (2016). Antioxidant activity of camel and bovine milk fermented by lactic acid bacteria isolated from traditional fermented camel milk (Chal). In Dairy Science & Technology (Vol. 96, Issue 4, pp. 443–457). Springer Science and Business Media LLC. https://doi.org/10.1007/s13594-016-0278-1

96. Jans, C., Bugnard, J., Njage, P. M. K., Lacroix, C., & Meile, L. (2012). Lactic acid bacteria diversity of African raw and fermented camel milk products reveals a highly competitive, potentially health-threatening predominant microflora. In LWT (Vol. 47, Issue 2, pp. 371–379). Elsevier BV. https://doi.org/10.1016/j.lwt.2012.01.034

97. Ashmaig, A., Hasan, A. M., & Gaali, E. I. (2009). Identification of lactic acid bacteria isolated from traditional Sudanese fermented camel's milk (Gariss). In African Journal of Microbiology Research (Vol. 3, Issue 8, pp. 451–457). Academic Journals.

98. Carballo, J. (2016). Potential technological interest of indigenous lactic acid bacteria from algerian camel milk [JB]. In Italian Journal of Food Science (Vol. 28, Issue 4, pp. 598–611). Codon Publications. https://doi.org/10.14674/1120-1770/ijfs.v391

99. Mourad, K., & Nour-Eddine, K. (2006). Physicochemical and microbiological study of a traditional butter made from camel milk in the Sahara (Algeria): isolation and identification of lactic acid bacteria and yeasts. In Grasas y Aceites (Vol. 57, Issue 2). Editorial CSIC. https://doi.org/10.3989/gya.2006.v57.i2.37

100. Konuspayeva, G., Baubekova, A., Akhmetsadykova, S., & Faye, B. (2023). Traditional dairy fermented products in Central Asia. In International Dairy Journal (Vol. 137, p. 105514). Elsevier BV. https://doi.org/10.1016/j.idairyj.2022.105514

101. Serikbayeva, A., Konuspayeva, G., Faye, B., Loiseau, G., Narmuratova, M. K., & Esenov, P. (2005). Probiotic properties of a sour-milk product: shubat from the camel milk. In Proceedings of the NATO Advanced Research Workshop, 19-21 April 2004, Ashgabad, Turkmenistan (Vol. 362, pp. 187–191). IOS press.

102. Rahman, I. E., Dirar, H. A., & Osman, M. A. (2009). Microbiological and biochemical changes and sensory evaluation of camel milk fermented by selected bacterial starter cultures. In African Journal of Food Science (Vol. 3, pp. 398–405). Academic Journals.

103. Manaer, T., Yu, L., Zhang, Y., Xiao, X.-J., & Nabi, X.-H. (2015). Anti-diabetic effects of shubat in type 2 diabetic rats induced by combination of high-glucose-fat diet and low-dose streptozotocin. In Journal of Ethnopharmacology (Vol. 169, pp. 269–274). Elsevier BV. https://doi.org/10.1016/j.jep.2015.04.032

104. Urazakov, N. U., & Baĭnazarov, S. H. (1974). "Tushibek" - pervaia v istorii shubatolechebnitsa ["Tushchibek" - the 1st clinic in history for the treatment of pulmonary tuberculosis with camel's sour milk]. In Problemy tuberkuleza (Vol. 2, pp. 89–90). Moskva : Meditsina, -2003.

105. Sulieman, A. M. E., & Alayan, A. A. (2022). Nutritional, Antimicrobial and Bioactive Components of Gariss, a Fermented Camel Milk Product. In African Fermented Food Products- New Trends (pp. 175–187). Springer International Publishing. https://doi.org/10.1007/978-3-030-82902-5_12

106. Shori, A. B. (2012). Comparative study of chemical composition, isolation and identification of micro-flora in traditional fermented camel milk products: Gariss, Suusac, and Shubat. In Journal of the Saudi Society of Agricultural Sciences (Vol. 11, Issue 2, pp. 79–88). Elsevier BV. https://doi.org/10.1016/j.jssas.2011.12.001

107. Lore, T. A., Mbugua, S. K., & Wangoh, J. (2005). Enumeration and identification of microflora in suusac, a Kenyan traditional fermented camel milk product. In LWT - Food Science and Technology (Vol. 38, Issue 2, pp. 125–130). Elsevier BV. https://doi.org/10.1016/j.lwt.2004.05.008

108. Konuspayeva, G. S. (2020). Manufacture and Challenges of Camel Milk Cheese. In Handbook of Research on Health and Environmental Benefits of Camel Products (pp. 110–122). IGI Global. https://doi.org/10.4018/978-1-7998-1604-1.ch006

109. Abdalla, A., Abu-Jdayil, B., AlMadhani, S., Hamed, F., Kamal-Eldin, A., Huppertz, T., & Ayyash, M. (2022). Low-fat akawi cheese made from bovine-camel milk blends: Rheological properties and microstructural characteristics. In Journal of Dairy Science (Vol. 105, Issue 6, pp. 4843–4856). American Dairy Science Association. https://doi.org/10.3168/jds.2021-21367

110. Ramet, J. P. (2001). The technology of making cheese from camel milk (Camelus dromedarius). In Food & Agriculture Organization (p. 62). FAO

111. Al-zoreky, N. S., & Almathen, F. S. (2021). Using recombinant camel chymosin to make white soft cheese from camel milk. In Food Chemistry (Vol. 337, p. 127994). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.127994

112. Bekele, B., Hansen, E. B., Eshetu, M., Ipsen, R., & Hailu, Y. (2019). Effect of starter cultures on properties of soft white cheese made from camel (Camelus dromedarius) milk. In Journal of Dairy Science (Vol. 102, Issue 2, pp. 1108–1115). American Dairy Science Association. https://doi.org/10.3168/jds.2018-15084

113. Mahgoub, S., & Sulieman, A. M. E. (2022). Production and Quality Assessment of Camel Milk Cheese. In African Fermented Food Products- New Trends (pp. 189–202). Springer International Publishing. https://doi.org/10.1007/978-3-030-82902-5_13

114. Khan, H. I., Athar, I. H., Aslam, M. Z., & Moorat, K. (2004). Evaluation of Cheese Prepared by Processing Camel Milk. In Pakistan Journal of Zoology (Vol. 36, Issue 4, pp. 323–326). University of Punjab (new Campus).

115. Inayat, S., Arain, M. A., Khaskheli, M., & Mali, A. H. (2003). Study of the Effect of Processing on the Chemical Quality of Soft Unripened Cheese Made from Camel Milk. In Pakistan Journal of Nutrition (Vol. 2, pp. 102–105). Asian Network for Scientific Information. https://doi.org/10.3923/pjn.2003.102.105

116. Chandramohan, V. P. (2022). Experimental Analysis of Freeze Drying and Estimating the Transient Moisture Contents of Food Products. In Energy and Exergy for Sustainable and Clean Environment, Volume 2 (pp. 535–546). Springer Nature Singapore. https://doi.org/10.1007/978-981-16-8274-2_36

117. Zou, Z., Duley, J. A., Cowley, D. M., Reed, S., Arachchige, B. J., Bhandari, B., Shaw, P. N., & Bansal, N. (2022). Physicochemical Properties and Whey Proteomes of Camel Milk Powders Produced by Different Concentration and Dehydration Processes. In Foods (Vol. 11, Issue 5, p. 727). MDPI AG. https://doi.org/10.3390/foods11050727

118. Deshwal, G. K., Singh, A. K., Kumar, D., & Sharma, H. (2020). Effect of spray and freeze drying on physico-chemical, functional, moisture sorption and morphological characteristics of camel milk powder. In LWT (Vol. 134, p. 110117). Elsevier BV. https://doi.org/10.1016/j.lwt.2020.110117

119. Habtegebriel, H., Wawire, M., & Sila, D. (2018). The Effect of Pretreatment (Spray Drying) on the Yield and Selected Nutritional Components of Whole Camel Milk Powder. In Journal of Food Science (Vol. 83, Issue 12, pp. 2983–2991). Wiley. https://doi.org/10.1111/1750-3841.14361

120. Rasika, D. M. D., Munasinghe, M. A. D. D., Vidanarachchi, J. K., da Cruz, A. G., Ajlouni, S., & Ranadheera, C. S. (2020). Probiotics and prebiotics in non-bovine milk. In Advances in Food and Nutrition Research (pp. 339–384). Elsevier. https://doi.org/10.1016/bs.afnr.2020.06.008

121. Abou-Soliman, N. H. I., Sakr, S. S., & Awad, S. (2017). Physico-chemical, microstructural and rheological properties of camel-milk yogurt as enhanced by microbial transglutaminase. In Journal of Food Science and Technology (Vol. 54, Issue 6, pp. 1616–1627). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-017-2593-9

122. Ali, A. H., Abu-Jdayil, B., Al Nabulsi, A., Osaili, T., Liu, S.-Q., Kamal-Eldin, A., & Ayyash, M. (2023). Fermented camel milk influenced by soy extract: Apparent viscosity, viscoelastic properties, thixotropic behavior, and biological activities. In Journal of Dairy Science (Vol. 106, Issue 10, pp. 6671–6687). American Dairy Science Association. https://doi.org/10.3168/jds.2023-23294

123. Arslan Amin, H. M., Inayat, S., Gulzar, N., Bhatti, J. A., Masood, S., Ayub, A., Kanwal, S., Batool, M., Ajmal, M., & Mustafa, G. (2024). Addition of transglutaminase enzyme in camel milk yoghurt to increase its sensorial aspects. In Brazilian Journal of Biology (Vol. 84). FapUNIFESP (SciELO). https://doi.org/10.1590/1519-6984.269043

124. Bhagiel, I., Mustafa, E.A., TabidiM., M., Ahmed, M. E., & Pha, D. (2015). Comparison Between The Physiochemical Attributes of Yogurt Processed From Camel Milk And That Processed From Cow Milk And The Effect of Storage Period on Ph And Acidity. In World Journal Of Pharmacy And Pharmaceutical Sciences (Vol. 4, Issue 8, pp.1530–1540). Atom and Cell Publishers.

125. Hashim, I. B., Khalil, A. H., & Habib, H. (2009). Quality and acceptability of a set-type yogurt made from camel milk. In Journal of Dairy Science (Vol. 92, Issue 3, pp. 857–862). American Dairy Science Association. https://doi.org/10.3168/jds.2008-1408

126. Konuspayeva, G., & Faye, B. (2004). A better knowledge of milk quality parameters: A preliminary step for improving the camel milk market opportunity in a transition economy–The case of Kazakhstan. Saving the Camel and Peoples' Livelihoods Building a Multi-Stakeholder Platform for the Conservation of the Camel in Rajasthan. In International Conference, (pp. 28–36).

127. Berhe, T., Seifu, E., & Kurtu, M. Y. (2013). Physicochemical properties of butter made from camel milk. In International Dairy Journal (Vol. 31, Issue 2, pp. 51–54). Elsevier BV. https://doi.org/10.1016/j.idairyj.2013.02.008

128. Seifu, E. (2023). Camel milk products: innovations, limitations and opportunities. In Food Production, Processing and Nutrition (Vol. 5, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s43014-023-00130-7

129. Mourad, K., & Nour-Eddine, K. (2006). Physicochemical and microbiological study of a traditional butter made from camel milk in the Sahara (Algeria): isolation and identification of lactic acid bacteria and yeasts. In Grasas y Aceites (Vol. 57, Issue 2). Editorial CSIC. https://doi.org/10.3989/gya.2006.v57.i2.37

130. Berhe, T., Seifu, E., & Kurtu, M. Y. (2013). Physicochemical properties of butter made from camel milk. In International Dairy Journal (Vol. 31, Issue 2, pp. 51–54). Elsevier BV. https://doi.org/10.1016/j.idairyj.2013.02.008

131. Hajian, N., Salami, M., Mohammadian, M., Moghadam, M., & Emam-Djomeh, Z. (2020). Production of Low-Fat Camel Milk Functional Ice creams Fortified with Camel Milk Casein and its Antioxidant Hydrolysates [JB]. Applied Food Biotechnology, 7(2). https://doi.org/10.22037/afb.v7i2.27779

132. Soni, V., & Goyal, M. (2013). Potential of using camel milk for ice cream making. In Journal of Camel Practice and Research (Vol. 20, Issue 2, pp. 271–275). Camel Publishing House.

133. Jafarpour, A. (2017). Feasibility of manufacture and investigation of physicochemical properties of camel milk – based ice cream. In Journal of Scientific Agriculture (Vol. 1, p. 300). Update Publishing House. https://doi.org/10.25081/jsa.2017.v1.838

134. Khosravi-Darani, K., Jahadi, M., Abbasi, H., Asgari, M., & Tarlak, F. (2022). Production of chocolate probiotic dessert based on camel milk using Lactocaseibacillus casei. In Carpathian Journal of Food Science and Technology (Vol. 14, Issue 2, pp. 189–206). Technical University of Cluj Napoca. https://doi.org/10.34302/crpjfst/2022.14.2.16

135. Brezovečki, A. (2015). Camel milk and milk products. In Mljekarstvo (pp. 81–90). Croatian Dairy Union. https://doi.org/10.15567/mljekarstvo.2015.0202

136. Zibaee, S., Hosseini, S. M. al-reza, Yousefi, M., Taghipour, A., Kiani, M. A., & Noras, M. R. (2015). Nutritional and Therapeutic Characteristics of Camel Milk in Children: A Systematic Review. In Electronic physician (Vol. 7, Issue 7, pp. 1523–1528). Knowledge Kingdom Publishing. https://doi.org/10.19082/1523

137. The Astana Times. Camel Breeding Emerges in Kazakhstan's Karagandy Region. 17th June, 2023. Retrieved from: https://astanatimes.com/2023/06/camel-breeding-emerges-in-kazakhstans-karagandy-region/.

138. Sfera.fm news. A camel milk powder plant was opened in Kazakhstan. 5th of August, 2022. Retrieved from: https://sfera.fm/news/v-kazakhstane-otkrylsya-zavod-po-proizvodstvu-verblyuzhego-moloka.

139. Advertisement on Dastarkhan24.kz. Retrieved from: https://dastarkhan24.kz/product/shubat_daulet_beket_0_0_5_l/.

Funds:

This research received no external funding.

Acknowledgments:

The authors would like to sincerely thank the Almaty University of Technology and Kazakh National Agrarian Research University for supporting the research.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

*Aikerim Zhumabay, Almaty Technological University, Almaty 050000, Kazakhstan,
Tel.: +7-778-312-5278
E-mail: ai94ka@mail.ru
ORCID: https://orcid.org/0000-0000-4743-8221

Assiya Serikbayeva, Kazakh National Agrarian Research University, Almaty 050010, Kazakhstan,
Tel.: +7-777-256-5375
E-mail: serikbayeva@yandex.ru
ORCID: https://orcid.org/0000-0003-4632-7343

Sabira Kozykan, Kazakh National Agrarian Research University, Almaty 050010, Kazakhstan,
Tel.: +7-747-389-7338
E-mail: sabira.kozykan@kaznaru.edu.kz
ORCID: https://orcid.org/0000-0002-2385-3917

Saule Sarimbekova, Kazakh National Agrarian Research University, Almaty 050010, Kazakhstan,
Tel.: +7-702-513-9198
E-mail: sarimbekova@yandex.ru
ORCID: https://orcid.org/0000-0002-9625-5467

Gaukhar Kossaliyeva, Northwest A&F University, Yangling, Xianyang 712100, China,
Tel.: +7-777-097-4674
E-mail: kosalieva.g@kaznmu.kz
ORCID: https://orcid.org/0009-0007-7171-4472

Aitbay Alimov, Kazakh National Agrarian Research University, Almaty 050010, Kazakhstan,
Tel.: +7-701-301-2447
E-mail: aitbai.65@mail.ru
ORCID: https://orcid.org/0000-0003-1028-0408

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 22.12.2023
Revised: 15.1.2024
Accepted: 18.1.2024
Published: 12.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 97-108
https://doi.org/10.5219/1945
ISSN: 1337-0960 online
www.potravinarstvo.com

The effect of yeast autolysis on the composition of wine

Jan Mikuš, Jiří Sochor, Štefan Ailer, Mojmír Baroň

ABSTRACT

The experiment aims to monitor the amino acid content, total polyphenolic components, and antioxidant activity values of wines that have matured on yeast and non-yeast sludge. The grape varieties used in the experiment, which lasted 300 days, were (*Vitis vinifera* L.) Chardonnay, Riesling rhinestone, and Veltliner green. During this time, both the measured parameters and the characteristics of the wine gradually changed. The total amount of amino acids in the wines aged on yeast sludge was more than 200% greater than that found in wines aged without yeast sludge. A 30% decrease in the total polyphenolic component content was noted for wines produced with yeast lees. The antioxidant activity levels correlated with the total polyphenol content, with the levels in wines made with yeast lees on average 13% lower. The experiment showed that wines produced by these different methods have different mutagenic characteristics. Sensory analysis of the wines demonstrated that wines matured on yeast sludge have better organoleptic properties. These wines were sturdier, fuller, and more harmonious than wines aged without a yeast sludge.

Keywords: autolysis, sludge, wine, amino acid, polyphenol

INTRODUCTION

The release of yeast compounds into wine during autolysis is a current major trend in the production of wine. The principle is that these wines remain in contact with the yeast for several months or years, as with Burgundy wines designated "sur lie" **[1]**. Autolysis is a very slow process of yeast degradation induced by increased temperature by adding plasmolysers through mechanical intervention or other factors that facilitate the activation of lytic cell enzymes **[2]**. Still, wines are not a suitable medium for the use of the autolysis process, which takes a long time due to their ethanol content (10-12%), low pH (3.0-3.5), and low maturation temperature (usually below 15 °C). The duration of autolysis depends on the yeast strain **[3]**. Most yeast autolysis studies are performed under conditions that accelerate the process to obtain results in a reasonable period. Tests almost always occur at relatively high temperatures, usually above 40 °C **[4]**. Temperature influences the process of yeast autolysis as the intracellular enzymatic activities are temperature-dependent. However, not all studies on this topic have produced final results that agree, and some have even been contradictory. For example, while **[5]** induced autolysis assays performed between 40 and 70 °C did not detect enzymes in the extracellular medium **[6]**, in autolysis assays with brewer's yeast at 45 °C and a pH of 6.5, say that it is clear that quite extensive proteolysis occurs outside the cell. During yeast autolysis, the individual constituents in the wine change. These substances have a major influence on the sensory profile of the wine. The compounds affected by autolysis include phenols, volatiles, polysaccharides, DNA, RNA, lipids, mannoproteins, amino acids, and other nitrogenous components **[3]**.

During fermentation, the yeasts use the amino acids present in the wine. After fermentation, when all the sugar has been consumed, the yeast releases the amino acids back into the wine. This movement of amino acids from the intracellular environment to the surrounding medium is passive and results in a higher amino acid content in the wine **[7]**. The amino acid content of the wine remains relatively stable, usually for three to four months. After this time, the amino acid content of the wine begins to increase. This increase is attributed to the autolytic process, where cellular proteins are degraded, dissolved, and released into the wine **[8]**. Prolonged maturation on lees with

regular stirring causes a reduction in the concentration of polyphenols in the wine. The yeast cell walls absorb the polyphenols, and the mannoproteins released during autolysis are incorporated into the lees. In this way, the yeast leaves act as a refining agent that reduces the tannin content of the wine **[9]**.

The present work aims to study the effect of autolysis on the content of selected substances found in the studied wines over 300 days and the changes in their sensory characteristics.

Scientific Hypothesis

After ethanol fermentation, the yeast dies and becomes a fermentation sludge. In an alcoholic environment, it decomposes and releases substances into the wine. These substances should bind polyphenolic compounds, increase the level of amino acids, and have a major influence on the sensory profile of the wine.

MATERIAL AND METHODOLOGY

Description of the experiment

Three varieties of grapes were used in the experiment: green Veltliner, Riesling, and Chardonnay, all came from the vineyards of the Institute of Viticulture and Enology (Faculty of Horticulture, Mendel University in Brno, Lednice, vineyard tract "Ve starých," Czech Republic) and were harvested by hand. The grapes were de-stemmed and crushed, and each variety's grape must be left to macerate for 2 days at 12 °C. Each must be then separated into two types of containers:

Variant A: the must was decanted into stainless steel containers, and the leaves were removed from the wine immediately after fermentation (control).

Variant B: the must was put into 600l wooden oak barrels, and the wine was stirred on the lees

Fermentation of all musts (spontaneous fermentation at 15 °C) continued for 20-25 days. In the stainless-steel containers (variant A), the wine was racked off the lees immediately after fermentation, and 40 $mg.l^{-1}$ of sulfur dioxide was added. The free sulfur dioxide was subsequently maintained at 25-30 $mg.l^{-1}$.

Variant B (wooden barrels), battonage began after the fermentation and continued for 300 days. The frequency of stirring was based on the sensory organoleptic characteristics of the wine (approximately weekly). Samples were taken from all wines and analyzed for the whole 300-day period.

Samples

Total number of variants: 2

Number of repetitions of each variant: 3

Number of repetitions: 20

For the first 63 days, samples were taken every 7 days.

After that, from day 64 to day 150, samples were taken every 14 days.

From day 150 to day 300, samples were taken every 30 days.

Biological Materials

Vitis vinifera L., variety Chardonnay, Veltiner green, Riesling.

Instruments

Gas chromatograph: Shimadzu (GC-17 A) equipped with an autosampler (AOC-5000) and connected to a QP detector (QP-5050 A) with GCsolution software (LabSolutions, version 1.20, Kyoto, Japan).

Spectrophotometer: (SPECORD 210, Carl-Zeiss, Jena, Germany).

Automatic spectrometric analyser: Miura one® (I.S.E. S.r.l. Via Luigi Einaudi, Italy).

Chemicals

Folin-Ciocalteu phenolic reagent (Sigma - Aldrich, St. Louis, Missouri, USA), sodium carbonate pa (99%) (Sigma - Aldrich, St. Louis, Missouri, USA), gallic acid (3, 4, 5-trihydroxybenzoic acid monohydrate, 99%; Alfa Aesar Thermo Fisher (Sigma Aldrich, St. luis, Missouri, USA)) were used to determine the total polyphenols. All reagents were dissolved in distilled water. The crucial reagent used in the measurement of total antioxidant activity was the Fe3+-2,4,6-tri(2-pyridyl-1,3,5-triazine) complex (Fe3+-TPTZ) (Sigma Aldrich, St. luis, Missouri, USA).

Laboratory Methods

Determination of the total quantity of polyphenolic compounds: The Folin-Ciocalteu method was used to determine the total quantity of polyphenolic compounds. All samples were analysed in triplicate; the final value was taken as the average of these measurements. A 40 µl sample was pipetted into a cuvette (3 ml) and diluted with 1960 µl of distilled water. Subsequently, 50 µl of Folin-Ciocalteu reagent was added to the cuvette. The mixture was thoroughly shaken. After three minutes, 300 µl of 20% sodium carbonate decahydrate solution (Na_2CO_3) was added. The reaction mixture was again shaken and then incubated at 22 °C for 120 minutes. Absorbance was measured using a dual beam spectrophotometer (SPECORD 210, Carl-Zeiss, Jena, Germany) at A = 750 nm. against a blank. The results were expressed as gallic acid equivalence.

Determination of the antioxidant activity by the FRAP method: Three solutions were used to determine the antioxidant activity by the FRAP (ferric reduction antioxidant power) method – (1) TPTZ solution: 10 mM TPTZ (m = 78.02 mg) dissolved in 25 ml of 40 mM HCl; (2) FeCl3 solution: 20 mM $FeCl_3$ (m = 135.13 mg) dissolved in 25 ml of distilled water; and (3) Acetate buffer solution: 0.02 M acetate buffer pH 3.6 (m = 775 mg sodium acetate dissolved in 250 ml of distilled water, pH 3.6 adjusted with acetic acid). The three solutions were mixed in a 1:1:10 ratio (TPTZ:$FeCl_3$:acetate buffer). 150 µl of the reagent was pipetted into plastic cuvettes, and 3 ul of the sample was added. The absorbance was measured for 12 min at A = 605 nm. The antioxidant activity was calculated from the calibration curve using gallic acid as a standard (10-200 $mg.l^{-1}$). The results were expressed as gallic acid equivalence.

Analysis of total amino acid content: Primary amino groups were derivatized using o-phtaldialdehyde and N-acetyl-L-cysteine (OPA/NAC) form isoindoles on a base medium. These derivatives underwent spectrophotometric detection at 340 nm. The absorbance is proportional to the total amino acid content in the sample. The analysis was performed using a Miura one® instrument (I.S.E. S.r.l. Via Luigi Einaudi, Italy), a spectrophotometer equipped with an autosampler. The determination was performed in triplicate on 2 ml collected samples and either immediately frozen or immediately analysed.

Number of samples analyzed: 120.

Number of repeated analyses: 120.

Number of experiment replication: 0.

Statistical Analysis

The results were processed in Statistica 10 (Czech Republic, Statsoft). The values obtained were plotted on graphs. The values used were the mean of the measurements of the three variants, and the standard deviation was calculated using a multivariate ANOVA analysis. Tukey's test and multivariate ANOVA were implemented.

RESULTS AND DISCUSSION

The tables and graphs below show the total amino acid content, antioxidant activity values, and phenolic compound content for the 300 days of the experiment for all three grape varieties that were divided into two types of containers:

(A) decalcified must was put into stainless steel containers, and immediately after fermentation, the leaves were withdrawn from the wine (control).

(B) the most were put into 600-litre wooden oak barrels, and the wine was stirred with the lees.

Amino acid content values

Amino acids can be released into the extracellular environment prior to autolysis. This is a cellular response to the lack of nutrients in the wine. Peptides with high molecular weight (mainly hydrophobic) are released in the first autolysis processes **[10]**. These peptides are subsequently hydrolysed, resulting in the production of smaller molecules and the release of amino acids. Therefore, the concentration of free amino acids decreases compared to the total amino acids as peptides are released into the wine and are only subsequently cleaved into amino acids **[11]**.

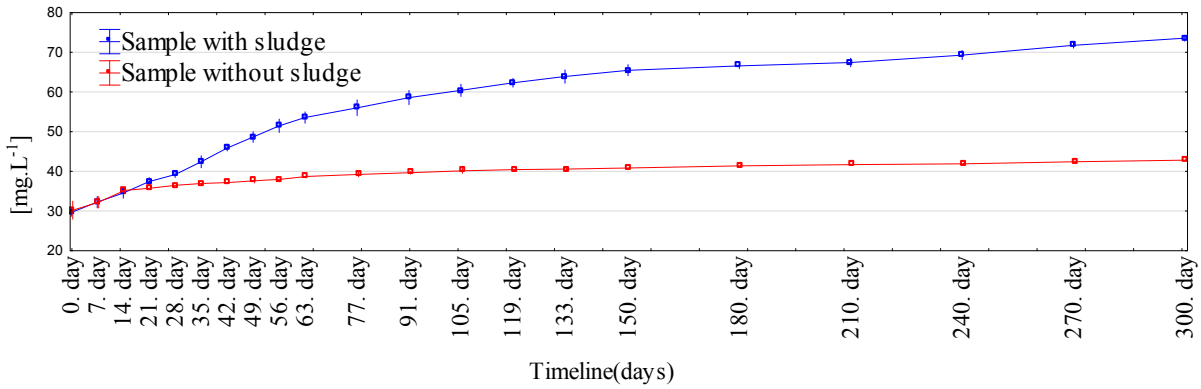


Figure 1 Total amino acid content of Green Veltliner during the maturation of wine on and off lees.

Figure 1 shows a large increase in the amino acid content of the variant with yeast sludge, which increased by 298% (day 0 versus day 300). However, for the variant without sludge, we can see a much smaller increase in value (a 33% increase from day 0 to day 300). The variant with yeast sludge had an amino acid content that was 265% higher on the last day of the experiment in comparison to the variant without sludge. The average total increase in the variant with sludge was 44 mg.l$^{-1,}$ while the variant without sludge only increased by 13 mg.l^{-1}.

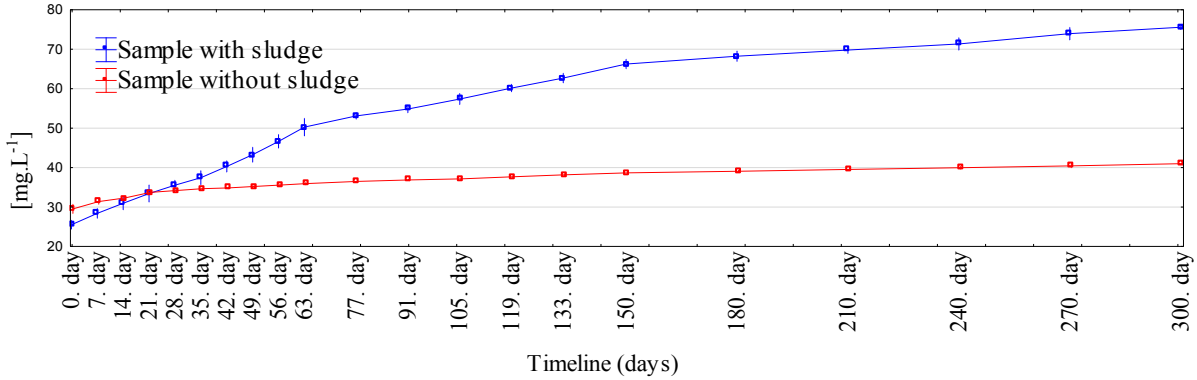


Figure 2 Total amino acid content of Riesling during the maturation of wine on and off lees.

Figure 2 shows a minimal increase of 37% in the amino acid content of the wine without lees (day 0 versus day 30). At the end of the experiment, the difference between the two variants was 164%. The amino acid content of the variant with lees increased by 202% (day 0 versus day 300). At the beginning of the experiment, the amino acid content measured in the variant with sludge was 25 mg.l^{-1}, and the variant without sludge was measured at 29 mg.l^{-1}. The average total increase for the with sludge variant was 50 mg.l^{-1} and for the without sludge variant, 11 mg.l^{-1}.

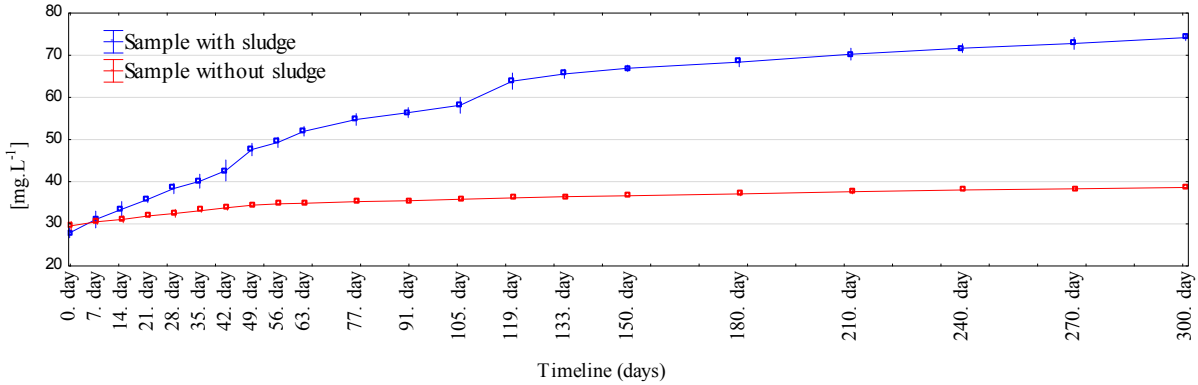


Figure 3 Total amino acid content of Chardonnay during the maturation of wine on and off lees.

It can be seen from Figure 3 that in the vessel without sludge (A), the amino acids increased by 31% (day 0 versus day 300), and in the vessel with sludge (B), the total amino acids increased by 254 % (day 0 versus day 300). The difference was 223% at the end of the experiment. At the beginning of the experiment (day 0), the measured value of amino acids was 28 mg.l^{-1} in the with sludge variant and 29 mg.l^{-1} in the without sludge variant. The average total increase was 46 mg.l^{-1} for the with sludge variant and 9 mg.l^{-1} for the without sludge variant.

The release of nitrogen in the form of amino acids is due to two factors: firstly, passive exorption of the internal contents of the yeast, and secondly, the process of proteolysis itself. Endogenous autolysis of wine yeasts during maturation on lees primarily involves the excretion of nitrogenous compounds, e.g., as the amino acid content gradually increases during lees aging, Sur-lie wines (analyzed at different stages of aging) can consider to be a suitable matrix for testing the impact of yeast autolysis **[12]**. Based on the ongoing autolysis that naturally occurs in wine with lees, it is a good matrix that may be useful for detecting emerging correlations due to changes in amino acid concentrations or other substances that may interfere **[13]**, **[14]**.

Determination of the total quantity of polyphenolic compounds

Polyphenols are the most important and very interesting compounds in the oenological aspects of wine. These compounds come from different parts of the grapevine and are extracted and moved into the wine during grape

processing and aging. Polyphenols and their compounds are directly linked to the wine's final quality. They contribute to the organoleptic characteristics of the wine and influence the color of the wine. Polyphenols are important antioxidants in wine **[15]**.

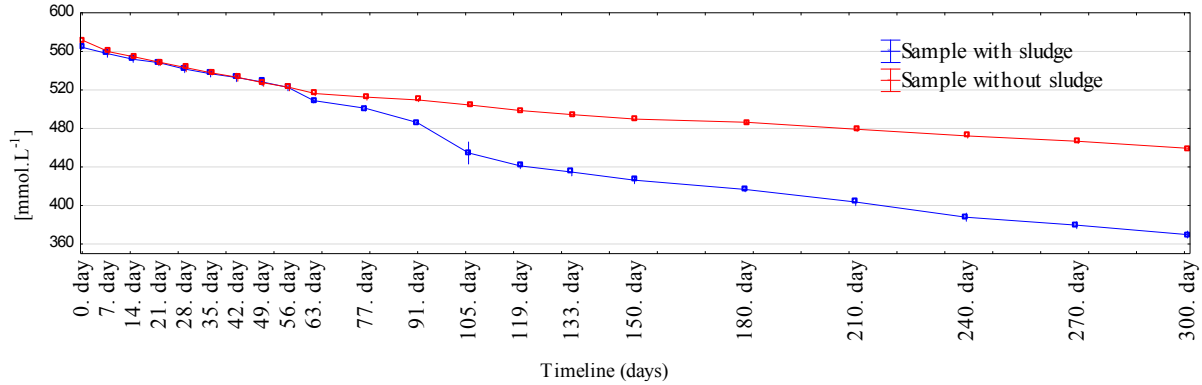


Figure 4 The measured values of total polyphenolic components of the Veltliner green variety, of both variants, with or without lees.

From Figure 4, at the end of the experiment (300 days), we can see an average value of 370 mmol.l^{-1} was measured for the sludge variant, whereas the value at the beginning was measured at 535 mmol.l^{-1}. In the without sludge variant, there was a more moderate decrease from a measured value of 550 mmol.l^{-1} at the beginning to a measured value of 459 mmol.l^{-1} at the end of the experiment.

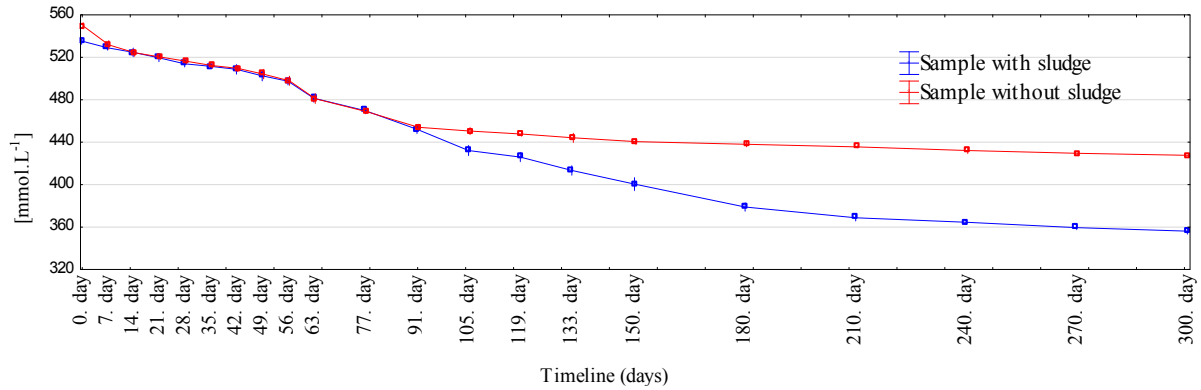


Figure 5 The measured values of total polyphenolic components in the Riesling variety of both variants, with or without lees.

Figure 5 shows that at the end of the experiment, the variant without sludge had an average measured value of 427 mmol.l^{-1} (556 mmol.l^{-1} at the beginning) and the variant with sludge had an average measured value of 587 mmol.l^{-1} at the beginning and 356 mmol.l^{-1} at the end of the experiment. Figure 5 shows a more moderate decrease observed in the variant without sludge (a 22% decrease in value at the end of the experiment compared to the first day). There was a more significant decrease of 33% in the sludge variant.

Figure 6 shows that at the beginning of the experiment (day 0), a high value of 587 mmol.l^{-1} was measured in the with sludge variant and 556 mmol.l^{-1} in the without sludge variant. The decrease in the total phenolic content of the with-sludge variant (B) was 34%; in the without sludge variant, there was a more moderate decrease of 22%. The average value, measured at the end of the experiment, in the with sludge variant, was 357 mmol.l^{-1}, and in the without sludge variant was 434 mmol.l^{-1}.

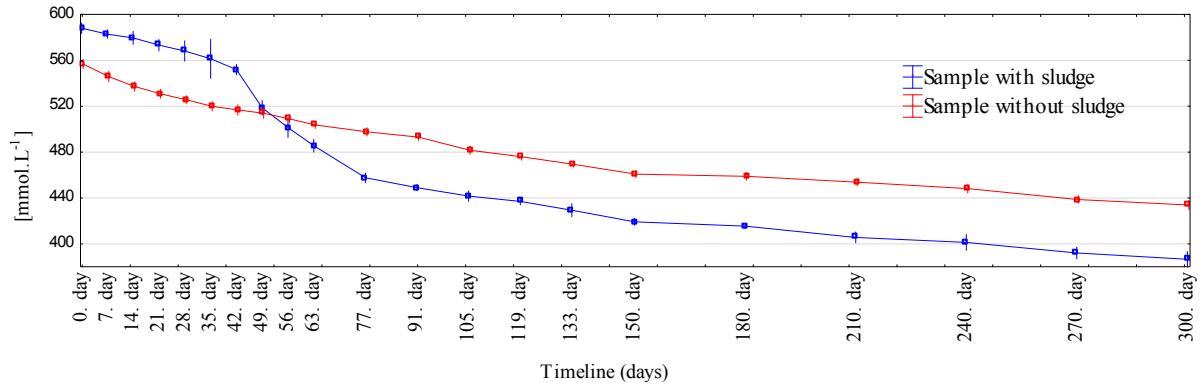


Figure 6 The measured values of total polyphenolic components in the Chardonnay variety of both variants, with or without lees.

The presence of lees protects the wine from oxidation, gives the wine its astringency, and increases the feeling of fullness in the mouth. This can be explained by the reaction of proanthocyanidins with compounds released by yeast autolysis, such as proteins and mannoproteins **[16]**, **[17]**. Figures 4-6 show the evolution of the total polyphenols during the experimental aging for variants with and without sludge over 300 days. The decrease in phenolics as the wine ages with lees could be due to the enzymatic activities of yeast and lactic acid bacteria from the lees **[18]**. Lees play an essential role in the aging of wine, mainly due to their ability to adsorb phenolic compounds **[19]**, **[20]** and release enzymes (after autolysis) that can modify the phenolic fraction **[14]**. Although wine polyphenols interact with yeast lees to a limited extent, such interactions have a significant impact on the reactivity of wine polyphenolic compounds and yeast lees with oxygen **[19]**.

Determination of antioxidant activity

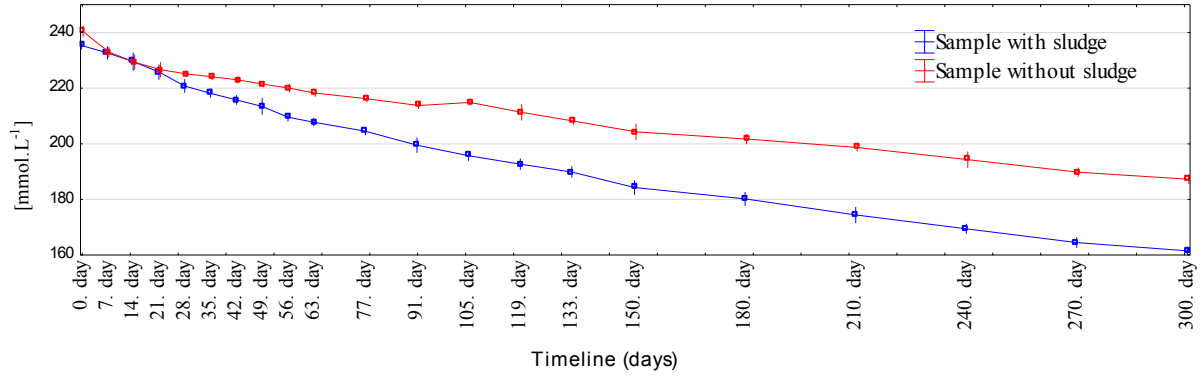


Figure 7 Development of the antioxidant activity in the Veltliner Green variety.

From Figure 7, we can see that the with sludge variant has a more significant decrease in antioxidant activity, of 32%, compared to the without sludge variant, which had a less marked reduction of only 20%. On experimental day 28, it can be seen that there was a very significant difference between the two variants of 5 mmol.l^{-1}, which steadily increased. By the end of the experiment, the value measured in the with sludge variant was 162 mmol.l^{-1} and the without sludge variant 187 mmol.l^{-1}.

Figure 8 shows that at the beginning of the experiment, the average value of antioxidant activity in the with-sludge variant (day 0) was 246 mmol.l^{-1}, and in the without sludge variant, it was 249 mmol.l^{-1}. At the end of the experiment (day 300), the average value in the with lees variant was 169 mmol.l^{-1} and in the without lees variant 198 mmol.l^{-1}.

From Figure 8, we can see that the without sludge variant exhibited a decrease in the antioxidant activity of 16%, but the variant with sludge exhibited a more significant drop of 32%. The average reduction in the value for the variant with sludge was 76 mmol.l^{-1}, and for the variant without sludge, 40 mmol.l^{-1}.

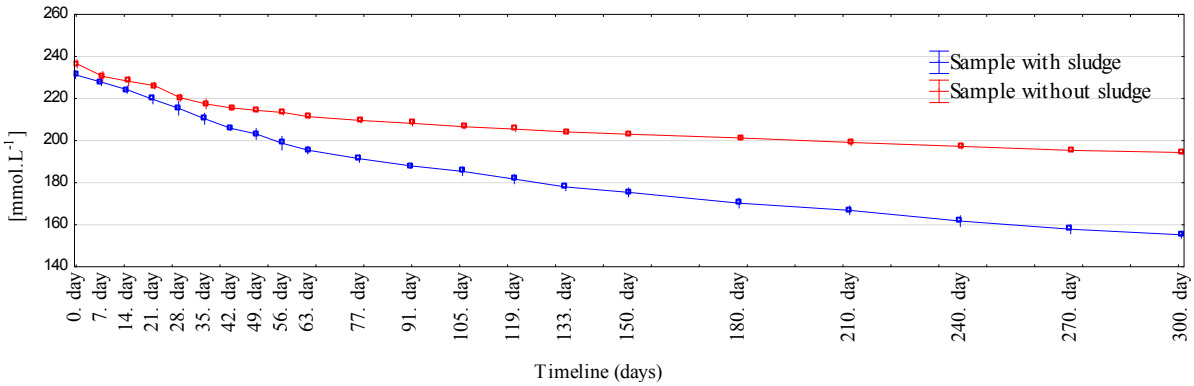


Figure 8 Development of antioxidant activity in the Riesling variety.

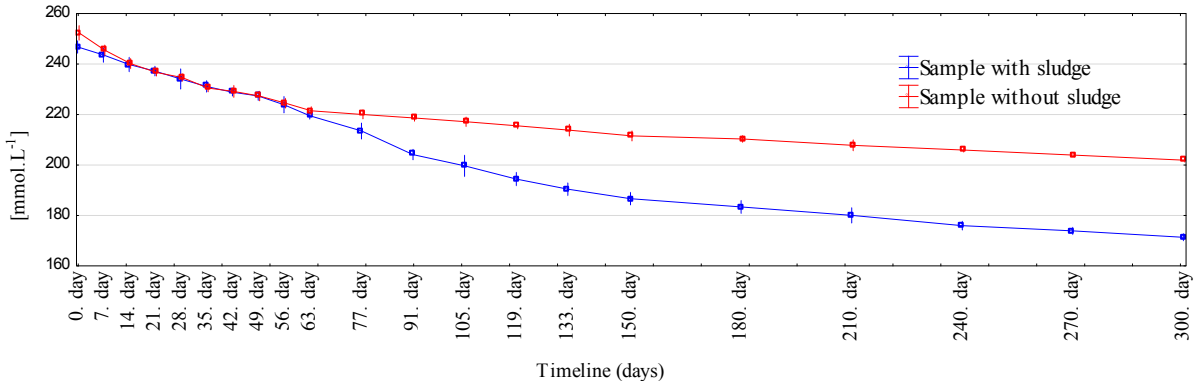


Figure 9 Development of antioxidant activity in the Chardonnay variety.

Figure 9 shows that at the beginning of the experiment, the average value of antioxidant activity in the with lees variant (day 0) was 235 mmol.l^{-1}; in the without lees variant, a value of 240 mmol.l^{-1} was measured. At the end of the experiment (day 300), the average value measured in the with lees variant was 161 mmol.l^{-1} and in the without lees variant 187 mmol.l^{-1}. Figure 9 shows an overall decrease in antioxidant activity in the without sludge variant of 19% and in the with sludge variant of 31%. The average overall reduction in the sludge variant was 70 mmol.l^{-1} and 50 mmol.l^{-1} for the without sludge variant.

Grapes are fruits with a high level of antioxidant activity **[21]**. However, after fermentation, phenolic compounds were lost, which are primarily responsible for antioxidant activity **[22]**, **[23]**. It can be seen from Figures 7-9 that the with sludge variants had a higher decrease in antioxidant activity than the without sludge variants. This can be explained by the fact that the sludge is mainly bound to polyphenols, which gradually oxidize **[24]**. This correlates with Figures 4-6, where a more significant decrease is observed for the sludge variant **[25]**.

Final evaluation

The tables and graphs below show the results of the measurement of total amino acid content, antioxidant activity values, and phenolic compounds at the beginning (immediately after fermentation – day zero) and after 300 days (the end of the experiment) for the three grape varieties divided into two types of containers.

Sensory evaluation of the strength and structure of the wines

The sensory analysis compared the different wine varieties after 300 days. A 10-point scale was selected for this evaluation to evaluate Aroma richness and intensity, flavor richness and intensity, aging potential, complexity, and balance.

Ageing the wine with lees and the associated autolysis of the yeast also impacted the wine's sensory characteristics.

Figure 12 shows, for the Chardonnay variety, that there was a statistically significant difference in all the evaluations between the two variants, but the most significant was in the Potential for maturation evaluation, which averaged 3.2 points. The smallest difference was for the Richness aroma and intensity evaluation, which was 1.2.

For Riesling, there was a statistically significant difference in the scores of all the evaluations of the two variants. The smallest difference was recorded for Richness aroma and intensity, only 0.6 points. However, the most statistically significant difference was recorded for the potential for maturation, which was 4.8 points.

The smallest difference between the two varieties was recorded for the Richness aroma and intensity evaluation, which was 0.4 points. The highest difference was recorded for the complexity descriptor in the variant with a level of 8.4.

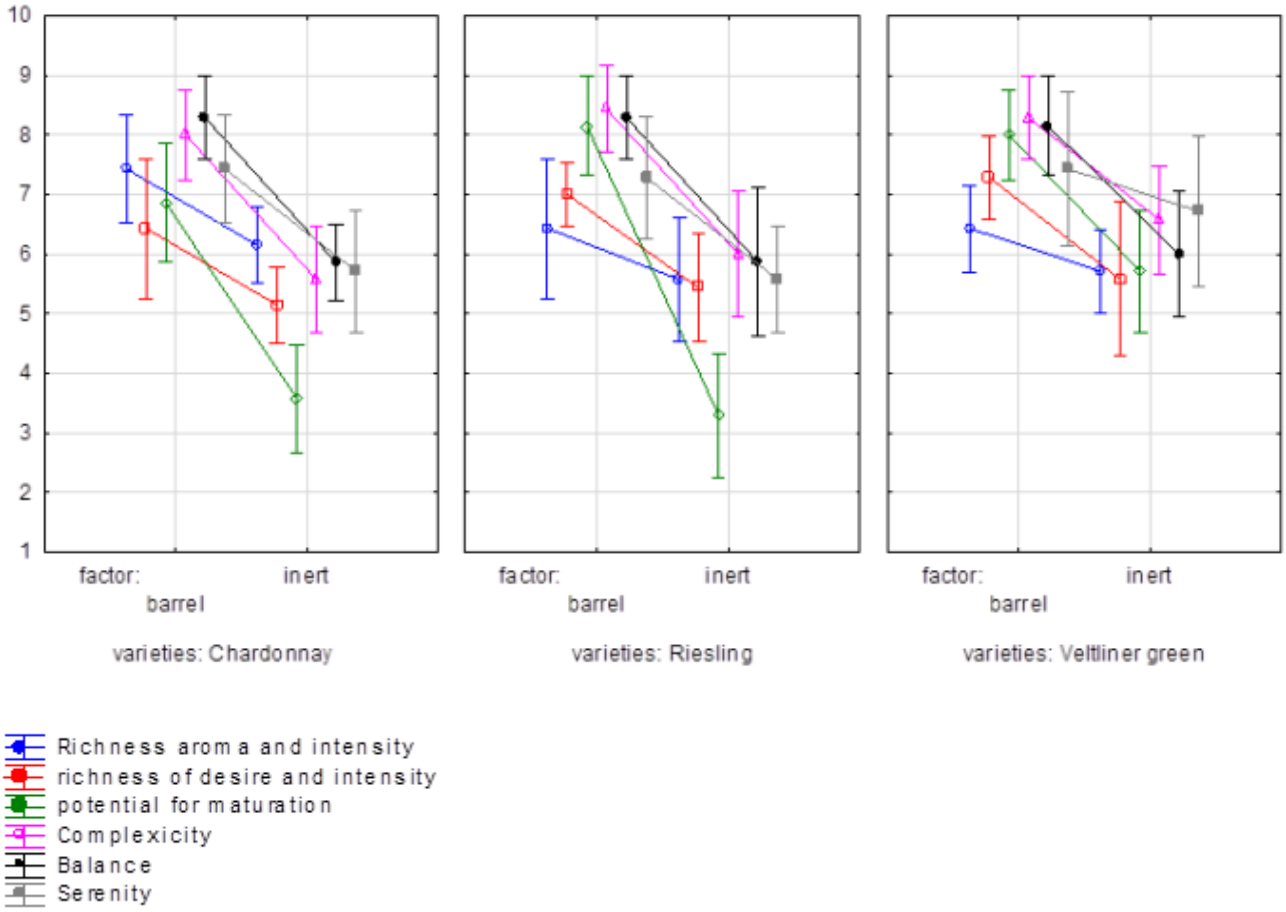


Figure 10 The strength and structure of wines.

All the wines aged on lees were perceived to be more robust, most likely due to interactions between the macromolecules released during cellular autolysis and the wine phenolics **[26]**, **[27]**. Fermentation lees have a high level of reactivity with salivary proteins and induce a feeling of astringency. However, in mannoproteins or polysaccharides, fermentation lees interact with them to form stable macrostructures that cannot react with salivary proteins, thus reducing the astringency **[28]**, **[29]**. Other authors report mannoproteins as active compounds that promote wine stability by reducing the particle size of aggregated tannins **[30]**, **[31]**. Therefore, this technique can be used to smooth these parameters during the aging of red wine. Along with the increased robustness that comes from aging with lees, the overall body and persistence of the wine improved for all varieties. In terms of color, all the wines aged on lees exhibited a greater degree of color intensity than the wines aged in inert containers without lees. This finding agrees with previous results suggesting that mannoproteins and cell wall polysaccharides protect anthocyanin monomers, making aging on lees a novel technique for color preservation **[32]**, **[33]**.

Based on the obtained results, it can be concluded that more biochemical processes took place in wines aged on lees compared to those aged without lees. It is important to note that many factors influence yeast autolysis's effects. The most important ones are temperature, yeast strain and population, ethanol content, vinification time on lees, and the pH of the wine **[34]**. When wines are vinified on lees, there is no need to sulphurise them, as the polyphenols protect them from oxidation through their own antioxidant activity. If wines are aged on lees, it is possible to have a very low sulfur dioxide content in the aged wine **[35]**, **[36]**, **[37]** describes wines, vinified on lees, as sophisticated, full-bodied wines. The sensory analysis of the wine confirmed this. During vinification, oak

lactones, furans, volatile phenols, and others may be released. In my opinion, these can interact with the compounds produced during autolysis. When vinifying on lees, it is also important to be aware of sulfur compounds that can adversely affect the sensory profile of the wine (hydrogen sulfide, mercaptans) **[38]**. In this context, it is important to pay attention to these compounds and take appropriate measures if they are present in the wine **[39]**, **[40]**. One option is to re-homogenize the lees with the wine. These compounds were not detected in the results of the sensory analysis. Otherwise, there are noticeable differences between wine produced with and without yeast lees **[41]**, **[42]**.

CONCLUSION

The experiment aimed to confirm the influence of yeast autolysis on the final composition of the wine. The experiment design was planned for a period when three grape varieties were available for wine production and bottling. Sensory analysis showed that wines that underwent autolysis were more robust, fuller, and harmonious and had a higher potential for archiving. On the other hand, in containers without lees, where autolysis has not taken place, the wines are lighter and fresher, more suitable for early consumption. The increase in amino acids was due to the decomposition of the yeast carcasses. Therefore, their concentration increased in the lees. Amino acids are precursors of aromatic substances, and therefore, wines aged on lees were fuller, more robust, and more harmonious. Polyphenols are antioxidants, and in the lees containers, they oxidized and protected the wine from complete oxidation. In inert containers, sulfur dioxide had to be used to keep the wine from oxidizing. The leaves are very important in the amount of dissolved oxygen in the wine. The experiment also determined the antioxidant activity of the wine. This correlated in all graphs with the polyphenol graphs. This is because polyphenols are the most abundant antioxidants in wine.

REFERENCES

1. Balík, J., & Stávek, J. (2017). Vinařská technologie. Národní vinařské centrum, ops.
2. Cebollero, E., & Gonzalez, R. (2006). Induction of Autophagy by Second-Fermentation Yeasts during Elaboration of Sparkling Wines. In Applied and Environmental Microbiology (Vol. 72, Issue 6, pp. 4121–4127). American Society for Microbiology. https://doi.org/10.1128/aem.02920-05
3. Kemp, B., Condé, B., Jégou, S., Howell, K., Vasserot, Y., & Marchal, R. (2018). Chemical compounds and mechanisms involved in the formation and stabilization of foam in sparkling wines. In Critical Reviews in Food Science and Nutrition (Vol. 59, Issue 13, pp. 2072–2094). Informa UK Limited. https://doi.org/10.1080/10408398.2018.1437535
4. Ma, T., Wang, J., Wang, H., Zhao, Q., Zhang, F., Ge, Q., Li, C., Gamboa, G. G., Fang, Y., & Sun, X. (2022). Wine aging and artificial simulated wine aging: Technologies, applications, challenges, and perspectives. In Food Research International (Vol. 153, p. 110953). Elsevier BV. https://doi.org/10.1016/j.foodres.2022.110953
5. Nunez, Y. P., Carrascosa, A. V., González, R., Polo, M. C., & Martínez-Rodríguez, A. J. (2005). Effect of Accelerated Autolysis of Yeast on the Composition and Foaming Properties of Sparkling Wines Elaborated by a Champenoise Method. In Journal of Agricultural and Food Chemistry (Vol. 53, Issue 18, pp. 7232–7237). American Chemical Society (ACS). https://doi.org/10.1021/jf050191v
6. Mina, M., & Tsaltas, D. (2017). Contribution of Yeast in Wine Aroma and Flavour. In Yeast - Industrial Applications. InTech. https://doi.org/10.5772/intechopen.70656
7. Pons-Mercadé, P., Giménez, P., Gombau, J., Vilomara, G., Conde, M., Cantos, A., Rozès, N., Canals, J.-M., & Zamora, F. (2021). Oxygen consumption rate of lees during sparkling wine (Cava) aging; influence of the aging time. In Food Chemistry (Vol. 342, p. 128238). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.128238
8. Gawel, R., Smith, P. A., Cicerale, S., & Keast, R. (2017). The mouthfeel of white wine. In Critical Reviews in Food Science and Nutrition (Vol. 58, Issue 17, pp. 2939–2956). Informa UK Limited. https://doi.org/10.1080/10408398.2017.1346584
9. Marchal, A., Marullo, P., Moine, V., & Dubourdieu, D. (2011). Influence of Yeast Macromolecules on Sweetness in Dry Wines: Role of the Saccharomyces cerevisiae Protein Hsp12. In Journal of Agricultural and Food Chemistry (Vol. 59, Issue 5, pp. 2004–2010). American Chemical Society (ACS). https://doi.org/10.1021/jf103710x
10. Pereira, C., Mendes, D., Dias, T., Garcia, R., da Silva, M. G., & Cabrita, M. J. (2021). Revealing the yeast modulation potential on amino acid composition and volatile profile of Arinto white wines by a combined chromatographic-based approach. In Journal of Chromatography A (Vol. 1641, p. 461991). Elsevier BV. https://doi.org/10.1016/j.chroma.2021.461991

11. Yılmaz, C., & Gökmen, V. (2021). Formation of amino acid derivatives in white and red wines during fermentation: Effects of non-Saccharomyces yeasts and Oenococcus oeni. In Food Chemistry (Vol. 343, p. 128415). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.128415
12. Mirás-Avalos, J. M., Bouzas-Cid, Y., Trigo-Córdoba, E., Orriols, I., & Falqué, E. (2019). Effects of Two Different Irrigation Systems on the Amino Acid Concentrations, Volatile Composition and Sensory Profiles of Godello Musts and Wines. In Foods (Vol. 8, Issue 4, p. 135). MDPI AG. https://doi.org/10.3390/foods8040135
13. Legras, J.-L., Moreno-Garcia, J., Zara, S., Zara, G., Garcia-Martinez, T., Mauricio, J. C., Mannazzu, I., Coi, A. L., Bou Zeidan, M., Dequin, S., Moreno, J., & Budroni, M. (2016). Flor Yeast: New Perspectives Beyond Wine Aging. In Frontiers in Microbiology (Vol. 7). Frontiers Media SA. https://doi.org/10.3389/fmicb.2016.00503
14. Ibern-Gómez, M., Andrés-Lacueva, C., Lamuela-Raventós, R. M., Buxaderas, S., Singleton, V. L., & de la Torre-Boronat, M. C. (2000). Browning of Cava (Sparkling Wine) During Aging in Contact with Lees Due to the Phenolic Composition. In American Journal of Enology and Viticulture (Vol. 51, Issue 1, pp. 29–36). American Society for Enology and Viticulture. https://doi.org/10.5344/ajev.2000.51.1.29
15. Kulkarni, P., Loira, I., Morata, A., Tesfaye, W., González, M. C., & Suárez-Lepe, J. A. (2015). Use of non-Saccharomyces yeast strains coupled with ultrasound treatment as a novel technique to accelerate ageing on lees of red wines and its repercussion in sensorial parameters. In LWT - Food Science and Technology (Vol. 64, Issue 2, pp. 1255–1262). Elsevier BV. https://doi.org/10.1016/j.lwt.2015.07.046
16. Thoma, V., Tampouris, K., & Petrou, A. L. (2008). Kinetics and Mechanism of the Reaction between Chromium(III) and 3,4-Dihydroxy-Phenyl-Propenoic Acid (Caffeic Acid) in Weak Acidic Aqueous Solutions. In Bioinorganic Chemistry and Applications (Vol. 2008, pp. 1–7). Hindawi Limited. https://doi.org/10.1155/2008/624583
17. Gutiérrez-Escobar, R., Aliaño-González, M. J., & Cantos-Villar, E. (2021). Wine Polyphenol Content and Its Influence on Wine Quality and Properties: A Review. In Molecules (Vol. 26, Issue 3, p. 718). MDPI AG. https://doi.org/10.3390/molecules26030718
18. Cerbu, M. I., Colibaba, C. L., Luchian, C., Zamfir, C.-I., Nechita, B. C., Cioroiu, I.-B., Niculaua, M., & Cotea, V. V. (2023). Effect of ageing on lees on the quality of white and rosé wines from Iași vineyard. In P. Roca (Ed.), BIO Web of Conferences (Vol. 56, p. 02022). EDP Sciences. https://doi.org/10.1051/bioconf/20235602022
19. Mazauric, J.-P., & Salmon, J.-M. (2005). Interactions between Yeast Lees and Wine Polyphenols during Simulation of Wine Aging: I. Analysis of Remnant Polyphenolic Compounds in the Resulting Wines. In Journal of Agricultural and Food Chemistry (Vol. 53, Issue 14, pp. 5647–5653). American Chemical Society (ACS). https://doi.org/10.1021/jf050308f
20. Tzachristas, A., Pasvanka, K., Calokerinos, A., & Proestos, C. (2020). Polyphenols: Natural Antioxidants to Be Used as a Quality Tool in Wine Authenticity. In Applied Sciences (Vol. 10, Issue 17, p. 5908). MDPI AG. https://doi.org/10.3390/app10175908
21. Zhu, F., Du, B., & Li, J. (2016). Aroma Compounds in Wine. In Grape and Wine Biotechnology. InTech. https://doi.org/10.5772/65102
22. Ortiz, J., Marín-Arroyo, M., Noriega-Domínguez, M., Navarro, M., & Arozarena, I. (2013). Color, Phenolics, and Antioxidant Activity of Blackberry (Rubus glaucus Benth.), Blueberry (Vaccinium floribundum Kunth.), and Apple Wines from Ecuador. In Journal of Food Science (Vol. 78, Issue 7). Wiley. https://doi.org/10.1111/1750-3841.12148
23. De Iseppi, A., Curioni, A., Marangon, M., Invincibile, D., Slaghenaufi, D., & Ugliano, M. (2023). Chemical and Electrochemical Assessment of Wine Lees Extracts Tested as Novel Antioxidant Additives in Model Wine. In Journal of Agricultural and Food Chemistry (Vol. 72, Issue 4, pp. 1969–1977). American Chemical Society (ACS). https://doi.org/10.1021/acs.jafc.3c00567
24. Buljeta, I., Pichler, A., Šimunović, J., & Kopjar, M. (2023). Beneficial Effects of Red Wine Polyphenols on Human Health: Comprehensive Review. In Current Issues in Molecular Biology (Vol. 45, Issue 2, pp. 782–798). MDPI AG. https://doi.org/10.3390/cimb45020052
25. Chassagne, D., Guilloux-Benatier, M., Alexandre, H., & Voilley, A. (2005). Sorption of wine volatile phenols by yeast lees. In Food Chemistry (Vol. 91, Issue 1, pp. 39–44). Elsevier BV. https://doi.org/10.1016/j.foodchem.2004.05.044
26. Cerbu, M. I., Colibaba, C. L., Luchian, C., Zamfir, C.-I., Nechita, B. C., Cioroiu, I.-B., Niculaua, M., & Cotea, V. V. (2023). Effect of ageing on lees on the quality of white and rosé wines from Iași vineyard. In P. Roca (Ed.), BIO Web of Conferences (Vol. 56, p. 02022). EDP Sciences. https://doi.org/10.1051/bioconf/20235602022

27. Rodriguez-Nogales, J. M., Fernández-Fernández, E., & Vila-Crespo, J. (2012). Effect of the addition of β-glucanases and commercial yeast preparations on the chemical and sensorial characteristics of traditional sparkling wine. In European Food Research and Technology (Vol. 235, Issue 4, pp. 729–744). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-012-1801-0
28. Tufariello, M., Fragasso, M., Pico, J., Panighel, A., Castellarin, S. D., Flamini, R., & Grieco, F. (2021). Influence of Non-Saccharomyces on Wine Chemistry: A Focus on Aroma-Related Compounds. In Molecules (Vol. 26, Issue 3, p. 644). MDPI AG. https://doi.org/10.3390/molecules26030644
29. Escot, S., Feuillat, M., Dulau, L., & Charpentier, C. (2001). Release of polysaccharides by yeasts and the influence of released polysaccharides on colour stability and wine astringency. In Australian Journal of Grape and Wine Research (Vol. 7, Issue 3, pp. 153–159). Hindawi Limited. https://doi.org/10.1111/j.1755-0238.2001.tb00204.x
30. Wang, C., Wang, C., Tang, K., Rao, Z., & Chen, J. (2022). Effects of Different Aging Methods on the Phenolic Compounds and Antioxidant Activity of Red Wine. In Fermentation (Vol. 8, Issue 11, p. 592). MDPI AG. https://doi.org/10.3390/fermentation8110592
31. Sawyer, S., Longo, R., Solomon, M., Nicolotti, L., Westmore, H., Merry, A., Gnoinski, G., Ylia, A., Dambergs, R., & Kerslake, F. (2021). Autolysis and the duration of ageing on lees independently influence the aroma composition of traditional method sparkling wine. In Australian Journal of Grape and Wine Research (Vol. 28, Issue 1, pp. 146–159). Hindawi Limited. https://doi.org/10.1111/ajgw.12527
32. Pollon, M., Bambina, P., Vitaggio, C., Cinquanta, L., & Corona, O. (2023). Sur lies élevage practice to modulate the features of red wines from calcareous soils with different textures. In Journal of the Science of Food and Agriculture. Wiley. https://doi.org/10.1002/jsfa.13104
33. Romero-Díez, R., Rodríguez-Rojo, S., Cocero, M. J., Duarte, C. M. M., Matias, A. A., & Bronze, M. R. (2018). Phenolic characterization of aging wine lees: Correlation with antioxidant activities. In Food Chemistry (Vol. 259, pp. 188–195). Elsevier BV. https://doi.org/10.1016/j.foodchem.2018.03.119
34. Wang, X., Fan, G., Peng, Y., Xu, N., Xie, Y., Zhou, H., Liang, H., Zhan, J., Huang, W., & You, Y. (2023). Mechanisms and effects of non-Saccharomyces yeast fermentation on the aromatic profile of wine. In Journal of Food Composition and Analysis (Vol. 124, p. 105660). Elsevier BV. https://doi.org/10.1016/j.jfca.2023.105660
35. Vázquez, J., Mislata, A. M., Vendrell, V., Moro, C., de Lamo, S., Ferrer-Gallego, R., & Andorrà, I. (2023). Enological Suitability of Indigenous Yeast Strains for 'Verdejo' Wine Production. In Foods (Vol. 12, Issue 9, p. 1888). MDPI AG. https://doi.org/10.3390/foods12091888
36. Crespo, J., García, M., Arroyo, T., Romero, V., & M. Cabellos, J. (2023). Effect of aging on lees on the volatile profile of Malvasia aromatica wines fermented with Saccharomyces native yeasts in PDO "Vinos de Madrid." In New Advances in Saccharomyces [Working Title]. IntechOpen. https://doi.org/10.5772/intechopen.1003667
37. Romano, P., Braschi, G., Siesto, G., Patrignani, F., & Lanciotti, R. (2022). Role of Yeasts on the Sensory Component of Wines. In Foods (Vol. 11, Issue 13, p. 1921). MDPI AG. https://doi.org/10.3390/foods11131921
38. Gonzalez, R., & Morales, P. (2021). Truth in wine yeast. In Microbial Biotechnology (Vol. 15, Issue 5, pp. 1339–1356). Wiley. https://doi.org/10.1111/1751-7915.13848
39. Palomero, F., Morata, A., Benito, S., Gonzalez, M., & Suarezlepe, J. (2007). Conventional and enzyme-assisted autolysis during ageing over lees in red wines: Influence on the release of polysaccharides from yeast cell walls and on wine monomeric anthocyanin content. In Food Chemistry (Vol. 105, Issue 2, pp. 838–846). Elsevier BV. https://doi.org/10.1016/j.foodchem.2007.01.062
40. Pollon, M., Bambina, P., Vitaggio, C., Cinquanta, L., & Corona, O. (2023). Sur lies élevage practice to modulate the features of red wines from calcareous soils with different textures. In Journal of the Science of Food and Agriculture. Wiley. https://doi.org/10.1002/jsfa.13104
41. Raveendran, S., Parameswaran, B., Ummalyma, S. B., Abraham, A., Mathew, A. K., Madhavan, A., Rebello, S., & Pandey, A. (2018). Applications of Microbial Enzymes in Food Industry. In Food Technology and Biotechnology (Vol. 56, Issue 1). Faculty of Food Technology and Biotechnology - University of Zagreb. https://doi.org/10.17113/ftb.56.01.18.5491
42. Pinu, F. R., Stuart, L., Topal, T., Albright, A., Martin, D., & Grose, C. (2023). The Effect of Yeast Inoculation Methods on the Metabolite Composition of Sauvignon Blanc Wines. In Fermentation (Vol. 9, Issue 8, p. 759). MDPI AG. https://doi.org/10.3390/fermentation9080759

Funds:

This paper was supported by the project "Research Infrastructure for Young Scientists, co-financed by Operational Programme Research, Development and Education" (CZ.02.1.01/0.0/0.0/16_017/0002334) and by the project "Study of the Effect of Yeast Autolysis on the Composition and Aromatic Profile of Wine" (IGA-ZF/2022-SI2-002).

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the authors.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Jan Mikuš**, Mendel University in Brno, Faculty of Horticulture, Department of Viticulture and Enology, Valtická, 531, 691 44, Lednice, Czech Republic,
Tel.: +420 724327902
E-mail: xmikus@mendelu.cz
ORCID: https://orcid.org/0000-0003-2582-1713

Jiří Sochor, Mendel University in Brno, Faculty of Horticulture, Department of Viticulture and Enology, Valtická, 531, 691 44, Lednice, Czech Republic,
Tel.: +420 777 648 937
E-mail: jiri.sochor@mendelu.cz
ORCID: https://orcid.org/0000-0001-7823-1544

Štefan Ailer, Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Institute of Horticulture, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia,
Tel.: +421 37 641 5804
E-mail: stefan.ailer@uniag.sk
ORCID: https://orcid.org/0000-0001-8447-5377

Mojmír Baroň, Mendel University in Brno,Faculty of Horticulture, Department of Viticulture and Enology, Valtická, 531, 691 44, Lednice, Czech Republic,
Tel.: +420 519 367 252
E-mail: mojmir.baron@mendelu.cz
ORCID: https://orcid.org/0000-0003-1649-0537

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 11.7.2023
Revised: 7.1.2024
Accepted: 9.2.2024
Published: 19.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 109-121
https://doi.org/10.5219/1898
ISSN: 1337-0960 online
www.potravinarstvo.com

Characteristics of probiotic glutinous rice tapai with the addition of *Lactobacillus plantarum* 1 RN2-53 and some natural dyes

Yusmarini, Shanti Fitriani, Vonny Setiaries Johan, Netti Herawati, Irhamdi, Emma Riftyan, Usman Pato

ABSTRACT

Tapai is a traditional Indonesian food usually made from cassava and glutinous rice. The study aims to determine natural dyes that produce the highest quality probiotic glutinous rice tapai. The study was conducted experimentally using a complete randomized design consisting of four treatments, and each treatment was repeated four times. The treatment in this study was the soaking of glutinous rice into natural dye extracts, namely red dragon fruit extract, purple sweet potato extract, and suji leaf extract, with different concentrations, namely 10%, 15%, 20%, and 25%. The data obtained were statistically analyzed using variance analysis and continued with Duncan's new multiple-range test (DNMRT) at a level of 5%. The results showed that natural dye extracts with different concentrations have a noticeable influence on total lactic acid, alcohol content, total lactic acid bacteria, antioxidant activity, and sensory characteristics of color, aroma, taste, and hardness. The soaking treatment in red dragon fruit extract and purple sweet potato extract with a concentration of 20% resulted in glutinous rice tapai, preferred by panelists. Furthermore, the characteristic pH value of 3.35-3.41, total lactic acid 0.61-0.70%, alcohol content 0.33-0.42%, total lactic acid bacteria 9.11-9.40 CFU/mL, and antioxidant activity 167.35-102.51 ppm.

Keywords: Glutinous rice tapai, Probiotics, *Lactobacillus plantarum* 1 RN2-53, natural dyes

INTRODUCTION

Tapai is a traditional Indonesian food made from high-carbohydrate ingredients such as glutinous rice and cassava, with a fermentation process involving tapai yeast. Tapai can also be manufactured using a mixture of yeast and lactic acid bacteria (LAB). Khasanah and Prima **[1]** added *Lactobacillus plantarum* B1765 as much as 10^5 CFU/g with a fermentation time of 48 hours in making cassava tapai. Other studies were conducted by Yusmarini et al. **[2]** with a mixture of yeast and some types of BAL strain *Lactobacillus plantarum* 1 RN2-53 to manufacture white glutinous rice tapai. The results showed that tapai, with the addition of *Lactobacillus plantarum* 1 RN2-53, was preferred by panelists because of its physical properties and better quality. The resulting white glutinous rice tapai has a less attractive color, yellowish-white, so improving sensory quality and increasing consumer preferences can be done by adding dyes.

The use of natural color is prevalent in the food processing process in Indonesia. Natural dyes are pigments obtained from plants, animals, or mineral sources. Natural dyes have long been used for food coloring, and until recently, their use was considered safer than synthetic dyes. Some agricultural products are often used as natural dyes in food, including red dragon fruit, purple sweet potato, and suji leaves. These commodities have a striking color, so if used in food products, they will improve sensory quality, especially appearance. Besides being easy to get, red dragon fruit, purple sweet potato, and suji leaves contain antioxidant compounds. According to

Nataliani et al. **[3]**, Red Dragon Fruit Extract has an antioxidant activity of 87.11% heating process, and extended storage room temperature and cold temperature will cause a decrease in antioxidant activity.

Another plant that can also be used as a natural dye is the purple sweet potato, which has anthocyanin compounds that give it an intense color, and the compound is also an antioxidant. Based on the research of Husna et al. **[4]**, concentrated purple sweet potatoes have an anthocyanin content of 61.85 mg/100g, while light purple sweet potatoes have 3.51 mg/100g. Another natural dye often used is suji leaves, which contain the natural green chlorophyll pigment. It is one of the compounds that are antioxidants and have a total chlorophyll content of 3,773.9 ppm consisting of chlorophyll a of 2,523.6 ppm and chlorophyll b of 1,250.3 ppm **[5]**. Several studies have used natural dyes to manufacture food products, including those reported by Putri et al. **[6]**, which state that red dragon juice by as much as 15% can increase panelists' liking for yogurt. According to Yusmarini et al. **[7]**, natural dyes from purple sweet potatoes, red dragon fruit, and suji leaves with a 10-25% concentration to manufacture cassava tapai. The study's results showed that adding natural dyes gave a more attractive appearance to the cassava tapai color. The study aims to determine natural dyes that produce the highest quality probiotic glutinous rice tapai.

Scientific Hypothesis

The addition of natural coloring extracts (dragon fruit, purple sweet potato, and suji leaves) significantly affected the quality and antioxidant content of probiotic glutinous rice tapai.

MATERIAL AND METHODOLOGY

Samples

This study was conducted at Universitas Riau, Processing of Agricultural Products Laboratory and Agricultural Products Analysis Laboratory in Pekanbaru. Natural dye materials include red dragon fruit, purple sweet potato, and suji leaves. The researchers developed tapai from white glutinous rice.

Chemicals

The chemicals used consist of deMan rugosa sharp (MRS) broth and agar (Merck), NaCl (Merck), NaOH (Merck), alcohol, distilled water, phenolphthalein indicator, and 1,1-diphenyl-2-picryl hydroxyl solution (Sigma).

Animals, Plants, and Biological Materials

Lactobacillus plantarum 1 RN2-53 (personal collection of Dr. Yusmarini) and instant yeast (*Saccharomyces cerevisiae*) used a commercial brand Fermipan.

Instruments

The equipment comprises glassware, test tubes, Petri dishes, erlenmeyer, drip pipettes, stirrers, measuring cups, and cups. Other tools consist of digital scales (Shimadzu), pH meters (Loviband), autoclaves (All American), laminar airflow (Elisa), incubators (Memmert), vortex-mixers (Taiyo s-100), micropipettes (Soccorex), hot plates, bunsen lamps, and tips.

Laboratory Methods

Parameters were observed as pH value, total lactic acid, alcohol level, total LAB, total yeast, antioxidant activity, and sensory properties. Sensory testing includes a descriptive test to describe the tapai produced and a hedonic test to see the panelist's favorite response to tapai. The panelists for the descriptive test were 30 people; for the hedonic test, 80 were students of the Faculty of Agriculture, University of Riau, aged 20-23 years. The panelists tested each booth to avoid bias. At each sample change, the panelists neutralized their taste by drinking mineral water and their sense of smell by resting for ±10 seconds.

pH value: Determination of the degree of acidity (pH) refers to **[8]**. The pH value was measured using a pH meter. The electrodes are rinsed with distilled water and dried with a tissue. The pH meter tool is calibrated first using a buffer solution of 7.0 and 4.0 left to stabilize. The electrode on the pH meter is rinsed with distilled water. A Tapai sample of as much as 10 g was then crushed, and 100 ml of distilled water was stirred until homogeneous. Measurement of the sample by dipping the electrode into the sample and leaving it until a stable reading is obtained.

Total Lactic Acid: Determination of total acid content was carried out by titration, referring to **[9]**. As much as 10 mL of glutinous rice solution was put into the Erlenmeyer. Then, three drops of 1% phenolphthalein indicator were added to the sample and titrated with 0.1 N NaOH solution until the end point of the titration was reached, i.e., the formation of a pink color. Total acid (w/v) is calculated as percent acid lactate with the following formula:

$$Lactic\ Acid\ (\%) = \frac{V_{NaOH} \times N_{NaOH} \times {}^{90}/_{1000}}{V\ sample} \times 100$$

Alcohol Level: Measurement of alcohol content refers to **[10]**. Tapai 10 g was weighed, put into an Erlenmeyer, added with 50 mL distilled water, and added 1% phenolphthalein indicator as much as three drops. The sample is stirred and titrated with NaOH until the tapai solution turns pink. After When the titration changes color, stop and then look at the volume of the NaOH solution. Then the amount is used to calculate the alcohol content contained in tapai. Alcohol level (w/v) is calculated as percent alcohol with the following formula:

$$\textit{Alcohol Level}\ (\%) = \frac{a \times M \times Mr\ C_2H_5OH}{\textit{Fermentation Time}} \times 100$$

Where:
a = V NaOH; M = Concentration of NaOH (0,1 M); Mr = Mass Relative C_2H_5OH.

Antioxidant Activity: Analysis of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) refers to **[11]**. The sample is 0.5 g extracted in 5 mL of 95% ethanol and allowed to stand for 24 hours in a dark room. The extract was taken as much as 1.3 mL and reacted with 5 ml of ethanol 95% and 5 mL of DPPH solution prepared by dissolving 0.0001 g of DPPH in 100 mL of 95% ethanol. The samples were then incubated in a dark place for 30 minutes, and then the absorbance was measured at a wavelength of 517 nm. Antioxidant activity is expressed as % inhibitor, which is formulated as follows:

$$\%\ \textit{Inhibitor} = \left[{}^{(A_B - A_B)}/_{AB}\right] \times 100$$

Where:
A_A = Sample Absorbance; A_B = Control Absorbance.

Total Lactic Acid Bacteria: The procedure for calculating the total number of LAB refers to **[12]** – a microbiological test using the spread method (spread surface plate). Calculating the amount of LAB was carried out by taking 1 mL of tapai liquid sample using a micropipette, then put into 9 mL of 0.85% physiological saline for a 10^{-1} dilution and continued until the 10^{-8} dilution. Taken 0.1 mL from the 10^{-6} dilution to 10^{-8} for inoculating on MRS Agar medium by dropping the sample in a petri dish containing MRS Agar, and the sample is leveled over the entire surface with a hockey stick that has been sterilized by burning over a Bunsen burner. Inoculation was carried out in laminar flow. The inoculated Petri dishes were then incubated in the incubator for 24 hours at a temperature of 37 ºC in reverse to avoid water droplets that may adhere to the inner wall of the cup lid. Colony The growing LAB was counted directly. Total LAB is expressed in logs CFU/mL.

Description of the Experiment

Sample preparation: The sample preparation for probiotic glutinous rice tapai with natural dyes can be seen in Figure 1.

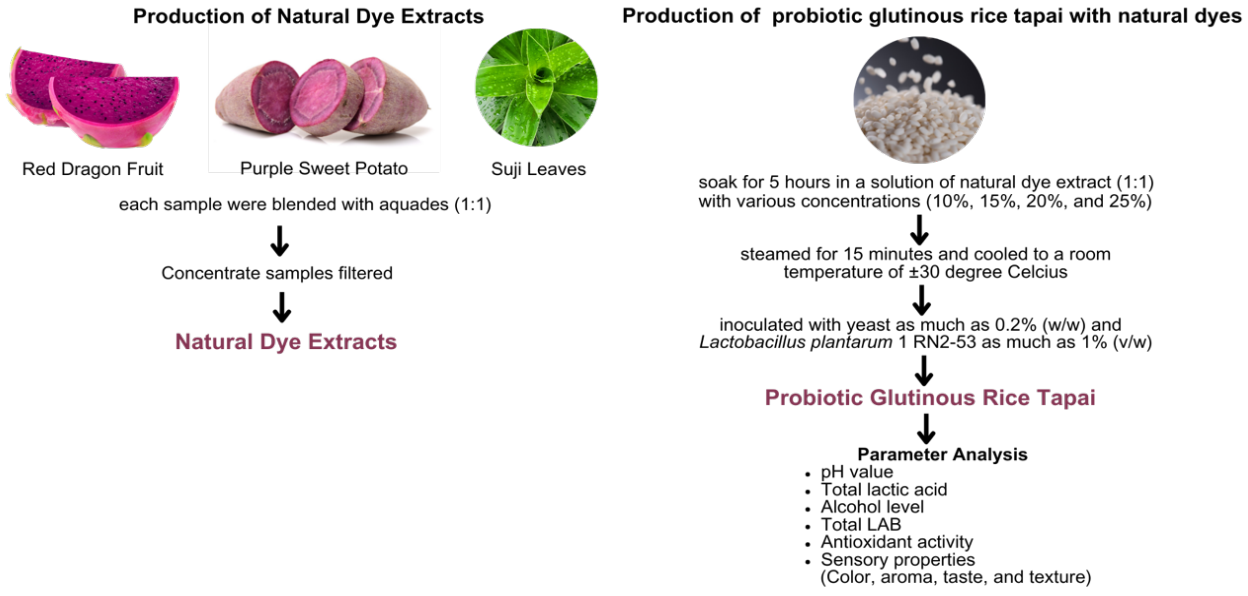


Figure 1 Production of natural dye extract and Probiotic glutinous rice tapai.

Production of natural dye extracts: The red dragon fruit, purple sweet potato, and suji leaves were washed clean. The flesh of samples was weighed as much as 500 g, cut into pieces to reduce the size, and then mashed using a blender by adding 500 mL of water (the ratio of ingredients and water is 1:1). Samples were filtered with a cloth for extraction.

Production of probiotic glutinous rice tapai with natural dyes: Probiotic glutinous rice tapai manufacture refers to **[2]** with minor modifications. The first step in making glutinous rice tapai is to weigh 500 g of glutinous rice, then wash and soak it for five hours in a solution of natural dye extract with various concentrations, namely 10%, 15%, 20%, and 25%. Comparison of glutinous rice and natural dye extract 1:2. Furthermore, glutinous rice is steamed for 15 minutes, and the glutinous rice that is still warm is soaked into a natural dye extract solution with a ratio of glutinous rice and a 1:1 dye extract solution. Soaking is carried out for 15 minutes. The soaked glutinous rice is then steamed for 15 minutes and cooled to a room temperature of ±30 °C. Glutinous rice is then inoculated with yeast as much as 0.2% (w/w) and *Lactobacillus plantarum* 1 RN2-53 as much as 1% (v/w). Glutinous rice inoculated starter is stirred thoroughly and incubated for 48 hours at ±30 °C.

The number of samples analyzed: We analyzed 48 samples, where three natural dyes were red dragon fruit extract, purple sweet potato extract, and suji leaf extract, and the four concentrations used were 10%,15%, 20%, and 25% (v/v). Each treatment was repeated for four replications.

Number of repeated analyses: All measurements of instrument readings were performed two times (Duplo).

Number of experiment replications: Each treatment was repeated four replications.

Design of the experiment: The study was conducted experimentally using a complete randomized design (CRD) with a two-factor experiment where Factor I: three natural dyes (red dragon fruit extract, purple sweet potato extract, and suji leaf extract) are applied with Factor II: four concentration (10%, 15%, 20% and 25%). This is a complete two-factor experiment with 4 x 3 = 12 combinations of the two factors (type of natural dyes and concentration). Analysis parameters are conducted with a CRD two-factor experiment, including pH, total LAB, and total alcohol. Hence, antioxidant, activity antioxidant, and sensory parameters were analyzed as descriptive.

Statistical Analysis

The results of chemical parameters, including pH and total alcohol, were analyzed by SPSS software (version 23) through one-way analysis of variance (ANOVA). Further testing was conducted using the Duncan New Multiple Range Test (DNMRT) at the confidence level of 5% (p <0.05), level to determine differences in each treatment if $Fcount \geq Ftable$.

RESULTS AND DISCUSSION

pH Value of Probiotic Glutinous Rice Tapai

A decreased pH value or degree of acidity in glutinous rice tapai indicates that yeast and lactic acid bacteria have been added to the fermentation process. The addition of natural dye extracts with different concentrations undeniably influences the pH value of glutinous rice tapai, as shown in Figure 1.

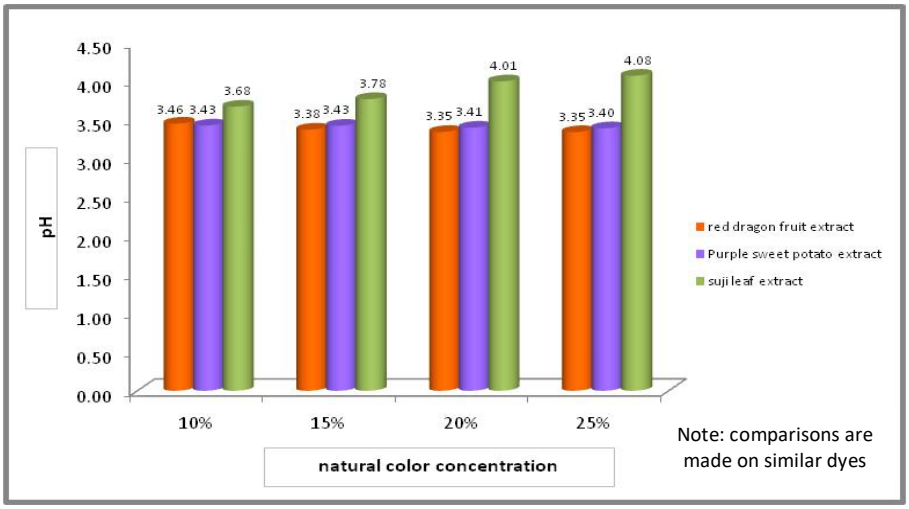


Figure 2 pH value of probiotic glutinous rice tapai.

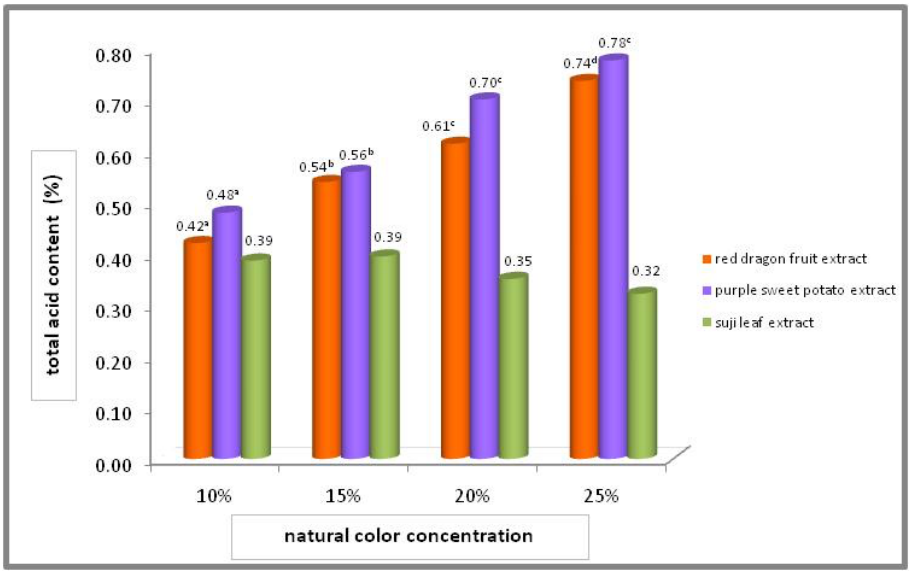
Figure 2 shows that the pH value of probiotic glutinous rice tapai tends to be the same for all natural dye concentration treatments added. Based on the ANOVA test results, it is known that the pH analysis between

factors does show no significance (p >0.05), as well as within factor I (type of natural dyes) and factor II (concentration) shows no significant results (p >0.05). The average pH value of glutinous rice tapai for natural dyes from red dragon fruit extract, purple sweet potato extract, and suji leaf extract, respectively, is between 3.35-3.46, 3.40-3.43, and 3.67-4.08. The resulting glutinous rice tapai has a low pH and an acidic taste. During the fermentation process, there is an overhaul of starch compounds by yeast and *L. plantarum* 1 RN-53 into organic acids, which causes a decrease in pH values. The *L. plantarum* 1 RN-53 used is amylolytic and can convert starch into simple sugars, which are then converted into organic acids. Adding *L. plantarum* 1 RN-53 will accelerate the decomposition of starch into organic acids. Yusmarini et al. **[2]** added *L. plantarum* 1 RN-53 resulted in a lower pH value than the treatment only added with yeast. The study indicates that adding *L. plantarum* 1 RN-53 will speed up fermentation. According to Yusmarini et al. **[13]**, *L. plantarum* 1 RN-53 is an amylolytic lactic acid bacterium. The decrease in pH value is also triggered by natural dye extracts that can be used as an energy source for yeast and *L. plantarum* 1 RN-53.

The pH value of the tapai sticky rice produced in this study was not too much different from the tapai white sticky rice added with dragon fruit extract as a colorant as a result of research by Isnaini et al. **[14]**, which ranged from 3.27 to 3.57, as well as the pH value of the tapai probiotics reported by Dede et al. **[15]** of 3.62.

Total Lactic Acid Tapai Glutinous Rice Probiotics

Total lactic acid is inversely proportional to the pH value. If high in total lactic acid, the pH value of the glutinous rice tapai produced will be lower. The total lactic acid of glutinous rice tapai is presented in Figure 2.



The different letters listed with the mean values in the graph represent statistically significant differences between the observed varieties (p <0.05).

Figure 3 Total lactic acid bacteria (LAB) tapai glutinous rice probiotics.

According to ANOVA results, it is known that the total LAB analysis between factors does show no significance (p >0.05), however, factor II (concentration of natural dyes) shows significant results (p <0.05). The data in Figure 2 shows that the more natural dye extracts derived from the red dragon fruit and purple sweet potatoes, the higher the total lactic acid produced, but this is not the case with suji leaf extract. Red dragon fruit extract and purple sweet potato extract are better able to provide substrates containing nutrients essential for yeast and LAB growth. These results align with the studies reported by Yusmarini et al. **[7]**, which state that cassava tapai with red dragon fruit extract and purple sweet potato extract has a higher total lactic acid than the addition of suji leaf extract. According to Nurul and Asmah **[16]**, red dragon fruit contains 12.97% of these carbohydrates containing glucose and fructose, which can be used by yeast and *L. plantarum* 1 to grow, multiply, and produce acid. Kakade et al. **[17]** state that red dragon fruit contains glucose of 5.70% and fructose of 3.20%. Kurnianingsig et al. **[18]** stated that purple sweet potatoes originating from the island of Java contain carbohydrates about 31.36-39.39% of 100 g. In addition, purple sweet potatoes also contain fairly complete amino acids needed by microbes to grow and multiply. Suji leaves contain more carbohydrates in the form of dietary fiber. According to Murtini et al. **[19]**, powdered suji leaves contain 42% dietary fiber, both soluble and insoluble, and generally cannot be utilized by LAB.

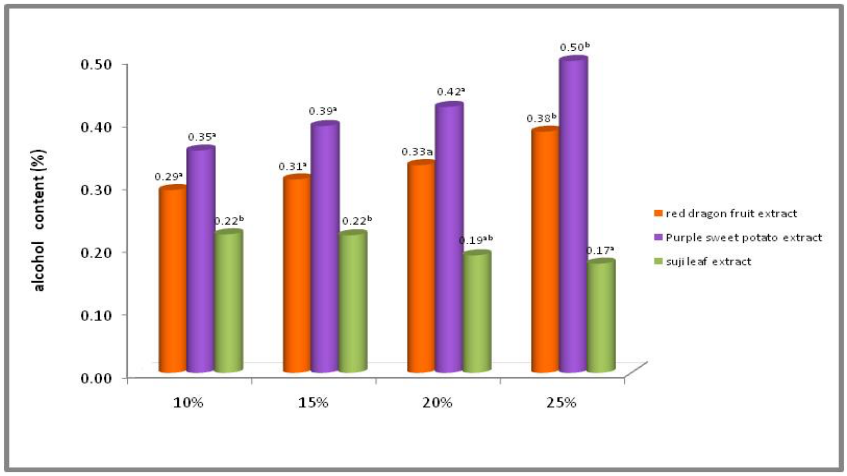
The total lactic acid of glutinous rice tapai soaked with red dragon fruit extract and purple sweet potato at a concentration of 20 to 25% ranged from 0.61-0.78% higher than the glutinous rice tapai reported by Kurniawan

et al. **[20]** and Yusmarini et al. **[2]** which was around 0.5%. Dede et al. **[15]** stated that sticky rice made by adding *Lactobacillus rhamnosus* SKG 34 had a total acidity of 0.57%. Based on the data presented, it can be seen that the addition of natural coloring extracts in the form of extracts of red dragon fruit and purple sweet potato can increase the total acid in tapai.

Total Alcohol Tapai Glutinous Rice Probiotics

Alcohol is one of the compounds produced during the process of making tapai. The more the use of natural dye extracts, there is tendency to increase alcohol levels in glutinous rice tapai, especially in the treatment with which red dragon fruit extract and purple sweet potato extract are added. Alcohol levels of glutinous rice tapai are presented in Figure 3.

The alcohol content in probiotic glutinous rice tapai ranges from 0.17-0.50%. The alcohol content in the treatment soaking with red dragon fruit extract and purple sweet potato was higher than in the treatment with suji leaf extract. The higher the concentration of natural dye extract used, the higher the alcohol content increases. It is closely related to the content of nutrients available to microorganisms used to manufacture tapai, especially yeast. Khamir plays a role in producing alcohol. Walker and Stewart **[21]** state that glucose, sucrose, fructose, maltose, and maltotriose are sugars that can be fermented into ethanol and carbon dioxide by Saccharomyces cerevisiae. According to Kurniawan et al. **[20]**, yeast will produce the zymase enzyme, which can break down sucrose into glucose and fructose, and the invertase enzyme, which plays a role in converting glucose into bioethanol. The lactic acid bacteria *L. plantarum* RN2-53 added to making tapai can help yeast decompose starch into glucose to produce alcohol. This result was approved by Yusmarini et al. **[2]**, who stated that yeast and lactic acid bacteria during fermentation are symbiotic mutualisms in utilizing the carbon source contained in the substrate. Amylolytic LAB hydrolyzes starch into simple sugars to produce lactic acid, while yeast will convert it into alcohol.



The different letters listed with the mean values in the graph represent statistically significant differences between the observed varieties (p <0.05).

Figure 4 Total alcohol tapai glutinous rice probiotics.

Based on the results of the ANOVA test, it is known that the overall LAB analysis between factors shows no significance (p >0.05). Still, factor I (type of natural dyes) shows significant results (p <0.05). The alcohol content of probiotic glutinous rice tapai with natural dye extract of red dragon fruit and purple sweet potato is higher than that of glutinous rice tapai. In the previous study Yusmarini et al. **[2]**, it is 0.22% but lower than the results of the study reported by Berlian et al. **[22]**, which is 0.58%, and the alcohol content reported by Marniza et al. **[23]** is 1.59%. Alcohol is produced by yeast, which is found in tapai yeast. The more yeast added and the longer the fermentation time, the higher the alcohol content of the tapai. Anisa et al. **[24]** stated that tapai made with 1% yeast produced much higher alcohol than tapai made with 0.25% yeast. Furthermore, it was stated by Marniza et al. **[23]** that the alcohol (ethanol) content of black sticky rice increased from 0.8% at 24 hours of incubation to 3.19% at 72 hours of incubation. This alcohol content is still classified as safe for consumption based on the Fatwa of the Indonesian Ulema Council **[25]**, which states that food and beverage products included in the khamr category or haram consumed are those that contain more than 0.5% alcohol.

Total Lactic Acid Bacteria

The calculation of total lactic acid bacteria (LAB) aims to determine the ability of *L. plantarum* 1 RN2-53 to grow and multiply together with yeast in making glutinous rice tapai soaked with natural dyes. The total LAB is presented in Table 1. The total LAB in the treatment soaked with red dragon fruit extract was 8.83-9.21 CFU/g, soaking in purple sweet potato extract was 8.99-9.53 CFU/g, and soaking in suji leaf extract was 8.17-8.84 CFU/g. Using natural dye extract from red dragon fruit and purple sweet potato does not inhibit the growth of *L. plantarum* 1 RN2-53. The result can be seen from the relatively high amount of LAB after glutinous rice is fermented for 48 hours at room temperature. The study results show that total LAB in the soaking treatment with red dragon fruit extract and purple sweet potato is higher than that with suji leaf extract, especially at 15%, 20%, and 25%. The extracts of red dragon fruit and purple sweet potatoes provide nutrients for the growth of LAB. The higher the concentration of natural dye extracts, the more LAB increases. It is proven that the natural dye extracts, in addition to improving the color of glutinous rice tapai, can also increase the amount of LAB.

Table 1 Total lactic acid bacteria of tapai glutinous rice probiotics.

Natural color concentration	Red dragon fruit extract (CFU/g)	Purple sweet potato Extract (CFU/g)	Suji leaf Extract (CFU/g)
10%	8.83	8.99	8.84
15%	9.10	9.10	8.68
20%	9.11	9.40	8.57
25%	9.21	9.53	8.17

Total LAB is closely related to the acid content and pH value. The more LAB in glutinous rice tapai, the more carbohydrates will be overhauled into organic acids. The lactic acid content will be higher and the pH value lower. Lactic acid bacteria will grow well if the need for nutrients is more available so that they can be utilized to grow and multiply.

Hasanah et al. **[26]** stated that sticky rice tapai produced by home industries in Bogor contains LAB of 7.9-8.5 CFU/g. The results of the research by Dede et al. **[15]** showed that the addition of *Lactobacillus rhamnosus* SKG 34 before it was fermented produced tapai sticky rice with a total LAB of 3.5 x 10^8 CFU/g or the equivalent of 8.53 CFU/g. Research conducted by Yusmarini et al. **[2]** regarding the production of glutinous rice tapai with the addition of *Lactobacillus plantarum* 1 produced tapai with a LAB content of 8.77 log CFU/ml. Several researchers reported results were slightly lower than that of glutinous rice made using natural dyes from red dragon fruit extract and purple sweet potato, but not much different from the suji leaf extract treatment. Total LAB is related to the additional nutritional content of red dragon fruit and purple sweet potato extracts. Furthermore, it is indicated that the added natural dyes do not inhibit LAB activity but can be additional nutrients for LAB. Moreover, *Lactobacillus plantarum* 1 is a type of lactic acid bacteria (LAB) that can be used as a probiotic culture or as a food additive. It can tolerate acidic and bile environments **[27]**, allowing it to survive and thrive in the digestive system. This strain has been found to have various therapeutic benefits, such as preventing mutations and inhibiting the growth of cancer cells **[28]**. It also possesses properties that help break down bile salts and bind cholesterol **[29]**. Additionally, it has been shown to modulate the immune system in the intestinal tract **[30]**.

However, the results of this study were encouraging as they showed that glutinous rice tapai with natural dyes still had a higher nutritional content than tapai made with suji leaf extract. According to this study, it can be seen that the addition of natural dye extracts, as much as 20%, has given good results; therefore, for testing antioxidant activity and sensory assessment is only carried out on the treatment of immersion in natural dye extracts as much as 20%.

Antioxidant Activity

The antioxidant activity found in glutinous rice tapai was made by adding *Lactobacillus plantarum* 1 RN2-53 and soaking in natural dye extract by 20%, ranging from 102.51-354.25 ppm. Data on antioxidant activity are presented in Table 2.

Table 2 Antioxidant activity of tapai glutinous rice probiotics.

Treatments	Antioxidant activity IC_{50} (ppm)
Addition of 20% red dragon fruit extract	167.345
Addition of 20% purple sweet potato extract	102.510
Addition of 20% suji leaf extract	354.248

Table 2 shows that soaking with purple sweet potato extract has a higher antioxidant activity than red dragon fruit extract and suji leaves. According to Molyneux **[31]** a compound is said to have robust group antioxidant activity if the IC_{50} value is less than 50 ppm, the strong group IC_{50} 50-100 ppm, the medium group IC_{50} 101-150 ppm, the vulnerable group IC_{50} 151-200 ppm, and more than 200 ppm are said to be weak. It can be stated that the treatment with purple sweet potato extract produces probiotic tapai with moderate antioxidant activity. Soaking with red dragon fruit extract and suji leaves produces tapai with fragile antioxidant activity. According to Kurnianingsih et al. **[18]**, purple sweet potatoes contain anthocyanins as a natural pigment of 283.1 mg/100 g, with antioxidant activity of 90.47% or equivalent to 9.047 ppm. During the processing process, antioxidant activity will decrease. The study results show that the antioxidant activity in glutinous rice powder is 102.514 ppm. The higher the IC_{50}, the smaller the antioxidant activity. Therefore, these results suggest that the antioxidant activity of purple sweet potatoes decreases significantly during the processing process. The research results of Salim et al. **[32]** showed that the steaming process in sweet potatoes would cause a reduction in antioxidant activity, which was initially 5 mg/L to 47.82 mg/L. Tapai making process involves a high temperature when glutinous rice is steamed for 30 minutes, which causes a decrease in antioxidant activity.

Red dragon fruit contains betacyanin, a natural dye and antioxidant. The super red-meat dragon fruit extract has antioxidant activity with an IC_{50} of 89.1 ppm **[33]**. The heating process will reduce the antioxidant activity of the red dragon fruit. Nataliani et al. **[3]** show that the longer and higher the heating temperature, the value of the antioxidant activity of the dragon fruit flesh natural dye solution decreases. Due to betacyanin found in the red dragon fruit, it cannot stand the high temperature. Furthermore, Reshmi et al. **[34]** show that betacyanin pigmentation occurs if heated at temperatures of 40, 50, and 60 °C.

Probiotic glutinous rice tapai on soaking treatment with suji leaf extract has lower antioxidant activity than soaking treatment in red dragon fruit and purple sweet potato extracts. Suji leaves contain chlorophyll, which acts as a dye and has antioxidant properties. The stability of the pigment chlorophyll is influenced by pH, temperature, and light **[35]**. The decrease in pH due to the fermentation process in the manufacture of glutinous rice tapai causes chlorophyll instability, which decreases its antioxidant activity.

Sensory Characteristics of Probiotics Glutinous Rice Tapai with the Addition of Natural Dyes

Glutinous rice tapai made with the addition of *Lactobacillus plantarum* 1 RN2-53 and immersion treatment into natural dye extracts have different sensory properties. Descriptive and hedonic assessments of glutinous rice tapai are presented in Figure 4.

Aroma: The results showed that soaking with natural dye extracts will produce tapai that smells of acid and alcohol. Hedonic panelists preferred treatment with immersion in red dragon fruit extract. Sour aromas of tapai with a slightly alcoholic aroma dominate the resulting aroma. While in other treatments, it has a more pungent smell of alcohol. Cassava tapai was added that the panelists liked the most, namely that the alcohol aroma was not too sharp **[36]**. Marniza et al. **[23]** stated that fermented glutinous rice for 2 days is preferable to fermented glutinous rice made with a 3-day fermentation period. Fermentation time is related to the high alcohol content in tapai. The longer the fermentation, the more sugar will be broken into alcohol. In addition, the purple sweet potato extract treatment produces another aroma, which is slightly off-flavor scented, while suji leaf extract still leaves the aroma of the leaves. The panelists liked the side scent.

Color: Color is one of the sensory characteristics that are very important because it can determine the level of consumer acceptance of a product. The research results by Widiyanti and Sukarta **[37]** state that tapai made from white glutinous rice has a white color and is not liked by panelists. One of the purposes of using natural dyes in making white sticky rice is to improve the color of the white sticky rice, which was initially yellowish-white. The color of probiotic glutinous rice tapai soaked with natural dye extract of as much as 20% can be seen in Figure 6.

Using 20% natural dye extract in soaking water produces glutinous rice tapai with an attractive color according to the type of natural dye used. The use of red dragon fruit extract produces tapai with a slightly orange-red color. In contrast, tapai in the soaking treatment in purple sweet potato extract produces purple tapai, and soaking in suji leaf extract produces green tapai. The cooking and fermentation process can cause a decrease in color density. Dragon fruit extract, which was originally a deep red color after undergoing a process with high temperature and an acidic atmosphere, has decreased its red concentration.

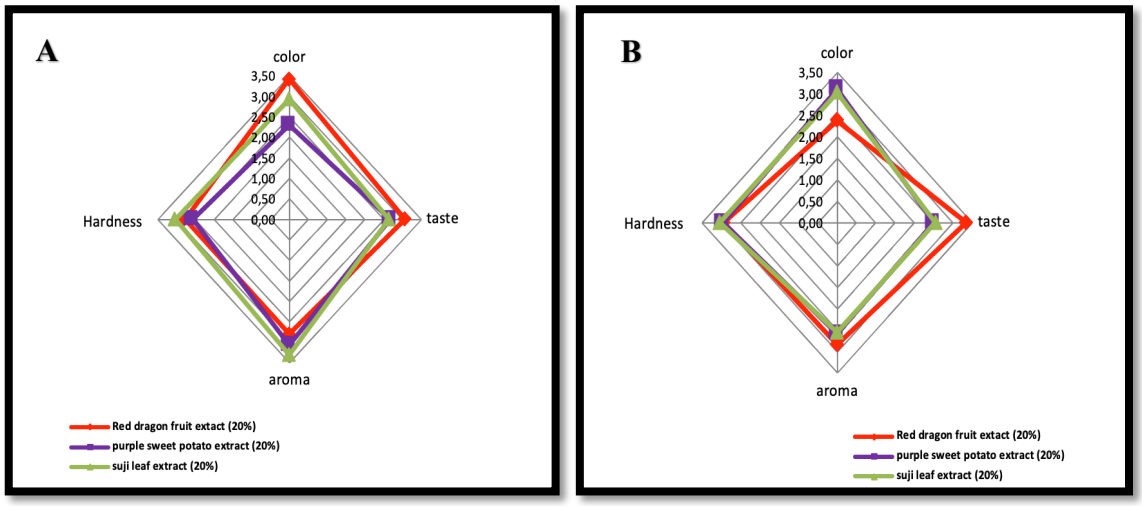


Figure 5 Sensory characteristics of probiotics glutinous rice tapai with the addition of natural dyes (A. Descriptive Analysis; B. Hedonic Analysis).

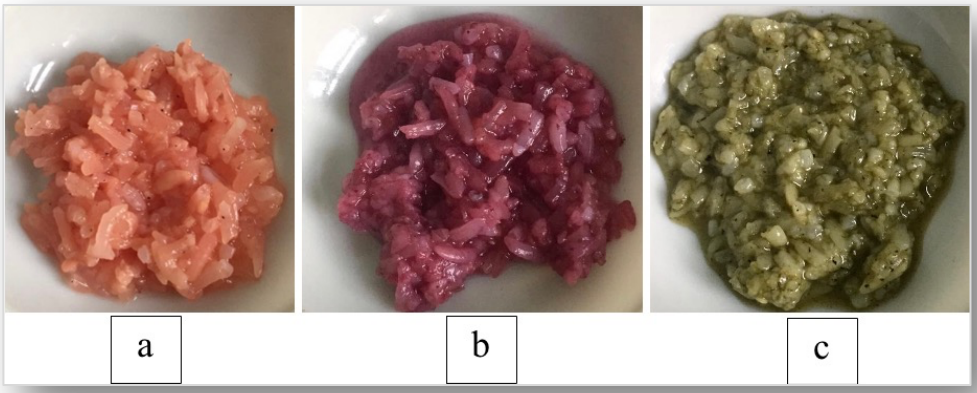


Figure 5 Color of probiotic glutinous rice (a) red dragon fruit extract (b) purple sweet.

According to Agne et al. **[38]**, temperatures above 40 °C will cause a change in betacyanin compounds from red to orange and then yellow. The research results of Husna et al. **[4]** state that the heating process by steaming purple sweet potatoes will result in a 34.14% decrease in anthocyanin pigment. Furthermore, Nugraheni **[5]** stated that heat treatment with a temperature of 100 °C degrades the pigment chlorophyll by 82.68%, and heating will convert chlorophyll into pheophytin by magnesium substitution by hydrogen so that the chlorophyll content becomes low. Based on the results of the hedonic sensory assessment, it is known that panelists prefer glutinous rice tapai color by soaking in purple sweet potato extract and suji leaf extract.

Taste: Glutinous rice tapai, soaking in natural dye extracts, generally has a mixture of sweet and sour tastes. However, the soaking treatment into purple sweet potato extract has a more robust sweetness than other treatments. The sweet and sour taste is produced during fermentation, remodeling complex carbohydrates into simple, sweet-tasting confectionery. Some simple sugars will be overhauled into organic acids that give tapai a sour taste. Furthermore, Barus and Wijaya **[36]** stated that the yeast's metabolic activities produce carbon dioxide gas that causes the dough to rise and produces alcohol and other flavor compounds. The fermentation process of tapai also produces ethanol and carbon dioxide gas, giving it an alcoholic aroma and a bubbly texture. Panelists preferred tapai glutinous rice probiotics with red dragon fruit extract to other treatments. The flavor characteristics were a balance of sweet and sour tastes that fit on the consumer's tongue and the absence of aftertaste after eating. Tapai with purple sweet potato extract dye still leaves the taste of yam, while suji leaf extract also leaves the particular flavor of suji leaves. Widiyanti and Sukarta **[37]** state that white sticky rice has a sweet taste and is not liked by panelists. Based on this, it can be seen that the panelists liked the balance of sweet, sour, and alcoholic flavors in tapai. Sweet, sour, and alcoholic tastes are influenced by the type of microbes used in making tapai.

Texture: Probiotic glutinous rice tapai generally has almost the same soft texture (hardness) in all treatments. However, in the treatment with suji leaf extract immersion, the tapai texture is slightly more complicated. The treatment concerns the more limited availability of nutrients in suji leaves. Nutrients are required by yeast and lactic acid bacteria during their growth. In the process of metabolism, the overhaul of complex compounds into simple compounds and the release of water causes the texture of tapai to become relatively soft. The texture is also affected by fermentation time. The longer the fermentation time, the softer the tapai texture becomes. Asnawi et al. **[39]** stated that fermented cassava tapai for 2 days was softer than 1 day of fermentation. The panelists' score on hardness was hedonic and ranged from 2.93-3.03, which is somewhat similar. Panelists prefer softer glutinous rice tapai over tapai with a soft or slightly soft texture. The results align with the research of Abdillah et al. **[40]**, which states that the panelists did not like wheat tapai with a very soft texture.

CONCLUSION

Based on the results of research that has been carried out by immersion with three different natural coloring extracts at a various concentration, tapai glutinous rice was selected from the tested parameters and fulfilled the food standards. standards that produced the best tapai quality based on the overall assessment. Tapai glutinous rice made with natural dyes has different physicochemical, microbiological, and sensory characteristics. The treatment in red dragon fruit extract (20%) and purple sweet potato extract (20%) resulted in the best treatment in glutinous rice tapai, which panelists preferred. Resulting in the characteristic pH value of 3.35-3.41, total lactic acid 0.61-0.70%, alcohol content 0.33-0.42%, total lactic acid bacteria 9.11-9.40 CFU/mL, and antioxidant activity 167.345-102.51 ppm.

REFERENCES

1. Khasanah, N., & Prima, R. W. (2014). Effect of fermentation time and addition of lactic acid bacteria Lactobacillus plantarum B1765 on the quality of cassava tapai product. In Unesa Journal of Chemistry (Vol. 3, Issue 1, pp. 78–84). Universitas Negeri Surabaya.
2. Yusmarini, J., V. S., Fitriani, S., Rahmayuni, Artanti, V. F., & Pato, U. (2019). Characteristics of probiotic tapai made by the addition of Lactobacillus plantarum 1. In International Journal of Agricultural Technology (Vol. 15, Issue 1, pp. 195–206). Association of Agricultural Technology in Southeast Asia (AATSEA).
3. Nataliani, M. M., Kosala, K., Fikriah, I., Isnuwardana, R., & Paramita, S. (2018). The Effect of Storage and Heating on Physical Stability and Antioxidant Activity of Natural Dye Solution from Dragon Fruit Flesh (Hylocereus costaricensis). In Jurnal Tumbuhan Obat Indonesia (Vol. 11, Issue 1, pp. 1–10). Badan Penelitian dan Pengembangan Kesehatan. https://doi.org/10.22435/toi.v11i1.8688.1-10
4. Husna, N. el, Novita, M., & Rohaya, S. (2013). Anthocyanins Content and Antioxidant Activity of Fresh Purple Fleshed Sweet Potato and Selected Products. In Agritech (Vol. 33, Issue 3, pp. 296–302). Faculty of Agricultural Technology, Universitas Gadjah Mada. https://doi.org/10.22146/agritech.9551
5. Nugraheni, M. (2014). Natural Dyes: Sources and Their Application to Food and Health (1st ed.). Graha Ilmu.
6. Putri, D. C. L. A., Putra, I. N. K., & Suparthana, I. P. (2019). The Effect of Adding Red Dragon Fruit (Hylocereus Polyrhizus) Juice for The Characteristics of Yogurt Mixed Milk and Red Beans (Phaseolus Vulgaris). In Jurnal Ilmu Dan Teknologi Pangan (Vol. 8, Issue 1, pp. 8–17). Faculty of Agricultural Technology, Udayana University. https://doi.org/10.24843/itepa.2019.v08.i01.p02
7. Yusmarini, Y., Fitriani, S., Johan, V. S., Rahmayuni, R., & Artanti, V. F. (2020). Utilization of Lactobacillus plantarum 1 RN2-53 and plant-based natural dyes to manufacture probiotic tapai. In Jurnal Teknologi Dan Industri Pertanian Indonesia (Vol. 12, Issue 1, pp. 9–15). Agricultural Product Technology Department, Faculty of Agriculture, Universitas Syiah Kuala. https://doi.org/10.17969/jtipi.v12i1.15777
8. Muchtadi, T. R., Sugiyono, & Ayustaningwarno, F. (2013). Food Science (4th ed.). CV. Alfabeta.
9. Cunniff, P., & Association of Official Analytical Chemists. (1995). Official methods of analysis of AOAC international (Association of Official Analytical Chemists, Ed.; 16th ed.). Association of Official Analytical Chemists.
10. Yulianti, C. H. (2014). Analysis of Different Alcohol Levels on Tape Rice, Black Glutinous Rice, and Cassava. In Jurnal Teknika (Vol. 6, Issue 1, pp. 531–536). Department of Informatics Engineering, Faculty of Engineering, Universitas Islam Lamongan.
11. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. In LWT - Food Science and Technology (Vol. 28, Issue 1, pp 25–30). Elsevier. https://doi.org/10.1016/S0023-6438(95)80008-5
12. Fardiaz, S. (1992). Food Microbiology. Gramedia Pustaka Utama. Jakarta.

13. Yusmarini, Y., Pato, U., Johan, V. S., Ali, A., & Kusumaningrum, K. (2017). Characterization of Amylolytic Lactic Acid Bacteria from Sago Starch Processing Industry. In AgriTECH (Vol. 37, Issue 1, pp. 95–100). Faculty of Agricultural Technology, Universitas Gadjah Mada. https://doi.org/10.22146/agritech.17014
14. Isnaini, H. N., Saputrayadi, A., & Sari, D. A. (2022). Quality of white glutinous rice tapai with the addition of dragon fruit as natural dye. In Food and Agro-Industry Journal (Vol. 3, Issue 2, pp. 199–211). Faculty of Agricultural Technology, Universitas Teknologi Sumbawa.
15. Dede, E. G., Nocianitri, K. A., & Darmayanti, L. P. T. (2018). The Effect of Time Addition Lactobacillus rhamnosus SKG 34 on Characteristics of Probiotic Fermented Sticky Riceduring Storage. Jurnal Ilmiah Teknologi Pertanian Agrotechno (Vol. 3, Issue 1, pp. 269-276). Faculty of Agricultural Technology, Universitas Udayana. https://doi.org/10.24843/JITPA.2018.v03.i01.p02
16. Nurul, S. R., & Asmah, R. (2014). Variability in nutritional composition and phytochemical properties of red pitaya (Hylocereus polyrhizus) from Malaysia and Australia. In International Food Research Journal (Vol. 21, Issue 4, pp 1689–1697). Faculty of Food Science and Techology, Universiti Putra Malaysia.
17. Kakade, V., Jinger, D., Chavan, S., Nangare, D. D., Wakchaure, G. C., & Dinesh, D. (2020). Dragon Fruit: Wholesome and remunerative fruit crop for India. In Food and Scientific Reports (Vol. 1, Issue 12, pp. 44–48). Raiganj: Food and Scientific Reports.
18. Kurnianingsih, N., Ratnawati, R., Nazwar, T. A., Ali, M., & Fatchiyah, F. (2020). A Comparative Study on Nutritional Value of Purple Sweet Potatoes from West Java and Central Java, Indonesia. In Journal of Physics: Conference Series (Vol. 1665, Issue 1). IOP Publishing. https://doi.org/10.1088/1742-6596/1665/1/012011
19. Murtini, E. S., Adhamatika, A., & Putri, D. A. (2021). The effects of powdered suji leaves (Dracaena angustifolia (medik.) roxb.) on the pasting properties of wheat flour and characteristics of steamed bun. In IOP Conference Series: Earth and Environmental Science (Vol. 733, Issue 1, p. 012092). IOP Publishing. https://doi.org/10.1088/1755-1315/733/1/012092
20. Kurniawan, T. B., Bintari, S.H., & Susanti, R. (2014). Interaction effects Tape and Bread Yeast on the Level of Bioethanol Cassava (Manihot utilissima, Pohl) Mukibat Varieties. In Jurnal Biosaintifika (Vol. 6, Issue 2, pp. 152–160). Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang. https://doi.org/10.15294/biosaintifika.v6i2.3783
21. Walker, G. M., & Stewart, G. G. (2016). Saccharomyces cerevisiae in the production of fermented beverages. In Beverages (Vol. 2, Issue 4, pp. 1–12). MDPI. https://doi.org/10.3390/beverages2040030
22. Berlian, Z., Aini, F., & Ulandari, R. (2016). Evaluation of the alcohol content of glutinous rice white and cassava through fermentation with different doses of yeast. In Jurnal Biota (Vol 2, Issue 1, pp. 106–111). Department of Biology Faculty Saints and Technology, Universitas Islam Negeri Raden Fatah Palembang.
23. Marniza, M., Syafnil, S., & Safitri, S. (2020). Characterization of Tapai Ketan Hitam with Various Cooking Methods. In Jurnal Teknologi Agro-Industri (Vol. 7, Issue 2, pp. 112–120). Center for Research and Community Service, Politeknik Negeri Tanah Laut. https://doi.org/10.34128/jtai.v7i2.124
24. Anisa, F. A., Bintoro, V. P., & Nurwantoro, N. (2017). Quality of Chemical and Organoleptic Tape from Fermented Taro Kimpul (Xanthosoma sagittifolium) with Various Concentrations of Yeast. In Jurnal Aplikasi Teknologi Pangan (Vol. 6, Issue 1, pp. 43–47). Faculty of Agriculture and Animal Husbandry, Universitas Diponegoro. https://doi.org/10.17728/jatp.207
25. Commission of the Indonesian Ulema Council. Commission of the Indonesian Ulema Council Number 10 Year 2018 Fatwa on Ethanol Content in Food and Beverage Product. Retrieved from https://www.halalmui.org/mui14/main/detail/memahami-fatwa-mui-tentang-kadar-etanol-pada-produk-makanan-dan-minuman.
26. Hasanah, U., Ratihwulan, H., & Nuraida, L. (2019). Sensory Profiles and Lactic Acid Bacteria Density of Tape Ketan and Tape Singkong in Bogor. In AgriTECH (Vol. 38, Issue 3, pp. 265-272). Faculty of Agricultural Technology, Universitas Gadjah Mada. https://doi.org/10.22146/agritech.30935
27. Pato, U. (2003). Bile and Acid Tolerance of Lactic Acid Bacteria Isolated from Dadih and Their Antimutagenicity against Mutagenic Heated Tauco. In Animal Bioscience (Vol. 16, Issue 11, pp. 1680–1685). https://doi.org/10.5713/ajas.2003.1680
28. Usman, P., & Hosono, A. (1997). Antimutagenic activity of maillard reaction products againts mutagenic heated tauco. In Italian Journal of Food Science (Vol. 9, Issue. 4, pp. 267–276). Codon Publications.
29. Usman, P., & Hosono, A. (1999). Binding of cholesterol to the cells and peptidoglycan of Lactobacillus gasseri. Milchwissenschaft (Vol. 54, Issue 9, pp. 495–498) AVA-Verlag.

30. Harzallah, D., & Belhadj, H. (2013). Lactic Acid Bacteria as Probiotics: Characteristics, Selection Criteria and Role in Immunomodulation of Human GI Muccosal Barrier. In Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes. InTech. https://doi.org/10.5772/50732
31. Molyneux, P. (2004). The Use of the Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity. In Songklanakarin Journal of Science and Technology (Vol. 26, Issue 2, pp. 211–219). INFORMS. https://doi.org/10.1287/isre.6.2.144
32. Salim, M., Dharma, A., Mardiah, E., & Oktoriza, G. (2017). Influence of Antosianin and Antioxidant Influences Onprocess of Processing of Purlpe Sweet Potato. In Jurnal Zarah (Vol. 5, Issue 2, pp. 7–12). Faculty of Education Universitas Maritim Raja Ali Haji. https://doi.org/10.31629/zarah.v5i2.209
33. González, E., Vaillant, F., & Pérez, A. (2012). In Vitro Cell-Mediated Antioxidant Protection of Human Erythrocytes by Some Common Tropical Fruits. In Journal of Nutrition & Food Sciences (Vol. 2, Issue 03, pp. 2–8). Longdom Publishing. https://doi.org/10.4172/2155-9600.1000139
34. Reshmi, S. K., Aravindhan, K. M., & Suganya Devi, P. (2012). The Effect of Light, Temperature, pH on Stability of Betacyanin Pigments in Basella alba Fruit. In Asian Journal of Pharmaceutical and Clinical Research (Vol. 5, Issue 4, pp. 107–110). Innovare Academic Sciences.
35. Hörtensteiner, S., & Kräutler, B. (2011). Chlorophyll breakdown in higher plants. In Biochimica et Biophysica Acta – Bioenergetics (Vol. 1807, Issue 8, pp. 977–988). Elsevier. https://doi.org/10.1016/j.bbabio.2010.12.007
36. Barus, T., & Wijaya, N. (2011). Dominant Microbiota and Their Role in Flavor of Cassava Tape. In Jurnal Biota (Vol. 16, Issue 2, pp. 354–361). Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta. https://doi.org/10.24002/biota.v16i2.119
37. Widiyanti, N. L. M., & Sukarta, I. N. (2022). Various Comparisons of White Sticky Rice with Red Rice Toward the Volume of Brem Drink and Organoleptic Test Tape Foods. In JST (Jurnal Sains dan Teknologi) (Vol. 11, Issue 2, pp. 274–282). Universitas Pendidikan Ganesha. https://doi.org/10.23887/jstundiksha.v11i2.51055
38. Agne, E. B. P., Hastuti, R., & Khabibi, K. (2010). Ekstraksi dan Uji Kestabilan Zat Warna Betasianin dari Kulit Buah Naga (Hylocereus polyrhizus) serta Aplikasinya sebagai Pewarna Alami Pangan. In Jurnal Kimia Sains dan Aplikasi (Vol. 13, Issue 2, pp. 51–56). Institute of Research and Community Services Diponegoro University (LPPM UNDIP). https://doi.org/10.14710/jksa.13.2.51-56
39. Asnawi, M., Sumarlan, S. H., & Bagus Hermanto, M. (2013). Characteristics Maturation Process of Cassava Tape (Manihot utilissima) Through the Use of Temperature Control. In Jurnal Bioproses Komoditas Tropis (Vol. 1, Issue 2, pp. 56–66) Department of Agricultural Engineering, Faculty of Agricultural Technology, Universitas Brawijaya.
40. Abdillah, J., Widyawati, N., & Suprihati. (2016). Pengaruh dosis ragi dan penambahan gula terhadap kualitas gizi dan organoleptik tape biji gandum. In Agric (Vol. 26, Issue 1, p. 75). Universitas Kristen Satya Wacana. https://doi.org/10.24246/agric.2014.v26.i1.p75-84

Funds:

Universitas Riau funded this research, Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia, with Contract Number 852/UN.19.5.1.3/PT.01.03/2020.

Acknowledgments:

We would like to thank to Research institutions and Community Service Universitas Riau.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Yusmarini**, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: yusmarini@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0002-8879-4030

Shanti Fitriani, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: shanti.fitriani@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0002-9693-7679

Vonny Setiaries Johan, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: vonny.setiaries@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0002-5140-5222

Netti Herawati, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: netti.herawati@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0002-8579-4748

Irhamdi, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: irhamdibendang@gmail.com
ORCID: https://orcid.org/0000-0003-1198-8467

Emma Riftyan, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: emma.riftyan@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0001-7919-4319

Usman Pato, Universitas Riau, Faculty of Agriculture, Department of Agricultural Technology, HR. Soebrantas KM. 12,5, 28293, Pekanbaru, Indonesia,
Tel.: (+62)761-63272
E-mail: usmanpato@yahoo.com
ORCID: https://orcid.org/0000-0001-7716-5282

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 29.10.2023
Revised: 14.1.2024
Accepted: 9.2.2024
Published: 19.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 122-138
https://doi.org/10.5219/1933
ISSN: 1337-0960 online
www.potravinarstvo.com

Quality characteristics of the shape preservation of cooked pasta with millet addition at different time intervals

Gulmaida Karimova, Rimma Niyazbekova, Khaldun Al Azzam, El-Sayed Negim

ABSTRACT

The current study presents the indicators of shape preservation of cooked pasta by adding 7.7% millet in various time intervals. The experiments were conducted immediately after the pasta cooking process and after two hours. In the Eurasian Economic Union and the Republic of Kazakhstan, the quality indicators were produced by considering the established methodologies stated in the standardized papers. The interstate standard GOST 31964-2012, "Pasta products. Acceptance rules and methods of quality determination," was used to determine the shape preservation of cooked pasta. The study aimed to determine and compare cooked pasta's shape preservation indicators (consumer properties), considering a certain time interval. Pasta needs five minutes to cook. These days, though, pasta manufacturers specify the required cooking time – eight, nine, ten, or eleven minutes – on their packages. The study's findings indicate that the safety of pasta containing "7.7% millet after 2 (hours) from the moment of cooking" and "with the addition of 7.7% millet after cooking" is negligible by 1-2 N; however, data from 4-24 N show a more notable difference in a comparable scenario. The experiments were conducted at the Federal State Autonomous Scientific Institution "Scientific Research Institute of the Bakery Industry", Moscow, Russian Federation. The corresponding data was obtained according to the results of the conducted studies.

Keywords: pasta, cooking, time, quality indicators, shape, safety, standardization documents

INTRODUCTION

Globally, health authorities advise reducing animal protein and fat consumption while increasing cereal consumption, which is an important source of dietary fiber. In addition, the World Health Organization (WHO) considers pasta, a cereal product, to be a good way to supplement nutrients **[1]**. Made from durum wheat flour, pasta is a classic dish staple in many nations. Customers choose it because of its inexpensive cost and low glycemic index, as well as its ease of handling, cooking, storing, and transportation (2-4) **[1]**, **[2]**.

Pasta is a widely used cereal cuisine that may be found in many different forms and sizes, including noodles, spaghetti, and vermicelli **[3]**, **[4]**. It is mainly produced from durum wheat because of its favorable viscoelastic behavior, which is in charge of good matrix networking and ideal dough formation during the mixing and extrusion phases. Pasta quality attributes that are cooked result from a well-designed matrix network **[3]**.

Several studies on the value addition of various millets have been reported **[5]**, **[6]**, **[7]**, **[8]**, but little effort has been made to prepare small millets-based pasta products, possibly due to a variety of factors, including a lack of technology.

After three months of storage, there were no discernible differences in the sensory qualities of cooked pasta produced using hot water extrusion, and cooking loss was minimal (12%). Since there hasn't been much research on the creation of snack products made from Kodo millets, there haven't been any studies on the packaging and storage of products that resemble pasta that is ready to cook but contain Kodo millet. As a result, a study was carried out developing Kodo millet-based extruded ready-to-cook pasta products under various formulations

packed in different packaging materials for three months of storage studies with biochemical (fat, protein, carbohydrate, crude fiber, ash, and moisture content) and quality analysis at monthly intervals, to provide a good processing, packaging, and storage technique for pasta-like ready-to-cook products **[9]**.

Promoting nutrition reform has increased millet (*Panicum miliaceum* L.), receiving a place in food processing today. The short growing season of millet and its variety of uses increase its value. The outcomes of numerous animal experiments demonstrate that millet has a cholesterol-lowering effect. Millet has several advantages for treating diabetes and is crucial for maintaining good health. Fluorine, iron, magnesium, calcium, zinc, potassium, and manganese are also significant in millet. The excellent yellow color of pasta results from its high carotenoid content, provided by millet use. Additionally, it lowers the finished products' GI (glycemic index) and contains a significant amount of vitamins, particularly β-group vitamins. People with coeliac disease can eat millet because it is gluten-free. Still, clean millet that any other cereals haven't contaminated can be difficult to produce in our country **[10]**.

Pasta is one of the favorite dishes in the world. They are traditionally made from wheat, but nowadays, there are many more types of pasta, for example, pasta made from peas, lentils, beans, and other gluten-free varieties **[11]**. Today, one of the priority quality indicators in pasta is preserving the shape after cooking. The consumer focuses his attention on this indicator. The method for determining the quality indicator is established in GOST 31964-2012 "Pasta products". Acceptance rules and methods of quality determination" **[12]**. Given this, experiments were conducted regarding the influence of the time factor on the indicators of the preservation of the shape of pasta. The study aimed to assess and compare cooked pasta's shape preservation indicators (consumer characteristics) over a specific time frame. The trials were conducted at the Federal State Autonomous Scientific Institution "Scientific Research Institute of the Bakery Industry" in Moscow, Russia. The related data was gathered based on the findings of the investigations.

Scientific Hypothesis

The major scientific hypothesis of the study is to determine and compare the shape preservation indicators (consumer properties) of cooked pasta, considering a certain time interval. We expect that the cooked pasta's consumer qualities (shape preservation indicators) over a given cooking period will affect its quality and thus affect the consumer's needs.

MATERIAL AND METHODOLOGY

Samples

The following items were bought from Almaty, Kazakhstan local markets: millets, wheat flour, starch, pea, soy, amaranth flour, gluten-free flour, durum wheat (genotype), and flax seeds. Pasta usually consists of two ingredients: water and wheat flour. Sampling pasta with millet 7.7% was conducted according to GOST 31964-2012. The process of cooking pasta with the addition of 7.7% millet, according to GOST 31964-2012. The samples (pasta) temperature was conducted at a range of 24-25 °C.

Chemicals

The chemical composition (starch and amaranth flour, gluten-free flour, soy, pea) was determined.

Animals, Plants and Biological Materials

This study did not use any biological or animal components.

Instruments

Russian Federation, Moscow, Eleks -7M (Manufacturer Limited Liability Company "Tagler"). Ukraine, Vinnytsia area, Mogilev-Podilskyi, instrument manufacturing facility, drying cabinet SESH-3M. Furthermore, during product input/output control and scientific research, the MA-150 "SARTORIUS" infrared humidity analyzer is designed to track the humidity level in liquid, bulk, solid, and emulsion substances. Göttingen, Germany, "Sartorius Weighing Technologies GmbH," manufacturer. 94-108 Weender Landstrasse. The Structurometer ST-1M may determine the rheological characteristics of raw materials, semi-finished items, and final goods – producer "Ochakov Combination of Food Ingredients," Moscow, Russian Federation.

To accommodate gluten-intolerant patrons, pasta was enhanced with gluten-free flour, such as rice, buckwheat, and maize. Other components such as amaranth flour, peas, starch, and soy were added to gluten-free flour. Pasta products gained biological value and acquired therapeutic and preventative qualities when flaxseed flour was partially substituted for the best wheat flour grade. Regarding amino acid composition, the proteins in flaxseed flour are far superior to those in wheat. The fiber in flaxseed flour can reach 30% of its weight. Additionally, incorporated in flaxseed flour are readily absorbed minerals and vitamins.

Many domestic and foreign researchers have discussed the need for this direction in their search for novel raw materials and functional additives for pasta production. Their goal is to produce pasta that is higher in nutritional

value, lower in calories, and enhanced with active ingredients **[13]**, **[14]**. We use millet in our research as a dietary supplement.

About 12-15% of the protein, 70% of the starch, and essential amino acids are found in millet. Cereals contain between 0.5 and 8% fiber, 2.6-3.7% fat, up to 2% sugar, vitamins PP, B1, and B2, and significant amounts of potassium, magnesium, and phosphorus. Magnesium and molybdenum concentrations are highest in millet. The grain crop that is thought to be the least allergic is millet. Even people with sensitive digestion can consume this cereal because the body easily absorbs it.

Laboratory Methods

According to the specifications of GOST 31743-2017 "Pasta products, General Technical Conditions" and GOST 31964-2012 "Pasta products, Acceptance Rules and Methods of Quality Determination," studies were conducted on organoleptic parameters (color, shape, taste, and smell), as well as physicochemical parameters, such as humidity. The temperature used was in the range of 24-25 °C.

There are the following methods for determining humidity according to GOST 31964-2012:

- by drying to a constant mass.
- accelerated drying method.
- by the express method.
- on the MA-30 "SARTORIUS".

The organoleptic indicators of millet, including indications such as color, smell, and taste, are considered by GOST 572-2016 "Millet grain ground. Technical conditions."

The millet groats should have a color that is "yellow of various hues," a fragrance that is "typical of millet groats, without foreign odors, not musty, not moldy," and a taste that is "typical of millet groats, without foreign tastes, not sour, not bitter."

In preparing pasta, high-grade grits, ground millet, and water were used. The water intended for kneading the dough is heated in heat exchangers and mixed with cold tap water to the temperature specified in the recipe. The longitudinal section of the wheat grain shown in Figure 1 consists of shells 1, aleurone layer 2, endosperm 3, and embryo 4. When moistened with cold water, starch grains partially swell, retaining their shape and not dissolving. When an aqueous suspension of wheat starch is heated, the volume of starch grains increases, swelling due to the absorption of a large amount of water. At 20-300 °C there is an increase in the volume of grains with the preservation of their individuality, but with the loss of the crystallinity of the structure; at a temperature of 62.5 °C, the process of gelatinization of wheat starch begins, accompanied by the absorption of a significant amount of water, the rupture of polysaccharide chains, and the transformation of starch grains into a single body-like, jelly-like mass – paste; with further heating of the paste, starch absorbs five times the amount of water or more **[15]**. By the requirements of GOST 31743-2017 "Pasta products. General technical conditions", GOST 31964-2012 "Pasta products. Acceptance rules and methods of quality determination"**[16]**. By GOST 572-2016 "Millet grain ground. Technical conditions"**[17]**. Equipment used during experiments according to GOST 31964-2012. Laboratory scales according to GOST 24104. Electric stove, according to GOST 14919. They are cooking vessels with a thick bottom with a diameter of 170 mm and a capacity of 2.0-2.5 mL. Pressure plate made of transparent plastic with a thickness of 3 mm. A sieve with a diameter of 200 and a hole size of 1-2 mm. A stopwatch with a permissible error when measuring the interval up to 30 min ± 3 s. Distilled water according to GOST 6709. The plates are white. "Structurometer ST-1M" is designed to determine the rheological characteristics of food media (Figure 2). The device's operation principle measures the mechanical load on the indenter nozzle when inserted at a given speed into the prepared product sample. The microprocessor system of the device provides a certain measurement algorithm and the output of measurement results in an alphanumeric indicator and a personal computer. The device Structrometer ST-1M determines one of the quality indicators of «shape preservation» of pasta. The structure meter is designed to determine the quality indicators of prescription ingredients, semi-finished products, and finished products in various branches of the food industry according to their classical and conditional rheological characteristics:

- elastic and plastic deformations;
- the work of elastic deformation;
- rigidity;
- ultimate strength;
- modulus of elasticity;
- the maximum loading force;
- adhesive stress;
- the maximum shear stress;
- viscosity;
- stress relaxation time;

- elastic after effect;
- strength of jelly (gelling ability of agaroids and pectin-containing raw materials);
- hardness of fatty foods;
- general deformation of gluten;
- strength of pasta, pastry, and chocolate products;
- the depth of implementation;
- swelling of bread and flour confectionery products.

Thus, the structure meter is an information and measurement system for complex measurement of the rheological properties of food products. With a high degree of automation of the measurement process, it provides control of a wide range of rheological parameters. Given the versatility of the device, and its ability to determine the rheological characteristics of food products quickly and accurately, it is possible to use it both during scientific research and during input control of raw materials entering enterprises and operational control of the flow of technological operations of food production.

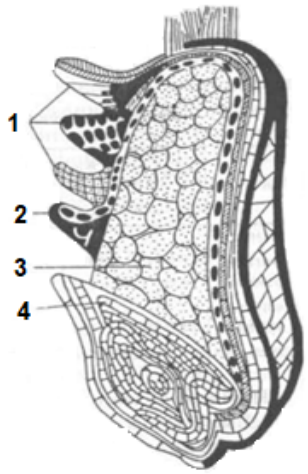


Figure 1 Longitudinal section of the wheat grain.

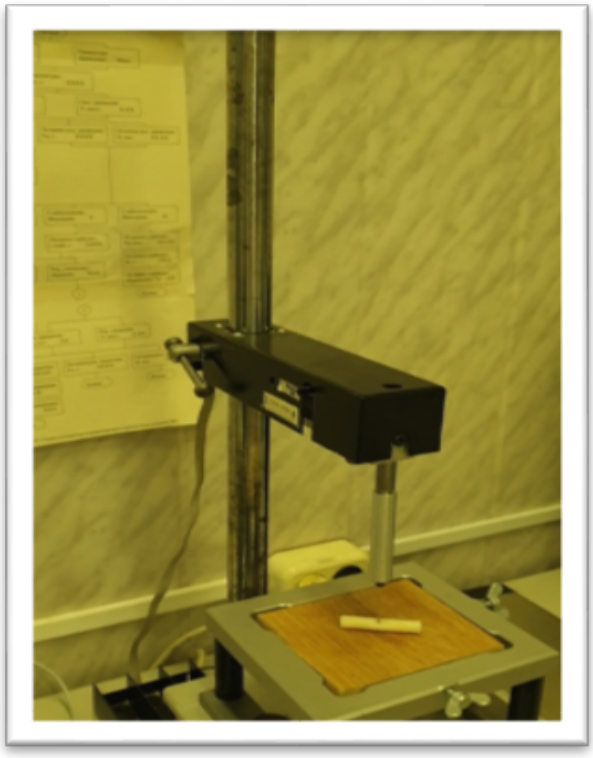


Figure 2 The device for determining the preservation of the form "Structrometer ST-1M", aimed at identifying the rheological properties of semi-finished, final, and raw materials; Moscow, Russian Federation; manufacturer: "Ochakov Combine of Food Ingredients."

Description of the Experiment

Sample preparation: The study was conducted at the Federal State Autonomous Scientific Institution "Research Institute of the Baking Industry," one of the top research facilities in Moscow, Russian Federation.

In a cooking vessel, 1000 mL of distilled water was added and then boiled. A sample for analysis weighing 50 g (based on the entire product) was cooked while being stirred in boiling water until the water reached boiling once again.

The product obtained was cooked in an open vessel at moderate heat, and their readiness was monitored with a pressure plate every minute after secondary boiling until the continuous white line visible in the centre of the plate disappeared. The time it takes for the product to be ready was set - from when the pasta is placed in boiling water until the continuous white line disappears. The cooked pasta was poured onto a sieve, allowing the cooking water to drain, and then put on a platter.

An apparatus made by "KERN & Sohn GmbH" in Balingen, Germany, model number KERN 440-45N, was used to weigh the grits. Using the Sandorina model, number 1861 equipment (made in Italy, 2002; Watt 400, Volts 220, Hz 50, Ph 1), the dough was kneaded with millet for around thirty minutes. The millet component's volume was determined to be 670 microns. As a control copy, water and premium grits were used.

Number of samples analyzed: 36.

Number of repeated analyses: 9.

Number of experiment replication: 3.

Statistical Analysis

The data are provided as the mean standard deviation (SD) of three experiments, with p <0.05 denoting significance. The study's data were statistically analyzed using Excel and STATISTICA 13 software (Dell, StatSoft). The difference between the values was considered probable p <0.05; 0.01 and 0.001.

RESULTS AND DISCUSSION

The shape preservation of pasta made of premium grade grits and adding 7.7% millet was determined on a texture analyzer device (structurometer ST-1M) (Figure 3). The moisture content of pasta was 28% at the pressing stage.

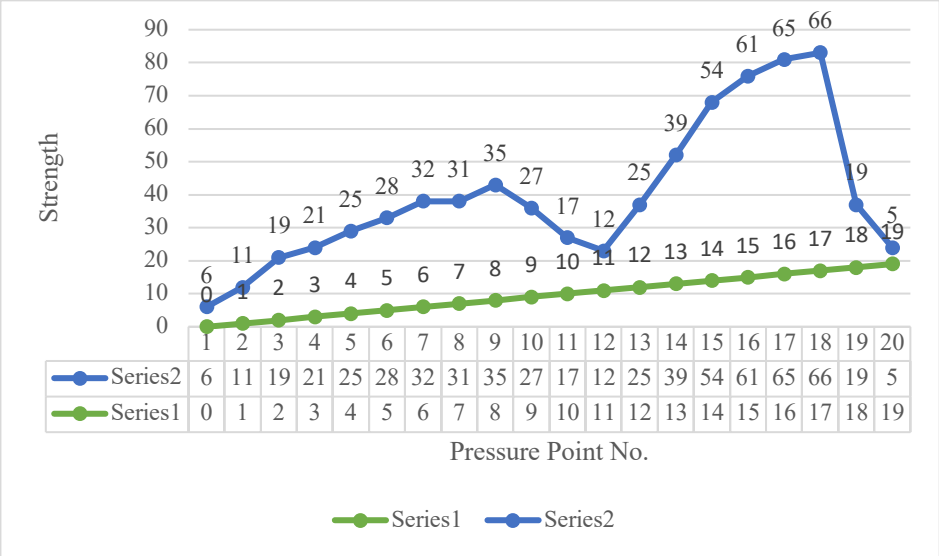


Figure 3 Strength indicators of pasta made from high-grade grits control (sample No. 1).

An essential component of assessing the product's overall quality and consumer acceptance is the evaluation of form preservation in pasta. The structure meter ST-1M, a texture analyzer equipment, was used in this investigation to examine the form preservation of pasta prepared from premium-grade grits and pasta with an addition of 7.7% millet (Figure 3). The selection of a texture analyzer highlights the sensitivity and accuracy of the test, enabling a thorough examination of the structural integrity of the pasta. Significantly, the pasta had a moisture level of 28% when it was pressed. Pasta's elasticity, hardness, and general structure are all greatly influenced by its moisture content. The designated moisture content is essential for understanding the texture analyzer results and adds important context to the experimental setup.

The utilization of the structurometer ST-1M, a widely recognized instrument for assessing the mechanical characteristics of food items, in this investigation suggests a thorough methodology to comprehend the influence of millet incorporation on pasta texture. The pasta's capacity to hold its shape while cooking and further processing largely depends on factors like hardness, chewiness, and resilience, all of which were probably evaluated by the device. The texture qualities of the premium grade grits-based pasta and the millet-fortified pasta are visually represented by the results in Figure 3. This comparison makes it possible to directly evaluate any differences in shape preservation between the two formulas.

The presence of 7.7% millet in the pasta recipe might have affected its textural characteristics and capacity to hold its shape while cooking. The structurometer ST-1M's data should provide information about how millet affects the hardness and resilience of pasta by interacting with the pasta matrix. Comprehending the alterations in the texture of the pasta is essential for forecasting its performance during cooking and other processing phases, in addition to setting consumer expectations. A thorough grasp of the impacts of millet addition on pasta shape maintenance is made possible by integrating data on moisture content, using an accurate texture analyzer, and the graphical display of results, all of which improve the study's robustness.

In summary, the careful method of analyzing the textural qualities of pasta containing millet is demonstrated using the structure meter ST-1M to measure form preservation and reveal moisture content. These findings establish the foundation for future developments in the creation of pasta products that satisfy both nutritional and textural expectations by deepening our understanding of how different grains affect the structural properties of pasta.

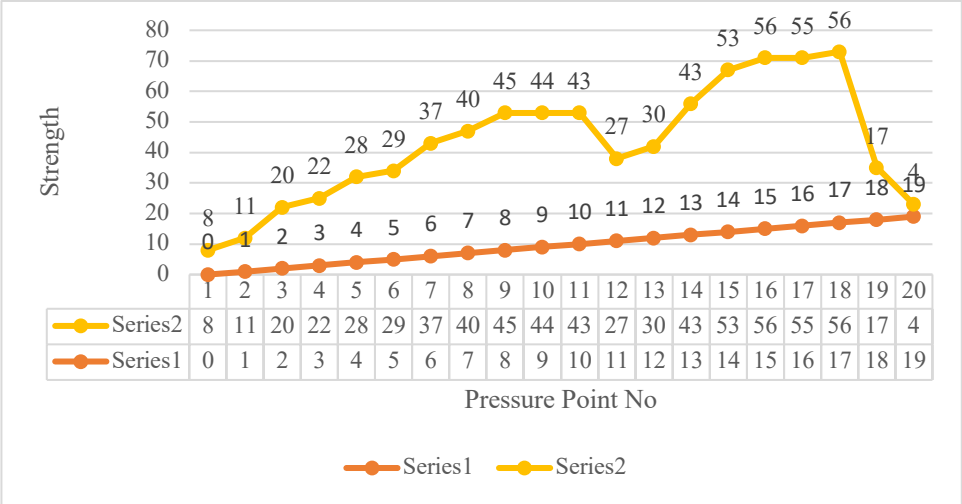


Figure 4 Strength indicators of pasta made from high-grade grits control (sample No. 2).

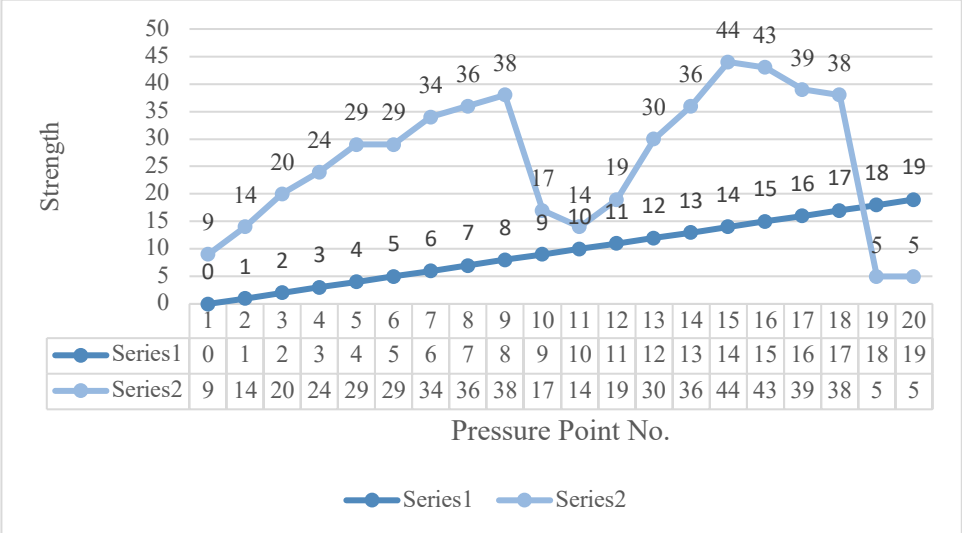


Figure 5 Strength indicators of pasta made from high-grade grits control (sample No. 3).

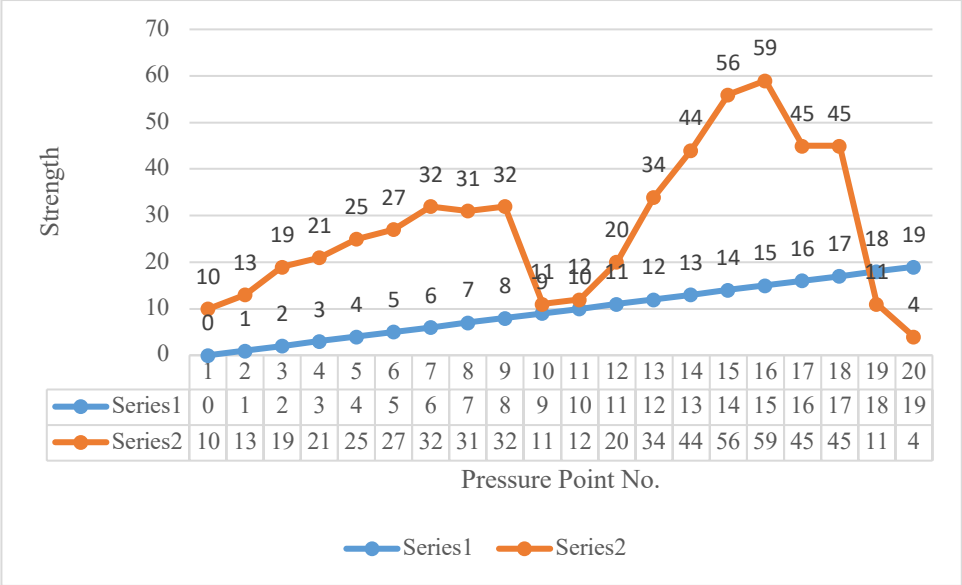


Figure 6 Strength indicators of pasta made from high-grade grits control (sample No. 4).

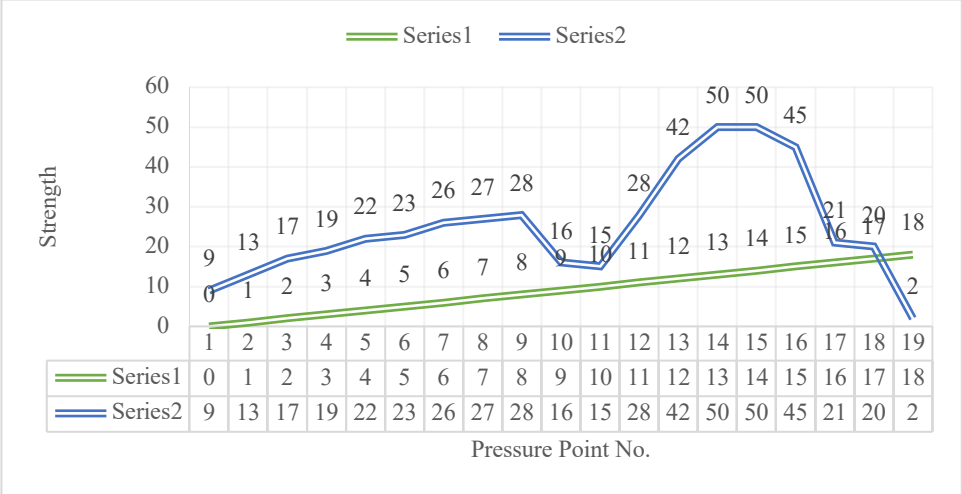


Figure 7 Strength indicators of pasta made from high-grade grits control (sample No. 5).

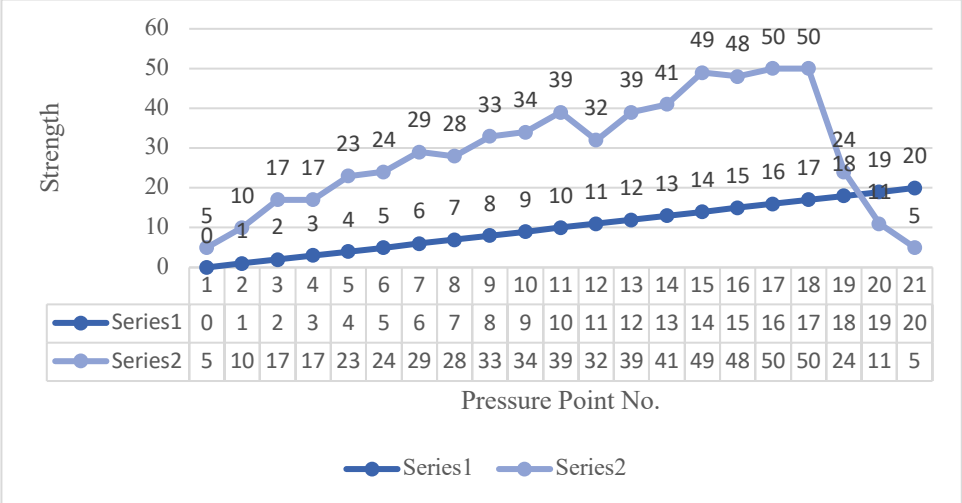


Figure 8 Strength indicators of pasta made from high-grade grits control (sample No. 6).

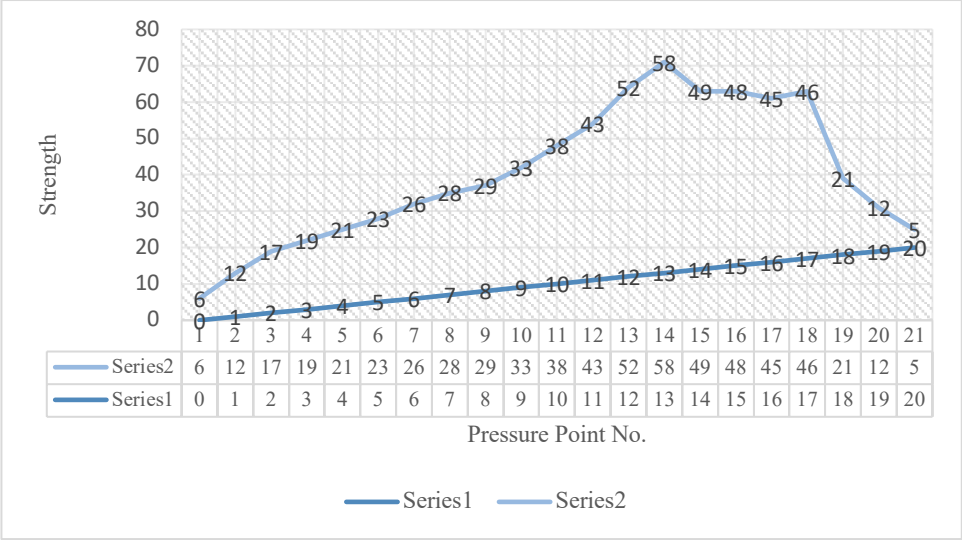


Figure 9 Strength indicators of pasta made from high-grade grits control (sample No. 7).

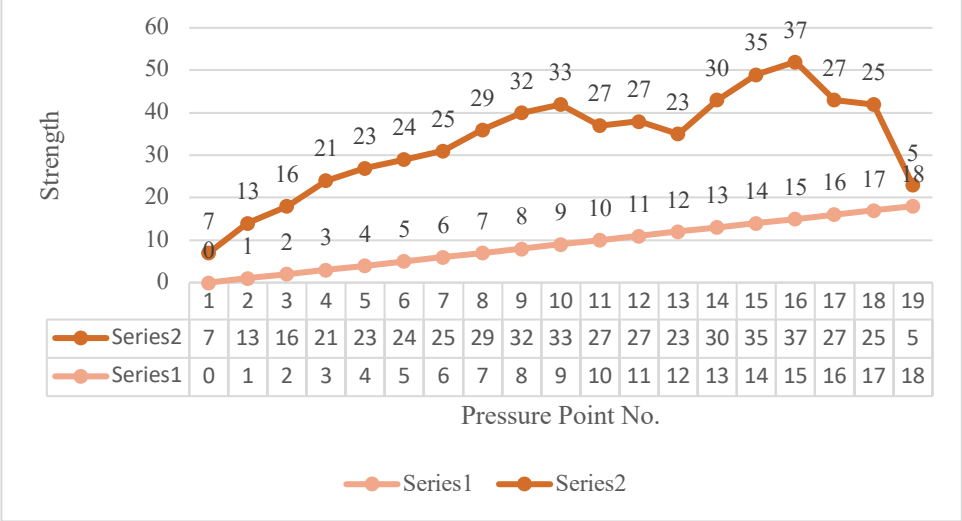


Figure 10 Strength indicators of pasta made from high-grade grits control (sample No. 8).

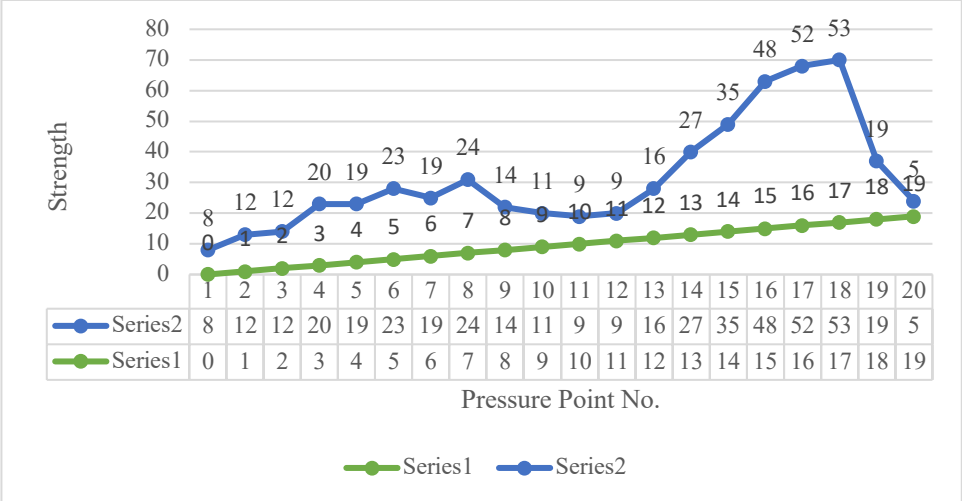


Figure 11 Strength indicators of pasta made from high-grade grits control (sample No. 9).

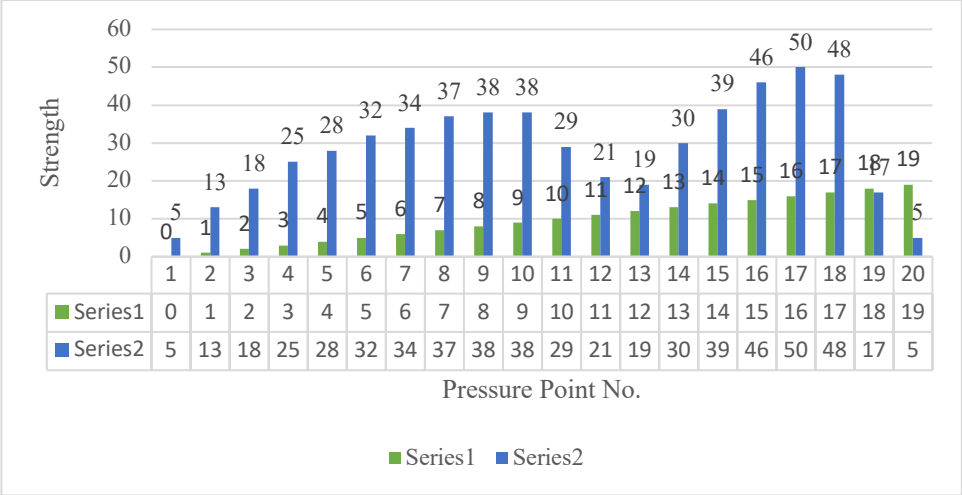


Figure 12 Strength indicators of pasta made with the addition of millet 7.7% after cooking (sample No. 9).

The strength of the pasta is measured by adding millet at 7.7% and 15.5% **[18]**, **[19]**, **[20]**, **[21]**, **[22]**. As shown in Figures 3–12, we first estimate the pasta strength of the high-grade grits control (Samples No. 1, 2, 3,4,5,6,7,8,9), which were used in the experiments.

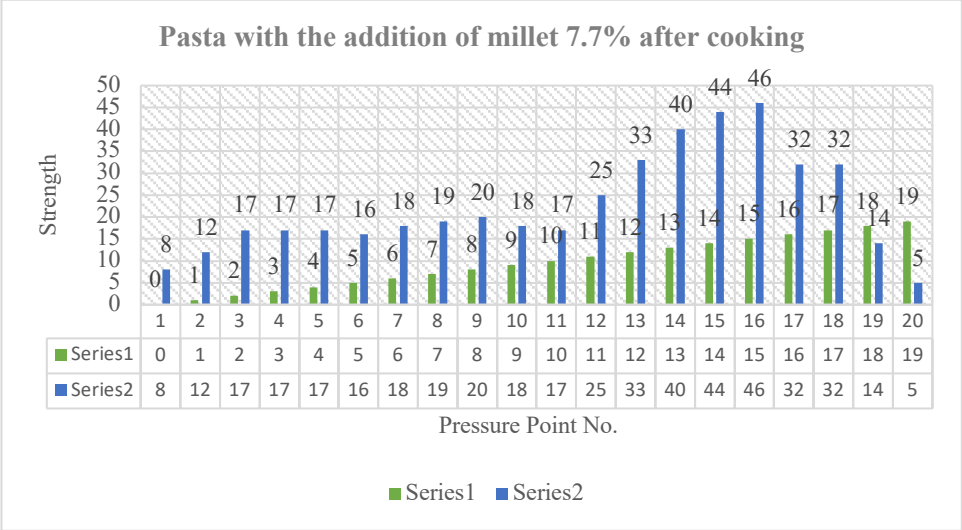


Figure 13 Data of the "Structurometer ST-1M" (sample No. 1).

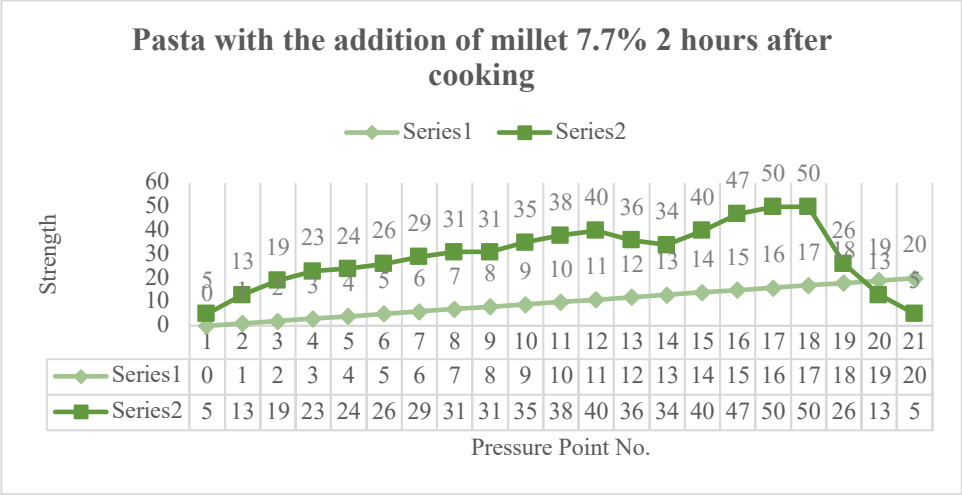


Figure 14 Data of the "Structurometer ST-1M" (sample No. 2).

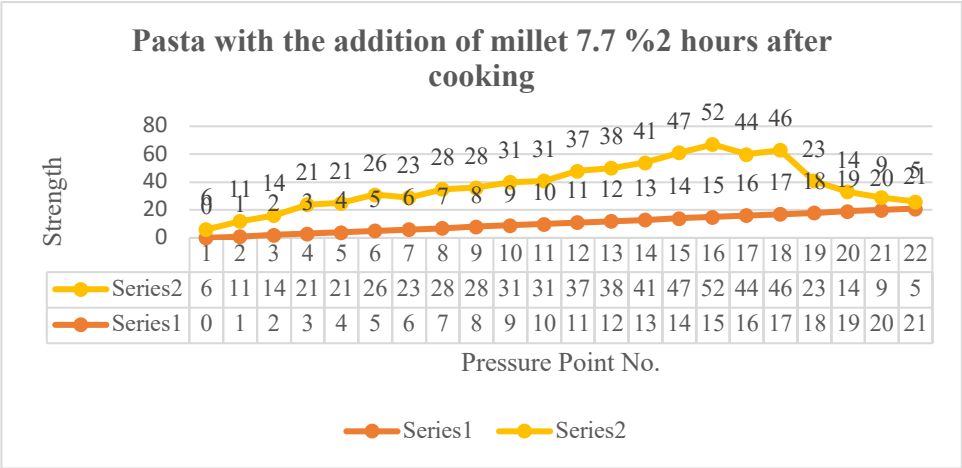


Figure 15 Data of the "Structurometer ST-1M" (sample No. 3).

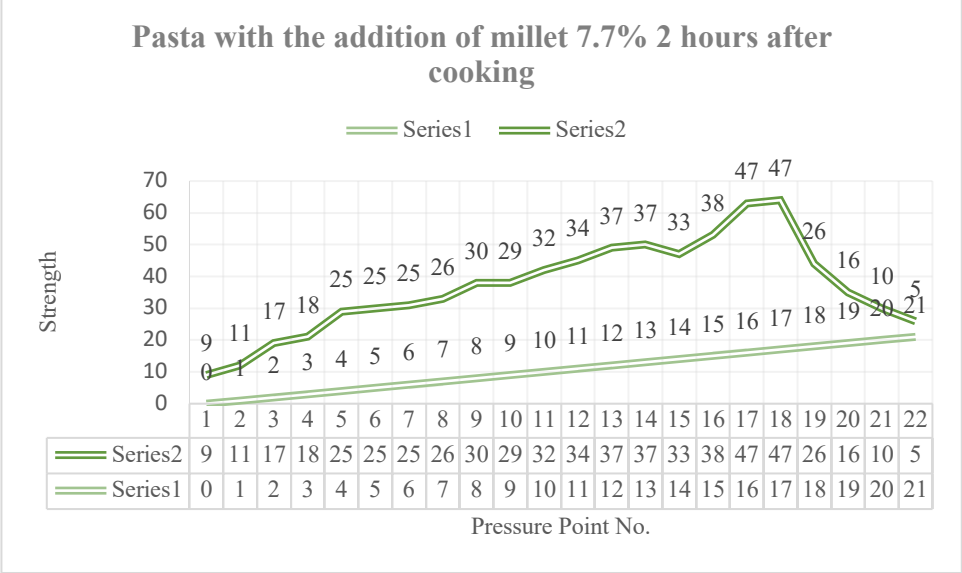


Figure 16 Data of the "Structurometer ST-1M" (sample No. 4).

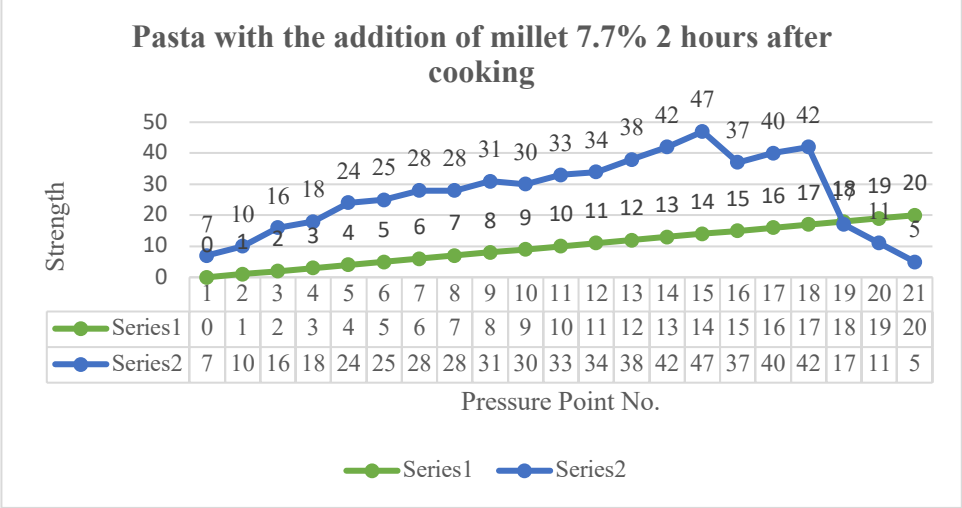


Figure 17 Data of the "Structurometer ST-1M" (sample No. 5).

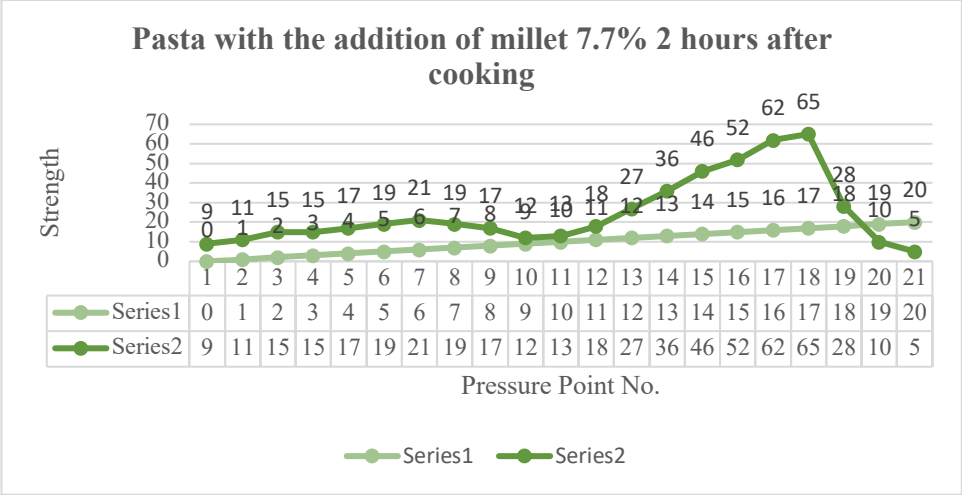


Figure 18 Data of the "Structurometer ST-1M" (sample No. 6).

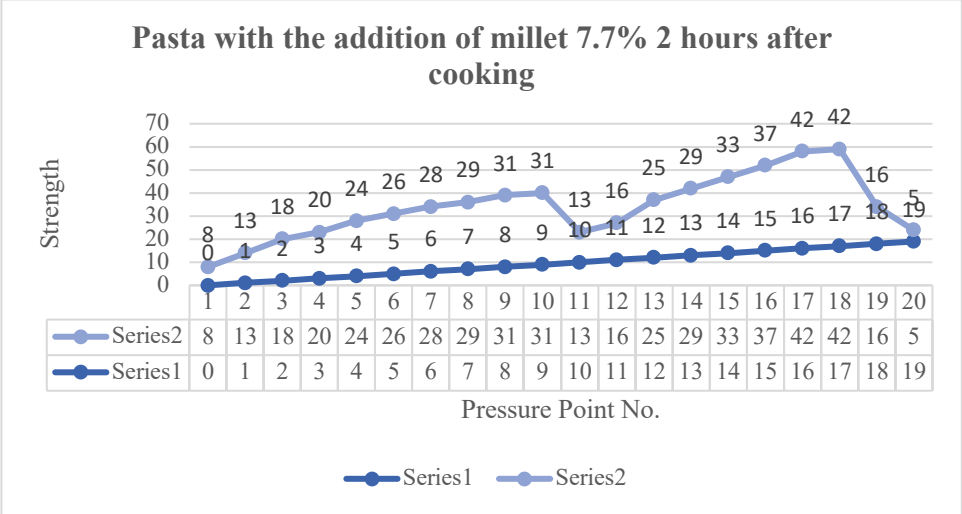


Figure 19 Data of the "Structurometer ST-1M" (sample No. 7).

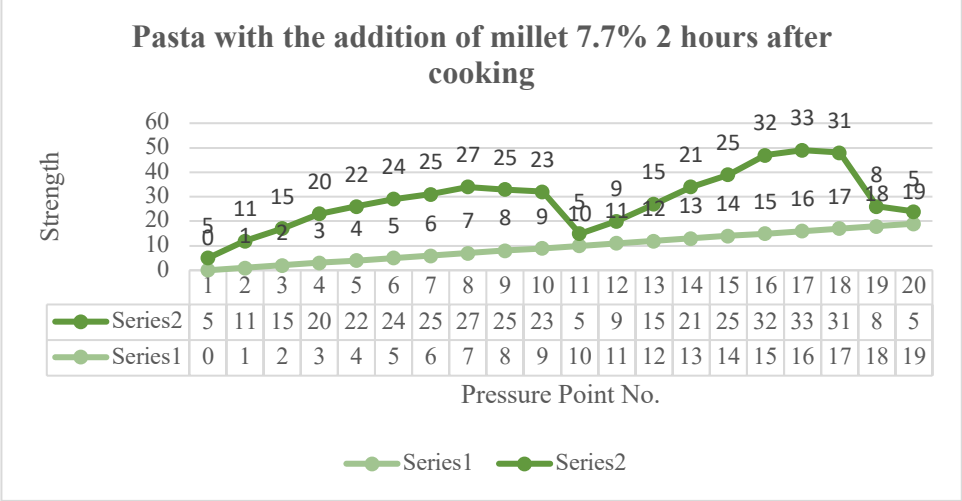


Figure 20 Data of the device "Structurometer ST-1M" (sample No. 8).

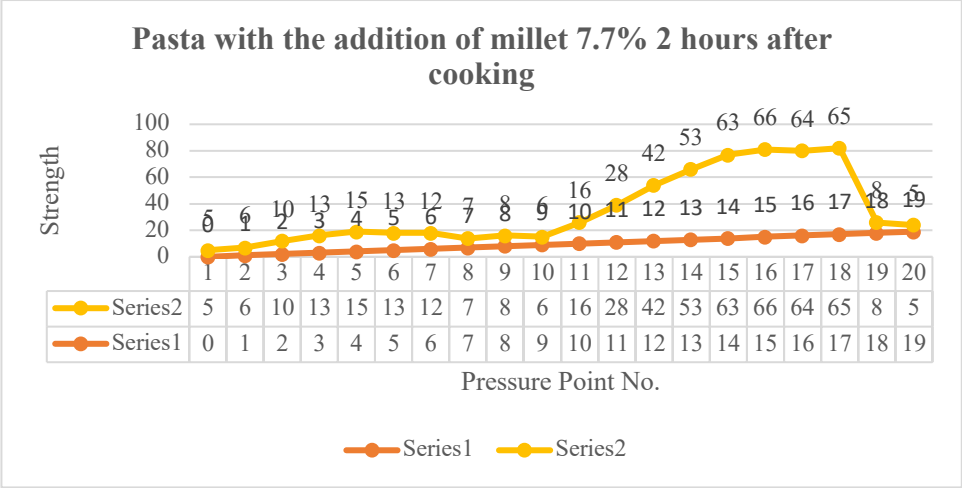


Figure 21 Data of the device "Structurometer ST-1M" (sample No. 9).

Standardized testing methods, specifically those described in GOST 31964-2012 clauses 7.7.1 and 7.7.2, are used in this study to demonstrate its dedication to thorough and trustworthy analytical preparation. A typical pasta sample (50 g) was used for testing, and the GOST standard's stipulated clauses were followed. This systematic approach is necessary to guarantee consistency and comparability in the outcomes, giving the following analytical procedures a strong basis **[23]**. Investigating pasta using the ST-1M structural meter increases the study's accuracy even more.

As was previously mentioned, the structurometer is a specialist tool that may offer in-depth information about the textural characteristics of food products. The random order in which the pasta was taken out of the vessel, in this instance, points to a careful analysis of several samples that captured a range of conditions and variances in the textural qualities of the pasta. Visualizations or graphical representations of the data from the structurometer analysis are probably shown in Figures 13-21. Understanding the patterns, trends, and variances in the texture and structure of the pasta samples is made much easier with the help of these figures. The results' visual component increases accessibility and clarity, making it easier for readers and researchers to understand the data **[24]**.

The structurometer analysis yields valuable information about the pasta samples' resilience, stiffness, and shape retention. It also enables a detailed comprehension of how adding millet affects these textural characteristics. The analysis is typical and considers any variances within the samples because of the random order in which the samples are removed from the vessel. These thorough analytical methods improve the study's validity and dependability. It exhibits a dedication to scientific rigor and a systematic approach in evaluating the effect of millet inclusion on pasta's textural characteristics **[25]**. The advancement of knowledge on incorporating alternative grains in pasta formulations depends on these in-depth analyses.

To sum up, using the structurometer ST-1M for pasta evaluation and the adherence to established testing procedures demonstrate a thorough and systematic approach to assessing the textural properties of pasta that has been millet added. Setting the bar high for similar research in the realm of food science and technology, the thorough analyses provide insightful information about the complex interactions between ingredients, processing parameters, and the structural qualities of the pasta as a result.

The research conformed to the approved methodology delineated in GOST 31964-2012, offering a uniform structure for assessing the safety and caliber of pasta compositions **[26]**. The study's findings provide intriguing new information about the mechanical characteristics of pasta that has 7.7% millet added to it, especially regarding safety as expressed in Newtons (N) **[27]**. Pasta added to the recipe "after 2 hours from the moment of cooking" and pasta added "with the addition of 7.7% millet after cooking" were the two different scenarios that were taken into consideration **[28]**.

The minor safety variations noted "after 2 hours from the moment of cooking" for pasta that had millet added indicate that the mechanical properties hold steady during this post-cooking phase. These variations range from 1-2 N. However, adding millet "after cooking" results in a more noticeable variation in safety data, ranging from 4 to 24 N. This difference could be explained by the pasta's varying moisture level at different times, affecting the work needed for mechanical testing **[29]**.

Moisture content plays an important function, particularly in the "after cooking" stages when the presence of water may impact the pasta's structural integrity. The ease of deformation is influenced by moisture content; in this case, less effort is required. The fact that driving is one of the outside elements influencing this reduction in effort highlights the importance of considering the environment while assessing the mechanical qualities of pasta **[28]**, **[29]**. The behavior of starch and gluten throughout the cooking process significantly influences the observed

differences. As water is absorbed, wheat starch in aqueous suspension swells and becomes softer **[30]**. Whereas starch granules would otherwise liquefy after boiling, gluten, a protein found in wheat, keeps the grains' mass. The starch matrix gets fixed and strengthened when gluten denaturation occurs during cooking **[31]**. The gluten denaturation process is responsible for the denser structure seen in pasta "after 2 hours from the moment of cooking". Given the impact of gluten on the mechanical characteristics of pasta, this structural densification leads to increased mechanical effort **[29]**.

In conclusion, the study explores the mechanical properties of pasta including millet, considering the influence of starch and gluten behavior during cooking and post-cooking times **[32]**. The intricate results illuminated the interactions between components and cooking methods, offering insightful knowledge for enhancing pasta recipes to satisfy safety and structural integrity standards.

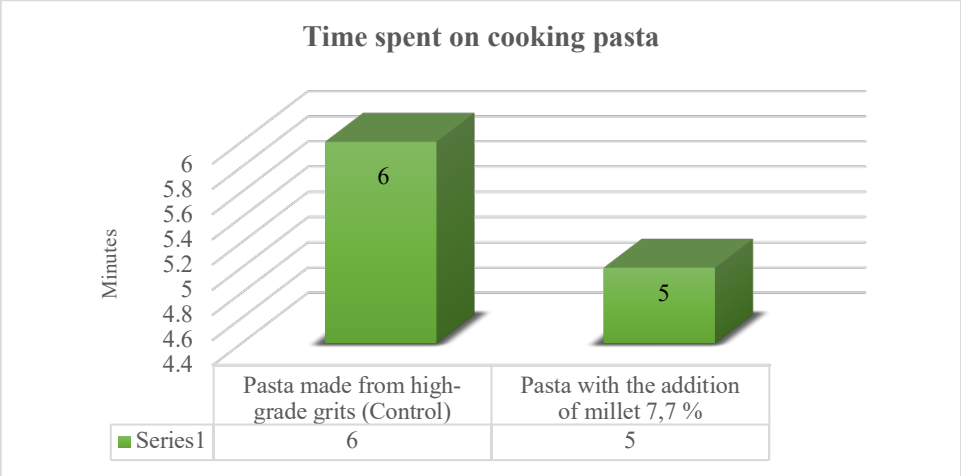


Figure 22 Time spent cooking pasta using pasta with millet 7.7% and control sample made from grade grits.

As previously mentioned, adding millet to pasta formulations improves mechanical qualities and significantly reduces cooking time **[33]**. The study emphasizes that adding 7.7% millet causes the pasta's ideal cooking time to decrease **[34]**. Given that pasta preparation efficiency heavily influences customer tastes and convenience, this shorter cooking time is a major and useful benefit **[35]**. As evaluating pasta requires maximizing its cooking properties, the results of this study support earlier research that indicates the ideal cooking time after pasta fortification should be lowered **[36]**. Here, the cooking time significantly improves over the lengthy cooking durations that product makers frequently list on labels (8 to 11 minutes) **[37]**. In this instance, the cooking time is considerably shortened to just 5 minutes. Customers' preparation time is streamlined, and there is also an energy savings benefit from this large reduction in cooking time **[38]**. Pasta readiness was evaluated following GOST 31964-2012, guaranteeing that the testing procedure complies with accepted norms. This standardized approach makes meaningful comparisons with industry norms possible, enabling consistent and reliable evaluation of pasta quality **[39]**.

The millet and pasta matrix interaction during cooking is probably the cause of the shorter cooking time. A quicker cooking procedure could result from the intrinsic qualities of millet, such as its composition and capacity for effective water absorption, which could hasten hydration and gelatinization **[40]**. This supports the reported decrease in cooking time and emphasizes millet's potential as a useful component to raise pasta products' overall cooking efficiency **[41]**. The study concludes that adding millet to pasta not only makes it safer and has better mechanical qualities but also considerably cuts down on cooking time, which helps customers and may save energy **[42]**. These findings highlight the importance of striking a balance between nutritional content, convenience, and cooking efficiency in the production of pasta products, which adds to the continuing investigation of alternative grains in pasta formulations **[42]**, **[43]**.

CONCLUSION

According to the research results, pasta with 7.7% millet added to it looks and preserves its form similarly to pasta prepared with premium quality grits, as long as the cooking time is shortened. We were able to effectively assess and compare the cooked pasta's consumer features (shape preservation indicators) over a certain length of time. Pasta takes less time to cook when millet is included. After pasta fortification, the ideal cooking time is typically shortened. Five minutes are needed for pasta cooking. However, these days, product makers list the amount of time that pasta needs to be cooked on their labels 8, 9, 10, or 11 minutes. In addition, studies on pasta were carried out based on the physicochemical characteristics (strength), and the outcomes were contrasted with a control sample (pasta prepared from premium grits). According to the investigation's findings, the strength indications are almost under control. These findings highlight the potential for adding other grains, such as millet, to improve the nutritional profile of pasta products and offer insightful information about optimizing pasta formulations for increased cooking efficiency. Intending to balance customer preferences, nutritional value, and cooking convenience in pasta formulas, the study's findings lay the groundwork for future research and product development in the food sector.

REFERENCES

1. Bchir, B., Karoui, R., Danthine, S., Blecker, C., Besbes, S., & Attia, H. (2022). Date, Apple, and Pear By-Products as Functional Ingredients in Pasta: Cooking Quality Attributes and Physicochemical, Rheological, and Sensorial Properties. In Foods (Vol. 11, Issue 10, p. 1393). MDPI AG. https://doi.org/10.3390/foods11101393
2. Minarovičová, L., Lauková, M., Kohajdová, Z., Karovičová, J., & Kuchtová, V. (2017). Effect of pumpkin powder incorporation on cooking and sensory parameters of pasta. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 11, Issue 1, pp. 373–379). HACCP Consulting. https://doi.org/10.5219/743
3. Yadav, D. N., Sharma, M., Chikara, N., Anand, T., & Bansal, S. (2014). Quality Characteristics of Vegetable-Blended Wheat–Pearl Millet Composite Pasta. In Agricultural Research (Vol. 3, Issue 3, pp. 263–270). Springer Science and Business Media LLC. https://doi.org/10.1007/s40003-014-0117-7
4. Linares-García, L., Repo-Carrasco-Valencia, R., Glorio Paulet, P., & Schoenlechner, R. (2019). Development of gluten-free and egg-free pasta based on quinoa (Chenopdium quinoa Willd) with addition of lupine flour, vegetable proteins and the oxidizing enzyme POx. In European Food Research and Technology (Vol. 245, Issue 10, pp. 2147–2156). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-019-03320-1
5. Viraktamath, C. S., Raghavendra, G., & Desikachar, H. S. R. (1971). Use of milling machinery for commercial pearling of grain sorghum (Jowar) and culinary uses for pearled sorghum products. In Journal of Food Science and Technology (Vol. 8, Issue 1, pp. 11–13). Springer Science+Business Media.
6. Purseglove, J. W. (1972). Tropical crops: Monocotyledons. Longman Group Limited edition, London, (pp. 334–336).
7. Vaidehi, M. P., Bharati, P., & Reddy, L. (1985). High-Protein Biscuits made with Ragi Flour and Oil-Seed Flour Blends. In Food and Nutrition Bulletin (Vol. 7, Issue 4, pp. 1–4). SAGE Publications. https://doi.org/10.1177/156482658500700412
8. Veena, B., Chimmad, B. V., Naik, R. K., & Shantakumar, G. (2004). Development of barnyard millet based traditional foods. In Karnataka Journal of Agricultural Sciences (Vol. 17, Issue 3, pp. 522–527). University of Agricultural Sciences.
9. Devi, G. S., Palanimuthu, V., & Arunkumar, P. (2014). Studies on development and storage of kodo millet-based Pasta. In International Journal of Processing and Post Harvest Technology (Vol. 5, Issue 1, pp. 33–40). Hind Agri-Horticultural Society.
10. Szedljak, I., Szántai-Kőhegyi, K., Tóth, M., & Bernhardt, B. (2014). Monitoring of chemical parameters of qualitative pasta samples containing millet flours during storage experiments. In Acta Alimentaria (Vol. 43, Issue Supplement 1, pp. 156–163). Akademiai Kiado Zrt. https://doi.org/10.1556/aalim.43.2014.suppl.22
11. Authority nutrition. (2023, October 20). In Authority Nutrition. Retrieved from https://www.authoritynutrition.net/pitanie/.
12. Macaroni products. GOST 31964-2012. Pasta products. Acceptance rules and methods of quality determination.
13. Lawal, O. M., Sanni, O., Oluwamukomi, M., Fogliano, V., & Linnemann, A. R. (2021). The addition of fluted pumpkin (Telfairia occidentalis) leaf powder improves the techno-functional properties of cassava pasta. In Food Structure (Vol. 30, p. 100241). Elsevier BV. https://doi.org/10.1016/j.foostr.2021.100241

14. Tagliasco, M., Fogliano, V., & Pellegrini, N. (2021). Pasta regrind: The effect of drying temperature on its functionality as a novel ingredient. In Food Structure (Vol. 30, p. 100230). Elsevier BV. https://doi.org/10.1016/j.foostr.2021.100230
15. Yan, W., Yin, L., Zhang, M., Zhang, M., & Jia, X. (2021). Gelatinization, Retrogradation and Gel Properties of Wheat Starch–Wheat Bran Arabinoxylan Complexes. In Gels (Vol. 7, Issue 4, p. 200). MDPI AG. https://doi.org/10.3390/gels7040200
16. Karimova, G., Niyazbekova, R., Al Azzam, K., Mashanova, N., Negim, E.-S., & Ibzhanova, A. (2023). Development of new technologies (recipes) to produce pasta with the addition of millet and the determination of organoleptic and physicochemical quality indicators. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 371–390). HACCP Consulting. https://doi.org/10.5219/1829
17. GOST 572-2016 "Millet grain ground. Technical conditions". Retrieved from
18. Schmid, T., Loschi, M., Hiltbrunner, J., & Müller, N. (2023). Assessment of the suitability of millet for the production of pasta. In Applied Food Research (Vol. 3, Issue 1, p. 100247). Elsevier BV. https://doi.org/10.1016/j.afres.2022.100247
19. Jalgaonkar, K., Jha, S. K., Mahawar, M. K., & Yadav, D. N. (2019). Pearl millet based pasta: optimization of extrusion process through response surface methodology. In Journal of Food Science and Technology (Vol. 56, Issue 3, pp. 1134–1144). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-019-03574-3
20. Wójtowicz, A., Combrzyński, M., Biernacka, B., Oniszczuk, T., Mitrus, M., Różyło, R., Gancarz, M., & Oniszczuk, A. (2023). Application of Edible Insect Flour as a Novel Ingredient in Fortified Snack Pellets: Processing Aspects and Physical Characteristics. In Processes (Vol. 11, Issue 9, p. 2561). MDPI AG. https://doi.org/10.3390/pr11092561
21. Jakab, I., Tormási, J., Dhaygude, V., Mednyánszky, Zs., Sipos, L., & Szedljak, I. (2020). Cricket flour-laden millet flour blends' physical and chemical composition and adaptation in dried pasta products. In Acta Alimentaria (Vol. 49, Issue 1, pp. 4–12). Akademiai Kiado Zrt. https://doi.org/10.1556/066.2020.49.1.2
22. Ho, I., Peterson, A., Madden, J., Huang, E., Amin, S., & Lammert, A. (2022). Will It Cricket? Product Development and Evaluation of Cricket (Acheta domesticus) Powder Replacement in Sausage, Pasta, and Brownies. In Foods (Vol. 11, Issue 19, p. 3128). MDPI AG. https://doi.org/10.3390/foods11193128
23. Carcea, M. (2020). Quality and Nutritional/Textural Properties of Durum Wheat Pasta Enriched with Cricket Powder. In Foods (Vol. 9, Issue 9, p. 1298). MDPI AG. https://doi.org/10.3390/foods9091298
24. Coello, K. E., Peñas, E., Martinez-Villaluenga, C., Elena Cartea, M., Velasco, P., & Frias, J. (2021). Pasta products enriched with moringa sprout powder as nutritive dense foods with bioactive potential. In Food Chemistry (Vol. 360, p. 130032). Elsevier BV. https://doi.org/10.1016/j.foodchem.2021.130032
25. Bchir, B., Karoui, R., Danthine, S., Blecker, C., Besbes, S., & Attia, H. (2022). Date, Apple, and Pear By-Products as Functional Ingredients in Pasta: Cooking Quality Attributes and Physicochemical, Rheological, and Sensorial Properties. In Foods (Vol. 11, Issue 10, p. 1393). MDPI AG.
26. Lisiecka, K., Wójtowicz, A., Dziki, D., & Gawlik-Dziki, U. (2019). The influence of Cistus incanus L. leaves on wheat pasta quality. In Journal of Food Science and Technology (Vol. 56, Issue 9, pp. 4311–4322). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-019-03900-9
27. Benhur, D. R., Bhargavi, G., Kalpana, K., Vishala, A. D., Ganapathy, K. N., & Patil, J. V. (2015). Development and standardization of sorghum pasta using extrusion technology. In Journal of Food Science and Technology (Vol. 52, Issue 10, pp. 6828–6833). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-015-1801-8
28. Bianchi, F., Tolve, R., Rainero, G., Bordiga, M., Brennan, C. S., & Simonato, B. (2021). Technological, nutritional and sensory properties of pasta fortified with agro-industrial by-products: a review. In International Journal of Food Science & Technology (Vol. 56, Issue 9, pp. 4356–4366). Wiley. https://doi.org/10.1111/ijfs.15168
29. Simonato, B., Trevisan, S., Tolve, R., Favati, F., & Pasini, G. (2019). Pasta fortification with olive pomace: Effects on the technological characteristics and nutritional properties. In LWT (Vol. 114, p. 108368). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.108368
30. Tolve, R., Pasini, G., Vignale, F., Favati, F., & Simonato, B. (2020). Effect of Grape Pomace Addition on the Technological, Sensory, and Nutritional Properties of Durum Wheat Pasta. In Foods (Vol. 9, Issue 3, p. 354). MDPI AG. https://doi.org/10.3390/foods9030354
31. Çabuk, B., & Yılmaz, B. (2020). Fortification of traditional egg pasta (erişte) with edible insects: nutritional quality, cooking properties and sensory characteristics evaluation. In Journal of Food Science and Technology (Vol. 57, Issue 7, pp. 2750–2757). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-020-04315-7

32. Tolve, R., Simonato, B., Rainero, G., Bianchi, F., Rizzi, C., Cervini, M., & Giuberti, G. (2021). Wheat Bread Fortification by Grape Pomace Powder: Nutritional, Technological, Antioxidant, and Sensory Properties. In Foods (Vol. 10, Issue 1, p. 75). MDPI AG. https://doi.org/10.3390/foods10010075
33. Ozgoren, E., & Yapar, A. (2018). Effect of the addition of smoked trout fillet powder to the quality properties of pastan[JB]. In Italian Journal of Food Science, 31(1). https://doi.org/10.14674/IJFS-1263
34. Hammad, E., & Abo-Zaid, E. (2020). Properties of Noodles Fortification with Turnip Leave powder. In Journal of Food and Dairy Sciences (Vol. 11, Issue 7, pp. 209–213). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/jfds.2020.111755
35. Kamble, V., Bhuvaneshwari, G., Jagadeesh, S. L., M. Ganiger, V., & Terdal, D. (2018). Development and Evaluation of Cooking Properties of Instant Noodles Incorporated with Drumstick Leaf Powder and Defatted Soybean Flour. In International Journal of Current Microbiology and Applied Sciences (Vol. 7, Issue 2, pp. 3642–3651). Excellent Publishers. https://doi.org/10.20546/ijcmas.2018.702.433
36. Yang, T., Wang, P., Zhou, Q., Zhong, Y., Wang, X., Cai, J., Huang, M., & Jiang, D. (2022). Effects of Different Gluten Proteins on Starch's Structural and Physicochemical Properties during Heating and Their Molecular Interactions. In International Journal of Molecular Sciences (Vol. 23, Issue 15, p. 8523). MDPI AG. https://doi.org/10.3390/ijms23158523
37. Fan, J.-L., Han, N., & Chen, H.-Q. (2021). Physicochemical and structural properties of wheat gluten/rice starch dough-like model. In Journal of Cereal Science (Vol. 98, p. 103181). Elsevier BV. https://doi.org/10.1016/j.jcs.2021.103181
38. Cappelli, A., Oliva, N., & Cini, E. (2020). A Systematic Review of Gluten-Free Dough and Bread: Dough Rheology, Bread Characteristics, and Improvement Strategies. In Applied Sciences (Vol. 10, Issue 18, p. 6559). MDPI AG. https://doi.org/10.3390/app10186559
39. Ren, Y., Linter, B. R., Linforth, R., & Foster, T. J. (2020). A comprehensive investigation of gluten free bread dough rheology, proving and baking performance and bread qualities by response surface design and principal component analysis. In Food & Function (Vol. 11, Issue 6, pp. 5333–5345). Royal Society of Chemistry (RSC). https://doi.org/10.1039/d0fo00115e
40. Brandner, S., Becker, T., & Jekle, M. (2019). Classification of starch-gluten networks into a viscoelastic liquid or solid, based on rheological aspects — A review. In International Journal of Biological Macromolecules (Vol. 136, pp. 1018–1025). Elsevier BV. https://doi.org/10.1016/j.ijbiomac.2019.06.160
41. Yano, H. (2019). Recent practical researches in the development of gluten-free breads. In npj Science of Food (Vol. 3, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41538-019-0040-1
42. Bresciani, A., Pagani, M. A., & Marti, A. (2022). Pasta-Making Process: A Narrative Review on the Relation between Process Variables and Pasta Quality. In Foods (Vol. 11, Issue 3, p. 256). MDPI AG. https://doi.org/10.3390/foods11030256
43. Ospanov, A., Muslimov, N., Timurbekova, A., Nurdan, D., & Zhalelov, D. (2022). Mixing of flour mixture components in the production of pasta from nontraditional raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 375–387). HACCP Consulting. https://doi.org/10.5219/1749

Funds:

This research received no external funding.

Acknowledgments:

eng - IRN BR12967830 "Development of technical regulation tools to improve the efficiency, safety, resource-saving of food production and eco-friendly packaging."

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Gulmaida Karimova**, Standardization, Certification and Metrology" (by industry), Technical Faculty, Department of Standardization, Metrology and Certification NAO, Kazakh Agrotechnical University named after S. Seifullin, Kazakhstan,
E-mail: Gulmaida@mail.ru
ORCID: https://orcid.org/0000-0002-7190-8847

Rimma Niyazbekova, Standardization, Certification and Metrology" (by industry), Technical Faculty, Department of Standardization, Metrology and Certification NAO, Kazakh Agrotechnical University named after S. Seifullin, Kazakhstan,
E-mail: rimma.n60@mail.ru
ORCID: https://orcid.org/0000-0001-8688-1408

Khaldun Al Azzam, The University of Jordan, Department of Chemistry, School of Science, 11942 Amman, Jordan,
E-mail: azzamkha@yahoo.com
ORCID: https://orcid.org/0000-0003-4097-6991

El-Sayed Negim, Satbayev University, School of Petroleum Engineering, 22 Satpayev Street, 050013 Almaty, Kazakhstan; School of Materials Science and Green Technologies, Kazakh-British Technical University, 59 Tole bi St., 050000, Almaty, Kazakhstan,
E-mail: elashmawi5@yahoo.com
ORCID: https://orcid.org/0000-0002-4370-8995

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 2.11.2023
Revised: 12.1.2024
Accepted: 1.2.2024
Published: 19.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 139-156
https://doi.org/10.5219/1935
ISSN: 1337-0960 online
www.potravinarstvo.com

Enhancing food packaging with nanofillers: properties, applications, and innovations

Elena Pavlenko, Evgenia Semkina, Alexander Pokhilko, Ekaterina Sukhanova, Victor Fursov, Natalia Lazareva, Alexander Pyanov, Igor Baklanov

ABSTRACT

Food packaging is undergoing a revolutionary change because of nanotechnology. This paper examines the influence of nanofillers on improving food packaging materials. Due to their distinctive qualities, nanofillers like nanoparticles and nanocomposites change food product protection, preservation, and appearance. The advantages of nanofillers, which may be controlled in terms of mechanical, barrier, thermal, optical, and surface characteristics, are described in the article along with their definition. Systematic examination exposes the many functions of nanofillers, from maintaining food safety with antibacterial Nano silver to increasing shelf life with better barriers. They also support sustainability and clever packaging. Innovative applications for real-time food monitoring are investigated, along with case examples demonstrating their efficacy, including nanocomposite materials, nanosensors, and nanotags. The use of nanofillers is evaluated concerning safety, compliance, cost-effectiveness, and environmental impact. Future research trends that will influence food packaging are outlined. A critical step toward developing safer, more robust, and environmentally friendly packaging solutions is represented by nanofillers. This review is a valuable tool for anyone involved in food packaging since it provides information on disruptive technology transforming food packaging into an intelligent, environmentally friendly guardian of food quality and safety.

Keywords: nano-biosensors, food safety, food quality, contaminants, pathogens, nanomaterials

INTRODUCTION

Food packaging has undergone a transformative evolution, adapting to the dynamic landscape of consumer preferences, industry regulations, and environmental imperatives. In this era of rapid technological advancement, a remarkable shift has occurred in how we conceptualize, design, and produce food packaging. Integrating nanofillers into food packaging materials is at the forefront of this paradigm shift, marking a noteworthy chapter in the annals of packaging science and technology **[1]**, **[2]**, **[3]**. Nanofillers, hailing from nanotechnology, have emerged as a transformative force in food packaging. Engineered at the nanoscale, these materials possess unparalleled properties that empower food packaging materials to rise above traditional constraints **[4]**.

The marriage of nanotechnology with food packaging has brought novel possibilities and transformative innovations. Nanofillers, nanoparticles, or nanocomposites integrated into packaging materials promise to improve the performance, safety, and sustainability of food packaging while concurrently addressing the contemporary challenges faced by the food industry. As a result, the intersection of nanoscience and food packaging has instigated a profound reimagining of how we preserve, transport, and consume our sustenance **[5]**, **[6]**.

This review paper embarks on a comprehensive exploration of the multifaceted realm of nanofillers in food packaging. By consolidating and synthesizing the wealth of research, development, and practical applications, we

endeavor to provide a holistic understanding of the opportunities and complexities engendered by nanofillers in the food packaging landscape.

Traditional food packaging materials, while widely used, exhibit several limitations that challenge their ability to meet evolving consumer demands and environmental considerations. These limitations have catalyzed the exploration of new materials and technologies in food packaging to address these shortcomings. Here, we elaborate on the constraints of traditional food packaging materials:

Environmental Impact: Traditional food packaging materials, particularly those derived from fossil-based plastics, contribute significantly to environmental issues such as plastic waste accumulation and pollution. These materials persist in the environment for extended periods, to ecosystems and human health **[7]**.

Limited Shelf-Life: Conventional food packaging materials may inadequately protect food products from environmental factors like moisture, oxygen, and light. This deficiency can result in a shorter shelf-life for packaged foods, impacting product quality and sustainability **[8]**.

Lack of Functionality: Traditional food packaging materials often lack the advanced functionality required to meet modern consumer needs. For instance, they may be unable to monitor food quality or provide real-time information about the product's condition, which has become increasingly important for consumers **[8]**.

Non-Biodegradable: Many traditional food packaging materials are non-biodegradable, contributing to long-term environmental consequences. Their persistence in landfills and natural environments exacerbates ecological concerns and resource depletion **[8]**.

Limited Mechanical Properties: Traditional food packaging materials may not always exhibit the necessary mechanical properties to withstand physical abuse during handling, transportation, and storage. Additionally, they may fall short of providing optimal barrier properties against external contaminants **[8]**.

Various innovations have emerged in the dynamic realm of food packaging to tackle industry challenges and drive progress. Materials, presenting a sustainable alternative to conventional plastics. These innovative packaging materials are meticulously designed to mitigate environmental impact and promote sustainability, aligning with the growing demand for eco-friendly solutions. Another transformative innovation is the integration of nanofillers, meticulously engineered at the nanoscale, into packaging materials. These nanofillers bolster critical attributes like mechanical strength and barrier properties, revolutionizing food packaging by making it safer and more efficient. Moreover, active packaging systems, specifically engineered to interact with packaged food, have emerged. These systems offer an array of functions, from moisture control to antimicrobial properties and gas scavenging, collectively extending the shelf life of products and elevating food safety standards. Complementing these innovations is the advent of innovative packaging technologies featuring the seamless integration of sensors and indicators within packaging materials. This technological leap enables real-time food quality and safety monitoring, providing consumers with invaluable information while contributing to a more informed and streamlined food supply chain **[9]**, **[10]**.

The limitations of traditional food packaging materials have led to a quest for innovation in food packaging. Nanofillers have the potential to enhance food packaging by improving mechanical strength and barrier properties. However, several challenges must be addressed, including cost considerations, regulatory compliance, compatibility, and toxicity concerns. Biopolymer-based packaging materials, including nanofillers and active agents, have become of greater interest due to their biodegradability, renewability, and biocompatibility. They offer potential solutions to the challenges of traditional packaging materials. Using natural polymer-based nanocomposites in food packaging and agriculture has also received significant attention. These nanocomposites consist of natural biopolymers and nanofillers, which can act as slow-release nanocarriers for delivering agrochemicals or as direct product coatings to extend product shelf life or improve seed germination or protection from pathogens and pests. Incorporating thermosensitive nanohydrogels in active packaging can also modulate the delivery mechanism of bioactive agents. These innovative packaging solutions aim to improve food chain sustainability, reduce food waste, and provide safer and higher-quality products **[11]**, **[12]**.

This document embarks on an in-depth exploration of nanofillers and their pivotal role in revolutionizing food packaging. Throughout this review, we will delve into the myriad facets of nanofillers, uncovering their intrinsic properties and the fundamental mechanisms by which they enhance food packaging materials. We will navigate the vast landscape of applications where nanofillers exhibit remarkable potential, from safeguarding the quality and shelf-life of food products to pioneering intelligent packaging that responds to environmental cues. Moreover, we will critically evaluate nanofiller integration's challenges and prospects, including cost considerations, regulatory compliance, and safety concerns.

What are nanofillers

Nanofillers are materials engineered and manipulated at the nanoscale, typically with dimensions ranging from one to 100 nanometers (nm). These materials are introduced into food packaging matrices to confer specific and

tailored properties to the packaging materials. The unique characteristics of nanofillers arise from their tiny size and high surface area-to-volume ratio, which can lead to extraordinary changes in the behavior and performance of the packaging material. The challenges associated with incorporating nanofillers into polymer matrices for food packaging include cost, regulations, compatibility, uniform dispersion, toxicity, and environmental impact. However, nanofillers can potentially improve the properties of polymer-based food packaging materials, such as barrier properties, shelf-life, mechanical properties, and sustainability. Ongoing research is focused on addressing these challenges to develop safe, effective, and sustainable nanofiller-based food packaging systems **[11]**, **[12]**, **[13]**, **[14]**, **[15]**.

At the nanoscale, materials exhibit unique properties and behaviors that differ from their bulk counterparts. Nanofillers, which can be nanoparticles or nanocomposites, are engineered and manipulated at the nanoscale and can interact more intimately with the packaging matrix due to their small size. The high surface area-to-volume ratio of nanofillers means that a significant proportion of their atoms or molecules are on the surface, leading to enhanced surface interactions, reactivity, and the ability to modify the surface properties of the packaging material. This advantage of nanofillers can be applied to various fields, such as dentistry, energy storage, and structural nanocomposites **[16]**, **[17]**. Figure 1 illustrates material uses in food packaging applications.

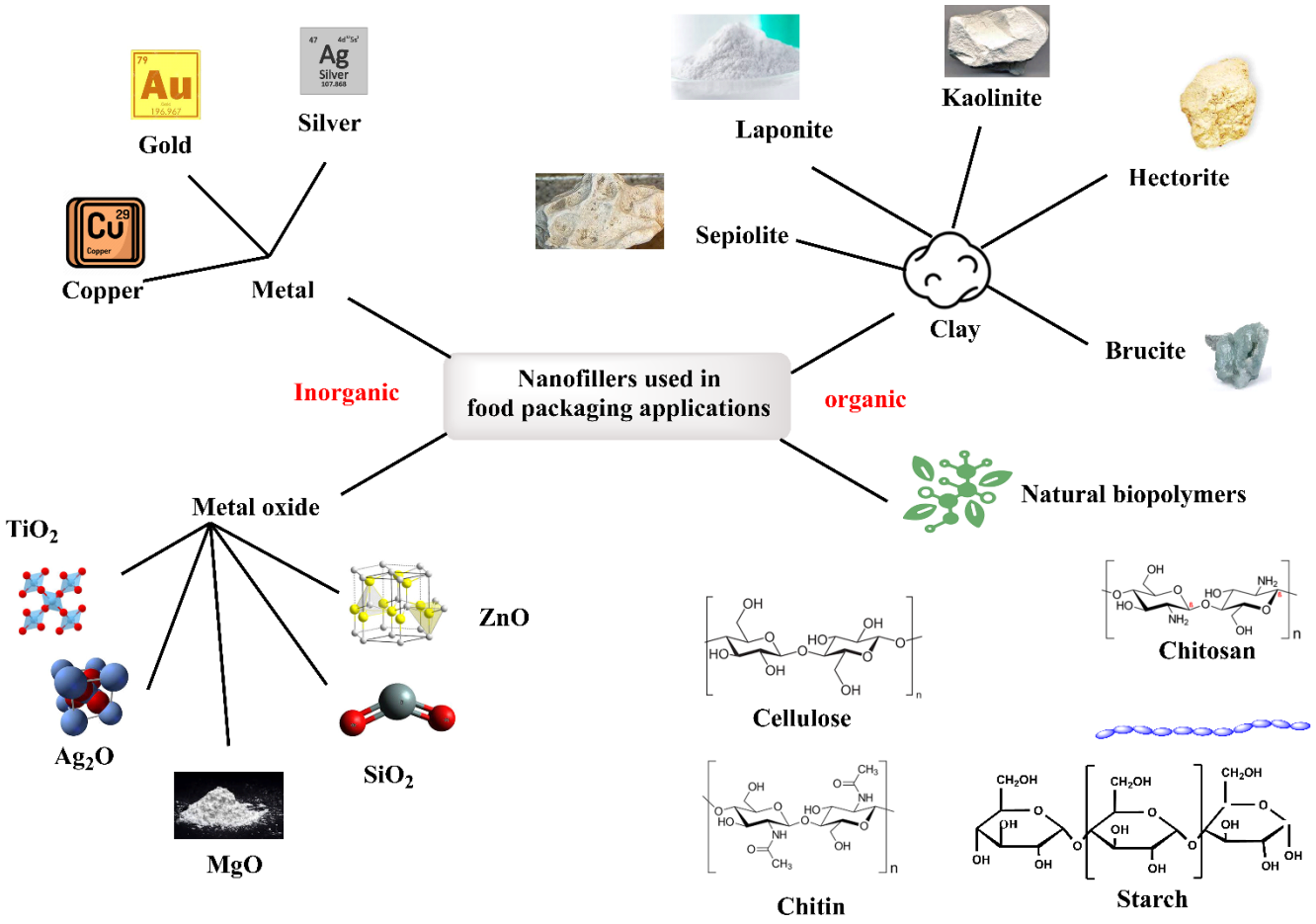


Figure 1 Various types of nanofillers are used in food packaging.

Nanofillers used in food packaging can be categorized into two main types:

Nanoparticles: Nanoparticles come in various forms, including metal nanoparticles (e.g., silver nanoparticles), metal oxide nanoparticles (e.g., titanium dioxide), and clay nanoparticles (e.g., montmorillonite). Each type of nanoparticle imparts distinct properties to the packaging material. For example, silver nanoparticles can provide antimicrobial properties, while titanium dioxide nanoparticles can enhance the packaging material's UV resistance.

Nanocomposites: Nanocomposites are created by dispersing nanoparticles within a polymer or other matrix material. This blending of nanoparticles with the matrix leads to enhanced mechanical strength, improved barrier properties, and other desirable characteristics. For instance, adding clay nanoparticles to a polymer matrix can improve the packaging material's resistance to moisture and oxygen, making it more effective in preserving food freshness **[18]**, **[19]**, **[20]**.

Nanofillers play a pivotal role in enhancing the performance of food packaging materials, contributing to various aspects of their functionality. They excel in augmenting barrier properties, bolstering mechanical strength, fortifying thermal stability, providing optical enhancements, and transforming surface characteristics. Nanofillers can exist as nanoparticles or nanocomposites in diverse forms such as metal nanoparticles, metal oxide nanoparticles, clay nanoparticles, and cellulose nanocrystals **[1]**, **[2]**, **[3]**, **[21]**.

Incorporating nanofillers into food packaging materials offers an avenue for substantial improvement in properties like water vapor permeability, hydrophilicity, and transparency, all while preserving visual appeal. However, it's important to note that while nanofillers can significantly enhance several attributes, they may have a more limited impact on the mechanical properties of the packaging material. The use of nanofillers in food packaging is subject to rigorous regulatory scrutiny, underscoring the need for extensive research to ensure their safety. Ongoing investigations are geared towards overcoming the challenges associated with integrating nanofillers into polymer matrices for food packaging, with the ultimate goal of developing safe, effective, and sustainable nanofiller-based food packaging systems. The array of nanofillers employed in food packaging encompasses nanoclays, nanosilver, nanofibers, nanotubes, and other emerging nanofillers. Nanoclays primarily enhance barrier properties and mechanical strength, while nanosilver particles showcase potent antimicrobial capabilities. Nanofibers and nanotubes are instrumental in elevating mechanical and barrier properties. Emerging nanofillers, characterized by their unique properties, continue to fuel innovations in food packaging **[11]**, **[22]**, **[23]**, **[24]**.

Mechanical Properties of Nanofillers

Due to their tiny size and distinctive structural characteristics, Nanofillers significantly enhance the mechanical properties of food packaging materials. Incorporating nanofillers enables packaging materials to exhibit superior strength, durability, and resistance to mechanical stresses. These enhancements ensure that packaging materials can withstand various challenges encountered during food processing, transportation, storage, and consumer handling. One significant improvement brought about by nanofillers is in the area of tensile strength. Nanofillers strengthen the packaging material's resistance to stretching or tension, improving tensile strength. This means the material is less likely to tear or deform under mechanical stress, which is particularly important during manufacturing processes, transportation, and consumer handling **[1]**, **[3]**, **[9]**, **[13]**, **[25]**, **[26]**, **[27]**.

Furthermore, nanofillers contribute to improved flexural strength. They enhance the material's ability to withstand bending or flexing without breaking. This property prevents packaging materials from cracking or fracturing when subjected to external forces. Packaging materials incorporating nanofillers also demonstrate increased impact resistance. This means they are better equipped to withstand sudden blows or impacts, which is critical for protecting the integrity of the food products contained within, especially during transit or when subject to accidental impact **[1]**, **[2]**, **[26]**, **[27]**.

Another essential mechanical property influenced by nanofillers is toughness. Nanofillers enhance the packaging material's overall toughness, combining strength and flexibility. This allows the material to absorb energy without catastrophic failure, making it less likely to rupture or break under stress. Additionally, nanofillers can contribute to reduced permeability to gases. Certain nanofillers, such as clay nanoparticles, can significantly reduce the permeability of packaging materials to gases like oxygen and carbon dioxide. This property is vital for extending the shelf life of food products by preventing gas exchange with the external environment. Moreover, nanofillers can improve abrasion resistance. This is particularly important for packaging materials subjected to repeated friction during handling and transportation. Enhanced resistance to abrasion reduces wear and tear, ensuring the packaging remains intact and effective. Nanofillers also offer the flexibility to tailor mechanical properties based on specific packaging requirements. By adjusting the type and concentration of nanofillers, packaging materials can be customized to meet the demands of different food products. This adaptability and resilience contribute to the overall performance and safety of food packaging, ensuring that products reach consumers in optimal condition **[3]**, **[9]**, **[13]**, **[28]**.

Thermal Properties of Nanofillers

The thermal properties of food packaging materials are vital for maintaining the quality and safety of food products during various stages, from processing to transportation, storage, and preparation. Nanofillers are crucial in influencing these thermal properties, offering significant advantages that enhance food packaging materials' overall performance and functionality. One primary benefit is the improved thermal stability provided by nanofillers. They bolster the packaging material's ability to withstand high-temperature processes such as hot filling, retort sterilization, and microwave heating. This heightened thermal stability ensures the packaging remains intact and effective throughout the product's lifecycle, contributing to food safety and quality **[29]**, **[30]**.

Another advantage is the resistance to heat transfer achieved through nanofillers. By reducing the heat transfer rate, nanofillers are particularly valuable in insulating or protecting heat-sensitive food products, including frozen or chilled items. This property helps maintain the desired temperature conditions within the packaging, ensuring the food's quality and safety. Furthermore, nanofillers can enhance thermal insulation properties in packaging materials. This is especially valuable for packaging designed to keep hot and cold foods cold, preserving the product's temperature for an extended period **[31]**.

Some nanofillers are tailored to interact with microwave radiation, making packaging materials suitable for microwave cooking or reheating. This feature adds convenience for consumers and expands the functionality of food packaging, facilitating quick and efficient meal preparation. Nanofillers can also promote uniform heat distribution within packaging materials, preventing hot spots and ensuring food products are heated or cooked evenly. This uniformity is essential for maintaining consistent product quality. Additionally, nanofillers enhance the thermal resistance of printing inks and labels on packaging materials. This ensures that crucial information and branding elements remain intact, even when exposed to high-temperature processes. Lastly, nanofillers extend the temperature tolerance range of packaging materials, making them suitable for a broader range of food applications, including those involving extreme temperatures. This adaptability and resilience contribute to the versatility and effectiveness of nanofiller-enhanced food packaging materials, addressing diverse food processing and storage needs while upholding food safety and quality standards.

Optical Properties of Nanofillers

Nanofillers significantly impact the optical properties of food packaging materials, improving transparency, UV resistance, and overall visual appeal. They enhance transparency, enabling consumers to view packaged food easily, which is vital for products requiring visual inspection. Some nanofillers possess UV-blocking properties, protecting light-sensitive products from UV radiation. Nanofillers can also reduce light scattering, resulting in a more uniform appearance, and they can enhance or modify the color of packaging material for branding and marketing purposes **[27]**, **[32]**.

Additionally, nanofillers can reduce glare, improve glossiness, enhance print quality, and maintain visual consistency. Beyond aesthetics, nanofillers play a functional role by providing UV protection and maintaining transparency, essential for preserving food quality and safety. These enhancements contribute to the overall effectiveness of nanofiller-enhanced food packaging materials and consumer appeal. Nanofillers can have a transformative effect on the surface properties of food packaging materials due to their nanoscale size and unique surface characteristics. They can modify surface wettability, making surfaces more hydrophobic or hydrophilic as needed for specific food products. Some nanofillers, like nanosilver particles, offer antimicrobial properties, inhibiting microorganism growth on the package's exterior **[1]**, **[33]**.

Nanofillers can influence surface texture, creating smoother or rougher surfaces based on size and distribution. Softer surfaces are easier to clean and are suitable for specific food applications, while rougher surfaces can enhance adhesion or provide tactile feedback. They can also improve printability, acting as taste and odor barriers and giving anti-fog properties for refrigerated or frozen food packaging. Furthermore, nanofillers enhance adhesion for labels and tapes, ensuring they remain securely attached throughout the product's lifecycle. The versatility of nanofillers allows for customized surface functionality tailored to specific food packaging requirements. Overall, nanofillers play a vital role in optimizing the surface properties of food packaging materials, enhancing their functionality and performance in various applications **[1]**, **[34]**.

Applications of Nanofillers in Food Packaging

Nanofillers have found a wide array of applications in food packaging, transforming conventional packaging materials into advanced systems that meet the evolving demands of the food industry. These applications harness the unique properties of nanofillers to enhance food safety, extend shelf life, and improve packaging functionality. Here, we explore the diverse spectrum of applications wherein nanofillers exhibit remarkable potential:

One of the primary applications of nanofillers in food packaging is extending shelf life. Nanofillers are instrumental in creating packaging materials that enhance barrier properties against gases, moisture, and microorganisms. This results in packaging that preserves the freshness and quality of food products, reducing food waste and ensuring longer shelf life. Nanofillers, particularly those with antimicrobial properties, enhance food safety and preservation. They inhibit the growth of bacteria, molds, and other pathogens on the packaging surface, reducing the risk of contamination. This application is critical for ensuring the safety of perishable food products. The integration of nanofillers enables the development of smart packaging solutions. These intelligent systems can respond to environmental conditions, such as temperature and moisture changes, and provide real-time information about the food product's freshness and safety. Smart packaging enhances consumer confidence. It ensures products are consumed at peak quality **[1]**, **[2]**, **[3]**, **[21]**.

Nanofillers contribute to the development of sustainable and eco-friendly food packaging materials. By reducing the environmental impact of packaging materials, such as fossil-based plastics, nanofillers align with the global push for eco-conscious packaging solutions. This application addresses ecological concerns and supports sustainable packaging practices. Nanofillers improve the mechanical properties of packaging materials, making them more durable and resistant to damage. This application is valuable for packaging that undergoes physical stress during handling, transportation, and storage, ensuring the packaging remains intact and effective. Nanofillers allow for the customization of barrier properties in packaging materials. This is particularly useful for products that require specific gas compositions or moisture levels inside the package, such as modified atmosphere packaging (MAP). Nanofillers enable packaging materials to maintain optimal conditions for different food products **[27]**, **[35]**.

Incorporating nanofillers can result in packaging materials with built-in freshness indicators. These indicators change color or provide visual cues when the food product inside the package deteriorates or becomes unsafe to consume. Freshness indicators enhance food safety and consumer confidence. Nanofillers contribute to the development of sustainable active packaging systems. These systems release preservatives, antioxidants, or antimicrobials into the package to protect the food product. Nanofiller-enhanced materials allow for controlled release, improving the efficiency of active packaging. Nanofillers enhance the printability and adhesion of labels, graphics, and branding elements on packaging materials. This ensures that essential information and marketing messages remain clear and legible, contributing to effective consumer communication. These applications highlight the versatility and transformative potential of nanofillers in food packaging. By harnessing their unique properties, packaging materials can meet the diverse requirements of the food industry, from ensuring food safety and preservation to extending shelf life and enhancing sustainability. Nanofillers pave the way for innovative packaging solutions that address the evolving needs of both consumers and the environment **[3]**, **[36]**.

Extended Shelf Life

Nanofillers play a crucial role in extending the shelf life of various food products by enhancing the barrier properties of packaging materials. They create effective shields against gases like oxygen, carbon dioxide, moisture, and volatile compounds, preventing food deterioration. This reduction in oxygen permeation is significant as oxygen can lead to flavor loss, rancidity, and nutrient degradation. Nanofillers also enhance resistance to moisture ingress, preventing condensation, mold growth, and textural changes in food items. Moreover, certain nanofillers, such as nanosilver, possess intrinsic antimicrobial properties. When integrated into packaging materials, they inhibit the growth of bacteria, molds, and other microorganisms on the package's surface. This microbial resistance reduces the risk of contamination, extending the shelf life of perishable foods. Nanofillers are also instrumental in protecting food products from the harmful effects of ultraviolet (UV) radiation, which can lead to flavor alteration, nutrient degradation, and changes in food texture. By blocking UV rays, nanofiller-enhanced packaging materials help preserve the product's quality **[1]**, **[2]**, **[3]**, **[21]**.

In the context of modified atmosphere packaging (MAP), nanofillers can be engineered to allow the selective passage of specific gases while blocking others. This particular gas permeability is essential for maintaining different food products' freshness and shelf life. Additionally, nanofillers minimize the escape of volatile organic compounds from food products, including aromas and flavors. This preservation of sensory attributes ensures that consumers experience the intended taste and aroma when they open the package. Nanofillers guard food quality, safety, and longevity within packaging materials. They contribute to reducing food waste by ensuring that consumers enjoy fresh and safe food products over an extended shelf life. This application of nanofillers aligns with sustainability goals and consumer expectations for high-quality food items **[37]**, **[38]**.

Food Safety and Preservation

Nanofillers, especially those endowed with antimicrobial properties, wield significant influence over food safety and preservation when integrated into food packaging materials. This application of nanofillers is instrumental in fortifying food safety protocols and extending the shelf life of a diverse range of food products. Here, we embark on an in-depth exploration of how nanofillers bolster food safety and preservation in the context of food packaging:

At the forefront of their capabilities, nanofillers, exemplified by nanosilver and other antimicrobial nanoparticles, display exceptional prowess in inhibiting the growth of a wide array of microorganisms. When seamlessly incorporated into packaging materials, they bestow them with surfaces that are formidable deterrents against microbial colonization and proliferation. This, in turn, leads to a tangible reduction in the risk of contamination and premature spoilage. Perhaps more notably, nanofillers, particularly those harnessing antimicrobial properties, emerge as stalwart sentinels in foodborne pathogen control. These nanoparticles fashion a protective shield that substantially hinders the proliferation of pathogenic microorganisms. The packaging

surface becomes a formidable barrier, making it arduous for these pathogens to establish a foothold on the packaging or infiltrate the food product **[11]**.

The implications of this microbial inhibition extend far and wide, especially in extending the shelf life of perishable food products. By their pathogen-control attributes, Nanofillers contribute significantly to extending the shelf life of items inherently prone to rapid deterioration, including fresh produce, meat, dairy products, and bakery goods. As an ancillary but substantial benefit, the interplay of nanofillers in food packaging substantially reduces food wastage. The enhanced food safety mechanisms and the prolonged shelf life ushered by nanofiller-enhanced packaging collectively significantly minimize food wastage. The conscientious management of food resources aligns harmoniously with global sustainability objectives and the collective resolve to address the burgeoning issue of food wastage worldwide **[2]**, **[3]**.

Equally significant is the role of nanofillers in preserving the sensory attributes of food products, a facet often overlooked but of paramount importance. As guardians of freshness, Nanofillers diligently keep the flavors, textures, colors, and nutritional content of food products over extended periods. This translates into a sensory experience for consumers that mirrors the intentions of food manufacturers, ensuring that food products are enjoyed in their pristine state. Moreover, the protective envelope woven by nanofillers within packaging materials is equally adept at shielding food products from cross-contamination. This is relevant in scenarios where multiple components or flavors coexist within the same package. Preventing inadvertent flavor mingling or cross-contamination underscores the meticulous nature of nanofiller-enhanced packaging **[39]**, **[40]**, **[41]**.

This leads us to the realm of consumer assurance and regulatory compliance. Food packaging materials fortified with nanofillers bolster food safety and provide an additional layer of consumer confidence. Consumers can rest assured that the enclosed food products are protected from microbial contamination and remain safe for consumption. Furthermore, these materials align seamlessly with stringent food safety regulations and standards, enabling food manufacturers and processors to navigate the intricacies of the food supply chain while maintaining unwavering standards of safety and quality. In summation, nanofillers, particularly those endowed with antimicrobial attributes, emerge as robust custodians of food safety and preservation within food packaging. Their proficiency in curbing microbial growth, prolonging shelf life, and preserving sensory attributes contributes to food products' safer and longer-lasting availability. It addresses the pressing global concerns of food wastage and foodborne illnesses. Nanofiller-enhanced food packaging materials stand as stalwart sentinels, ensuring that consumers receive food items that are also safe and of the highest quality.

Smart Packaging Technologies

Innovative packaging technologies powered by nanofillers represent a significant leap in food packaging, offering capabilities beyond traditional packaging. These innovative systems provide real-time monitoring, interactive features, and enhanced functionality. For instance, nanosensors within smart packaging can continuously monitor the freshness of food products by detecting changes in temperature, humidity, and gas composition within the package. When freshness deteriorates, indicators on the packaging may change color or provide visual cues to alert consumers. Temperature-sensitive packaging, made possible by nanofillers, can also change color or display temperature-related information, ensuring food safety during storage and transport. Additionally, nanofillers enable tamper-evident features, enhancing consumer confidence in product safety. QR codes and interactive labels created with nanofillers grant consumers access to comprehensive product information, including source, nutritional content, and recommended usage. Time-temperature indicators, active release systems, and shelf-life extension monitoring improve food safety and quality. Furthermore, nanofillers facilitate environmental sensors in smart packaging, monitoring factors like temperature and humidity during transportation and storage to ensure optimal handling. These innovations foster consumer confidence, engagement, and informed decision-making while maintaining food safety and quality from the manufacturer to the consumer's table. Smart packaging technologies powered by nanofillers represent a remarkable evolution in food packaging. Beyond their role as traditional containers, these innovative packaging systems introduce real-time monitoring, interactivity, and advanced functionalities that significantly enhance the consumer experience and food safety. The integration of nanosensors within smart packaging is a prime example. These sensors continuously monitor the freshness of food products by detecting changes in temperature, humidity, and gas composition within the package. As these critical factors fluctuate, indicators on the packaging can change color or provide visible cues, enabling consumers to make informed choices about product quality **[42]**, **[43]**, **[44]**, **[45]**.

Moreover, nanofiller-enabled temperature-sensitive packaging offers an additional layer of food safety assurance. These packages can react to specific temperature ranges by changing color or displaying temperature-related information, effectively alerting consumers to potential temperature-related issues during storage and transportation. Enhancing consumer confidence is another facet of smart packaging, with nanofillers contributing to tamper-evident features. This additional level of security helps consumers trust the safety and integrity of the

enclosed food product. Interactive features like QR codes and labels enriched by nanofillers bridge the physical and digital worlds. Consumers can easily access comprehensive product information, including the product's source, nutritional content, recommended usage, and even recipes, all through their smartphones. This level of engagement fosters a deeper connection between consumers and products, enhancing their overall experience.

Furthermore, nanofillers play a pivotal role in developing time-temperature indicators (TTIs) that provide real-time monitoring of product freshness. These indicators change color or offer visual cues based on the product's cumulative time and temperature exposure. Such systems empower consumers to make more informed decisions about the safety and freshness of the food item they are considering. Active release systems, facilitated by nanofillers, represent a proactive approach to preserving food quality. These systems can release preservatives, antioxidants, or antimicrobials into the package, ensuring the food remains safe and fresh over an extended period. Nanofiller-enhanced smart packaging also extends its capabilities to address authenticity concerns. QR codes, empowered by nanofillers, can incorporate authentication features. Consumers can quickly scan these codes to verify the product's authenticity and origin, mitigating the risk of counterfeit or adulterated food items. Lastly, thanks to nanofillers, environmental sensors integrated into smart packaging materials continuously monitor temperature, humidity, and light exposure during transportation and storage. This data ensures that products are handled and stored optimally, minimizing the risk of quality degradation **[46]**, **[47]**, **[48]**, **[49]**.

Sustainability in Food Packaging

Sustainability is a paramount concern in the modern food packaging industry, driven by the need to reduce environmental impacts and meet consumer demands for eco-friendly packaging solutions. Nanofillers contribute significantly to the development of sustainable food packaging materials and practices. Nanofillers enable the creation of sustainable and biodegradable food packaging materials, reducing reliance on non-renewable resources and fossil-based plastics. These address environmental concerns related to resource depletion and plastic pollution. Sustainable packaging materials incorporating nanofillers are often designed to be recyclable or compostable, reducing packaging waste and promoting a circular economy. Lightweighting is another advantage, as nanofillers enhance the strength and durability of packaging materials while reducing their weight. Lightweight packaging requires fewer raw materials for production and consumes less energy during transportation, contributing to reduced carbon emissions and resource conservation **[13]**, **[30]**, **[50]**, **[51]**.

Extending the shelf life of food products through nanofiller-enhanced packaging reduces food waste, benefiting both the environment and the economy. Nanofillers also enable the development of sustainable alternatives to traditional plastic films, offering biodegradability, compostability, and reduced environmental impact. By improving packaging efficiency and reducing damage during transportation, nanofillers minimize the need for over-packaging. Moreover, nanofiller-enhanced packaging materials can be designed to facilitate recycling and reprocessing, promoting the closed-loop recycling of packaging materials. Energy efficiency is a significant advantage, as lightweight packaging materials with nanofillers require less energy for production, transportation, and disposal. This contributes to overall energy efficiency in the packaging industry, reducing its carbon footprint. Nanofillers align with green chemistry principles, emphasizing environmentally friendly and sustainable processes and materials. Their incorporation promotes sustainable practices in food packaging. Lastly, nanofillers derived from sustainable and renewable sources, such as nanocellulose from wood pulp, further support sustainability goals by reducing the environmental impact of raw material extraction (figure 2). Incorporating nanofillers into food packaging materials represents a significant step toward achieving sustainable packaging solutions. These materials address critical environmental challenges, such as plastic pollution and resource depletion, while ensuring that food products remain protected, safe, and high-quality. Nanofiller-enhanced sustainable packaging aligns with the broader goals of reducing the environmental footprint of the food packaging industry and promoting responsible packaging practices **[11]**, **[52]**, **[53]**.

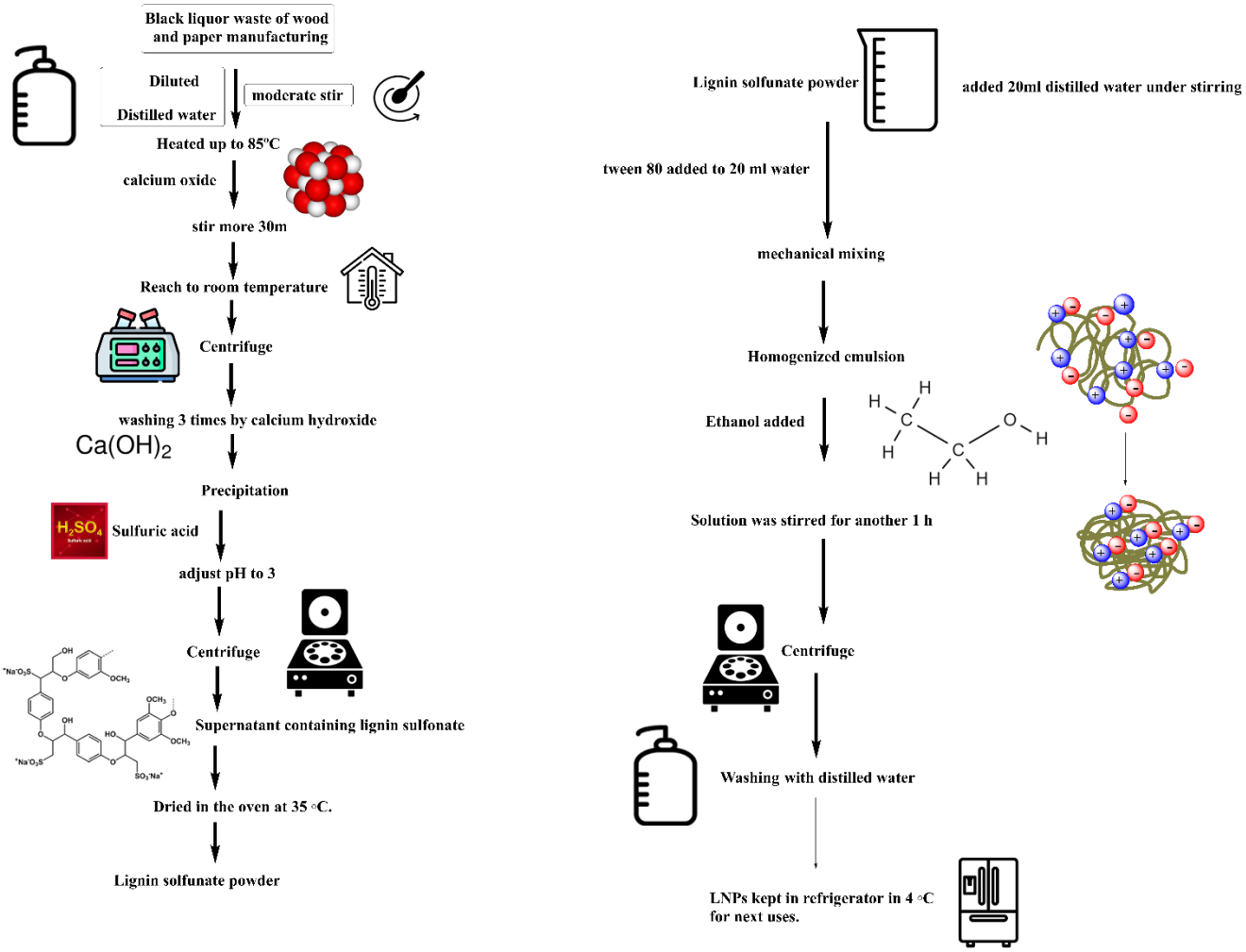


Figure 2 Mechanism of production of nanofiller from wood waste.

Innovations in Nanofiller-Based Food Packaging

Integrating nanofillers into food packaging materials has led to a wave of groundbreaking innovations. These innovations leverage the unique properties of nanofillers to address various challenges and opportunities in the food packaging industry. Nanosensor integration is a prime example, allowing nanosensors to detect changes in temperature, humidity, gas composition, and freshness in real time. When combined with smart packaging, these sensors provide consumers with vital information about a food product's condition and safety. Active release systems are another significant advancement. Nanofiller-enhanced packaging materials can incorporate these systems to release preservatives, antioxidants, or antimicrobials into the package. This controlled release ensures food products remain safe and fresh throughout their shelf life. Nanocoatings, created using nanofillers, form ultra-thin barriers on packaging surfaces. These coatings protect against external factors like moisture, oxygen, and contaminants while maintaining transparency and flexibility. Nanocomposite films blend nanofillers with biodegradable polymers, offering superior mechanical strength, barrier properties, and sustainability for various food packaging applications **[9]**, **[54]**.

Edible nanocomposites, made with food-grade polymers and nanofillers, enhance food safety, reduce packaging waste, and extend shelf life. Antimicrobial nanofillers like nanosilver inhibit microbial growth on packaging surfaces, improving food safety and shelf life **[55]**. Nanoparticle inks, employing nanofillers, enhance print quality, durability, and visual appeal on food packaging materials. Nanoscale barcodes on packaging labels provide secure product authentication, reducing counterfeiting risks. Personalized packaging solutions optimized for freshness, safety, and shelf life are achievable with nanofillers. Sustainable nanopackaging materials, combining biodegradability and enhanced performance, align with eco-conscious consumer preferences. Self-healing packaging materials, incorporating nanofillers, can repair small holes or tears, extending shelf life and protecting enclosed food products. Nanofillers also enhance smart labels, enabling features like QR code authentication and real-time freshness monitoring. These innovations exemplify the transformative potential of nanofillers in food packaging. They address critical challenges in food safety, sustainability, and consumer

engagement while opening new avenues for packaging functionality and performance. This dynamic landscape in the food packaging industry is driven by nanoscience and technology **[1]**, **[2]**, **[3]**, **[21]**, **[56]**.

Nanocomposite Materials in Food Packaging

Nanocomposite materials have emerged as a significant innovation in food packaging, capitalizing on the unique properties of nanofillers to enhance performance, safety, and sustainability. These materials find application in various aspects of food packaging:

Nanocomposite materials leverage nanofillers to create superior barrier properties against gases like oxygen, carbon dioxide, moisture, and volatile compounds. This extended the shelf life of food products by reducing factors that lead to spoilage and deterioration. Nanofillers reinforce the structural integrity of food packaging materials, making them more robust and resistant to damage during handling and transportation. This results in intact and practical packaging, reducing the risk of food product damage. Furthermore, nanocomposite materials often require less raw material to achieve the same or better performance than traditional packaging materials. This lightweight reduces resource consumption, transportation costs, and environmental impact **[1]**, **[14]**, **[27]**.

These materials can be engineered to be biodegradable or compostable, aligning with sustainability goals and reducing the environmental burden of packaging waste. Incorporating nanofillers allows for the customization of packaging material properties to suit the specific needs of food products. This includes tailored barrier properties, transparency, and mechanical strength. Some nanocomposite materials are designed to actively scavenge oxygen from the package's interior, creating an oxygen-free environment that extends the shelf life of oxygen-sensitive food products. Moreover, nanocomposite materials with flavor and aroma barrier properties prevent the escape of volatile compounds, preserving the sensory attributes of food products and ensuring they retain their intended taste and aroma. These materials can be part of active packaging systems that release preservatives, antioxidants, or antimicrobials into the package to protect the food product. This controlled release enhances food safety and freshness. Nanocomposites can provide thermal stability, protecting food products from temperature fluctuations during storage and transportation. This is particularly important for sensitive products that can degrade or spoil under varying temperature conditions **[57]**, **[58]**.

Additionally, nanocomposite materials can be created from biobased polymers and nanofillers derived from renewable sources, further promoting sustainability and reducing the reliance on fossil-based plastics. Lastly, nanocomposite materials offer improved printability for labels, graphics, and branding elements on packaging, ensuring that essential information and marketing messages remain clear and legible. In summary, nanocomposite materials represent a versatile and innovative approach to food packaging. They empower the food industry to develop packaging solutions that protect and preserve food products and align with sustainability objectives and consumer preferences for eco-friendly, high-performance packaging materials. Nanofiller-enhanced nanocomposites are at the forefront of modern food packaging, driving advancements in safety, sustainability, and functionality **[56]**, **[57]**, **[58]**, **[59]**.

Nanosensors and Nanotags in Food Packaging

Integrating nanosensors and nanotags into food packaging materials is a groundbreaking development with multifaceted advantages. These nanoscale technologies harness the unique properties of nanomaterials to offer real-time monitoring, traceability, and enhanced consumer interaction. Nanosensors discreetly embedded within packaging materials perform continuous surveillance of the package's internal environments. Parameters like temperature, humidity, and gas composition are meticulously tracked in real-time, ensuring that food products remain under optimal conditions throughout storage and transportation. Nanosensors also excel at detecting subtle shifts in the freshness of food products. They provide consumers with visual or digital indicators on the packaging, facilitating a swift product quality assessment. These indicators change color or display freshness levels, aiding consumers in making informed decisions. Furthermore, nanosensors offer precise temperature sensing capabilities, detecting even minor temperature fluctuations. In the event of exposure to unfavourable temperature conditions that could compromise food safety or quality, nanosensors provide timely alerts to consumers and supply chain stakeholders **[60]**, **[61]**.

Nanosensors also contribute to improved gas sensing within the packaging. They can detect and quantify specific gases, such as oxygen or carbon dioxide, essential for products requiring controlled atmospheres, like modified atmosphere packaging (MAP). This capability enhances product preservation and shelf life. Moreover, advanced nanosensors can be engineered to detect the presence of foodborne pathogens or contaminants within the package. This early warning system enhances food safety by promptly identifying potential issues and allowing for corrective action. Nanotags, often in QR codes or other barcodes, provide product authentication. Consumers can scan these codes to verify the authenticity and origin of the food product, bolstering consumer trust and countering counterfeiting. Supply chain traceability is another application enabled by nanotags. They

facilitate tracking food products from production to consumption, enhancing transparency and accountability throughout the supply chain. Additionally, nanotags strengthen the functionality of packaging labels. They allow consumers to access comprehensive information about the product, including its source, production date, nutritional content, and recommended usage. This information is easily accessible through consumers' smartphones or other devices. Nanotags also serve as an anti-counterfeiting measure. Their unique identifiers make it challenging for counterfeit products to mimic authentic ones, thereby protecting consumers and brands. Personalized packaging solutions become feasible with nanosensors and nanotags. Packaging can display dietary information tailored to individual preferences or restrictions, providing a more personalized consumer experience. Furthermore, nanosensors contribute to sustainability efforts by monitoring and reporting on the environmental sustainability of the packaging material or the product itself. This data empowers consumers to make informed choices aligned with sustainability criteria **[62]**, **[63]**.

Active and Intelligent Packaging in Food Packaging

Active and intelligent packaging systems represent a sophisticated approach to food packaging, incorporating various technologies, including nanofillers, to enhance food safety, quality, and consumer experience. These systems actively interact with the packaged food or provide intelligent information to consumers and stakeholders throughout the supply chain. Here, we delve into the applications and benefits of active and intelligent packaging in food packaging:

Active Packaging Systems: Active packaging systems enhanced with nanofillers deliver a suite of advantages for food preservation. Oxygen scavenging, achieved through nanofillers, creates an oxygen-free environment within the package, significantly extending the shelf life of oxygen-sensitive food products like snacks, grains, and dried fruits. Furthermore, active packaging materials incorporating nanofillers can efficiently absorb ethylene gas, accelerating the ripening process in some fruits and vegetables. This capability contributes to extending the freshness and overall shelf life of produce. Additionally, nanofillers empower active packaging materials to release antimicrobial agents or natural preservatives into the package contents, a feature particularly valuable for maintaining the quality and safety of fresh meat, poultry, and seafood. These systems also excel in aroma preservation, as nanofillers capture and release food aromas, enhancing the sensory experience and flavor of the enclosed food product. Furthermore, active packaging materials enriched with nanofillers exhibit moisture-regulating properties. This enables them to maintain the optimal moisture levels for specific food products, such as bakery items. By preventing excessive dryness or moisture, these materials play a pivotal role in preserving product quality throughout their shelf life, ensuring consumers enjoy the products as intended **[3]**, **[64]**, **[65]**, **[66]**.

Intelligent Packaging Systems: Intelligent packaging technologies in food packaging offer a range of benefits. Freshness indicators, equipped with nanosensors and nanotags, provide real-time freshness indicators to consumers. These indicators change color or display freshness levels, allowing consumers to assess the product's quality. Additionally, temperature monitoring is facilitated by integrated nanosensors that continuously track temperature conditions during storage and transportation. Should the product be exposed to unfavorable temperatures, consumers receive alerts concerning potential safety or quality issues. Intelligent packaging also enhances supply chain traceability through nanotags, enabling stakeholders to track a product's journey from production to consumption. This transparency not only improves food safety but also fosters consumer trust. Interactive labels featuring nanotags offer consumers comprehensive product information, including its source, production date, nutritional content, and recommended usage. This information is easily accessible through smartphones or other devices. Furthermore, nanotags serve as an effective anti-counterfeiting measure in intelligent packaging. They provide unique identifiers that help consumers verify the product's authenticity and distinguish it from counterfeit versions. Personalized packaging is another noteworthy aspect of intelligent packaging, allowing consumers to receive tailored content, promotions, and recommendations based on their preferences and dietary restrictions. Lastly, nanosensors within intelligent packaging materials can monitor and report on the environmental sustainability of the packaging material or the product itself. This data empowers consumers to make choices aligned with sustainability criteria, contributing to a more eco-conscious approach to food packaging. Active and intelligent packaging systems, certified by nanofillers and nanoscale technologies, bring a new level of functionality, safety, and consumer engagement to food packaging. These systems help ensure that food products remain fresh, safe, and high-quality while providing valuable information to consumers and stakeholders. They represent a convergence of advanced materials, sensor technologies, and data-driven insights that enhance the food packaging experience.

Challenges and Considerations

Incorporating nanofillers into food packaging materials undoubtedly brings transformative benefits, but it also necessitates a thorough examination of critical challenges and considerations.

Safety and Regulatory Issues: Nanofillers, especially those with distinctive properties, can prompt concerns about their potential toxicity and impact on food safety. Conducting comprehensive toxicity studies and risk assessments becomes imperative to ensure the safe utilization of nanofillers in food packaging. Moreover, navigating the complex regulatory landscapes as regulatory agencies worldwide assess the safety and approval of nanomaterials in food contact applications is a paramount concern. Packaging manufacturers must remain vigilant to maintain compliance with established guidelines. Understanding the potential for nanofiller migration or leaching into food products is crucial, necessitating the design of packaging materials that minimize such risks. Concurrently, developing and validating analytical methods for detecting any migration or leaching are essential.

Cost-Effectiveness: While integrating nanofillers can yield significant benefits, it may also increase the production costs of packaging materials. Thus, manufacturers must conduct meticulous cost-effectiveness evaluations, considering extended shelf life, reduced food waste, and improved product quality. Additionally, ensuring the economic viability of nanofiller-based packaging is critical. Smaller manufacturers and businesses may face challenges due to initial investment costs, necessitating research into cost-effective production methods and scalability to encourage broader adoption.

Environmental Concerns: The disposal of packaging materials containing nanofillers, especially non-biodegradable variants, raises concerns regarding their long-term environmental impact. Strategies for sustainable end-of-life management, recycling, or composting are essential considerations. Moreover, ecological evaluation of the environmental impact of nanomaterial production and extraction is essential, as the reduced material usage in nanofiller-based packaging must be balanced against potential adverse effects. Sustainable sourcing and production practices play a pivotal role in mitigating these concerns.

Future Directions and Research Needs: Several key areas warrant attention for future development in nanofiller-based food packaging. Standardization is essential, encompassing the creation of standardized testing methods and regulations specific to these materials. Consistent methodologies for evaluating safety, performance, and environmental impact are pivotal. Furthermore, consumer education is paramount to fostering trust and acceptance of nanofiller-based packaging. Clear labelling and communication of benefits and safety measures can alleviate consumer concerns. Collaborative research involving nanoscience, food science, material science, and regulatory expertise is necessary to address multifaceted challenges and opportunities in nanofiller-based food packaging. Continued research into biodegradable nanofillers holds promise for more sustainable packaging solutions, provided these materials align with existing waste management practices. Finally, comprehensive lifecycle assessments of nanofiller-based packaging materials are essential to gain insights into their environmental impact, including resource consumption and emissions.

CONCLUSION

Nanofillers have emerged as a transformative technology in food packaging, offering a myriad of possibilities to enhance food safety, extend shelf life, improve packaging efficiency, and promote sustainability. This comprehensive review has delved into the multifaceted world of nanofillers in food packaging, exploring their properties, applications, innovations, and challenges. Nanofillers, with their unique mechanical, barrier, thermal, optical, and surface properties, serve as catalysts for innovation in food packaging materials. They enable the creation of packaging solutions that address the limitations of traditional materials, providing enhanced protection for food products while reducing waste and environmental impact. Intelligent nanofillers exhibit remarkable versatility, from extending the shelf life of perishable goods to ensuring food safety and integrity and from pioneering smart packaging solutions to bolstering the sustainability of food packaging materials. They empower food manufacturers and packaging companies to meet the evolving demands of the food industry and consumer preferences. However, embracing nanofillers in food packaging comes with its own set of challenges and considerations. Safety and regulatory issues demand rigorous scrutiny, cost-effectiveness must be evaluated, environmental concerns require attention, and future research needs to be directed toward standardization, consumer education, and sustainable practices. As we conclude this review, it is evident that nanofillers have opened new horizons in food packaging, ushering in an era where packaging is not merely a passive vessel but an intelligent, sustainable, and protective guardian of our nourishment. The journey of nanofillers in food packaging is ongoing, with ongoing research and innovation poised further to shape the future of food packaging materials and practices. Collaboration among scientists, industry professionals, regulators, and consumers is essential in this dynamic landscape. By navigating the intricate terrain of nanofillers responsibly, we can harness the full potential of this technology to ensure the safety, quality, and sustainability of the food products that nourish us and protect the planet we call home.

REFERENCES

1. Souza, V., Mello, I., Khalid, O., Pires, J., Rodrigues, C., Alves, M., Santos, C., Fernando, A., & Coelhoso, I. (2022). Strategies to Improve the Barrier and Mechanical Properties of Pectin Films for Food Packaging: Comparing Nanocomposites with Bilayers. In Coatings (Vol. 12, Issue 2, p. 108). MDPI AG. https://doi.org/10.3390/coatings12020108
2. Pires, J., Paula, C. D. de, Souza, V. G. L., Fernando, A. L., & Coelhoso, I. (2021). Understanding the Barrier and Mechanical Behavior of Different Nanofillers in Chitosan Films for Food Packaging. In Polymers (Vol. 13, Issue 5, p. 721). MDPI AG. https://doi.org/10.3390/polym13050721
3. Wu, W., Liu, L., Goksen, G., Demir, D., & Shao, P. (2022). Multidimensional (0D-3D) nanofillers: Fascinating materials in bio-based food active packaging. In Food Research International (Vol. 157, p. 111446). Elsevier BV. https://doi.org/10.1016/j.foodres.2022.111446
4. Peidaei, F., Ahari, H., Anvar, S. A. A., & Ataee, M. (2021). Nanotechnology in Food Packaging and Storage: A Review. In Iranian Journal of Veterinary Medicine (Vol. 15, Issue 2, pp. 122–156). Faculty of Veterinary Medicine of the University of Tehran and Negah Institute for Social Research and Scientific Communication. https://doi.org/10.22059/ijvm.2021.310466.1005130
5. Primožič, M., Knez, Ž., & Leitgeb, M. (2021). (Bio)Nanotechnology in Food Science—Food Packaging. In Nanomaterials (Vol. 11, Issue 2, p. 292). MDPI AG. https://doi.org/10.3390/nano11020292
6. Duncan, T. V. (2011). Nanotechnology applications in food packaging and safety: Barrier materials, antimicrobials and sensors. In Journal of Colloid and Interface Science (Vol. 363, Issue 1, pp. 1–24). Elsevier BV. https://doi.org/10.1016/j.jcis.2011.07.017
7. Duncan, T. V. (2011). Nanotechnology applications in food packaging and safety: Barrier materials, antimicrobials and sensors. In Journal of Colloid and Interface Science (Vol. 363, Issue 1, pp. 1–24). Elsevier BV. https://doi.org/10.1016/j.jcis.2011.07.017
8. Mandai, P. K., Choi, K., Min, S., & Lee, C.-H. (2009). Application of Nanotechnology in Food Packaging: An Overview. In Korean Journal for Food Science of Animal Resources (Vol. 29, Issue 4, pp. 403–408). Korean Society for Food Science of Animal Resources.
9. Idumah, C. I., Zurina, M., Ogbu, J., Ndem, J. U., & Igba, E. C. (2019). A review of innovations in polymeric nanocomposite packaging materials and electrical sensors for food and agriculture. In Composite Interfaces (Vol. 27, Issue 1, pp. 1–72). Informa UK Limited. https://doi.org/10.1080/09276440.2019.1600972
10. Versino, F., Ortega, F., Monroy, Y., Rivero, S., López, O. V., & García, M. A. (2023). Sustainable and Bio-Based Food Packaging: A Review on Past and Current Design Innovations. In Foods (Vol. 12, Issue 5, p. 1057). MDPI AG. https://doi.org/10.3390/foods12051057
11. Amalnerkar, D. P., Pawar, J. R., Phatak, R. S., Qureshi, N. M., Singh, A. E., Shinde, M. D., & Doh, J. (2023). Contemporary Trends In Active And Intelligent (Polymer) Nanocomposite-Based Food Packaging Systems For Food Safety And Sustainability In The Modern Aeon. In Current Materials Science (Vol. 17). Bentham Science Publishers Ltd. https://doi.org/10.2174/2666145417666230712122748
12. Wypij, M., Trzcińska-Wencel, J., Golińska, P., Avila-Quezada, G. D., Ingle, A. P., & Rai, M. (2023). The strategic applications of natural polymer nanocomposites in food packaging and agriculture: Chances, challenges, and consumers' perception. In Frontiers in Chemistry (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fchem.2022.1106230
13. Perera, K. Y., Jaiswal, A. K., & Jaiswal, S. (2023). Biopolymer-Based Sustainable Food Packaging Materials: Challenges, Solutions, and Applications. In Foods (Vol. 12, Issue 12, p. 2422). MDPI AG. https://doi.org/10.3390/foods12122422
14. Liu, G., Shi, K., & Sun, H. (2023). Research Progress in Hemicellulose-Based Nanocomposite Film as Food Packaging. In Polymers (Vol. 15, Issue 4, p. 979). MDPI AG. https://doi.org/10.3390/polym15040979
15. Singha, S., & Hedenqvist, M. S. (2020). A Review on Barrier Properties of Poly(Lactic Acid)/Clay Nanocomposites. In Polymers (Vol. 12, Issue 5, p. 1095). MDPI AG. https://doi.org/10.3390/polym12051095
16. Guchait, A., Saxena, A., Chattopadhyay, S., & Mondal, T. (2022). Influence of Nanofillers on Adhesion Properties of Polymeric Composites. In ACS Omega (Vol. 7, Issue 5, pp. 3844–3859). American Chemical Society (ACS). https://doi.org/10.1021/acsomega.1c05448
17. Bikiaris, N. D., Koumentakou, I., Samiotaki, C., Meimaroglou, D., Varytimidou, D., Karatza, A., Kalantzis, Z., Roussou, M., Bikiaris, R. D., & Papageorgiou, G. Z. (2023). Recent Advances in the Investigation of

Poly(lactic acid) (PLA) Nanocomposites: Incorporation of Various Nanofillers and their Properties and Applications. In Polymers (Vol. 15, Issue 5, p. 1196). MDPI AG. https://doi.org/10.3390/polym15051196

18. Shahverdi-Shahraki, K. (2014). Developement of Pet/Kaolin Nanocomposites with Improved Mechanical Properties [Doctoral dissertation, École Polytechnique de Montréal].

19. Radusin, T., Pilić, B., Ristić, I., Bera, O., Baloš, S., & Aleksandra Novaković, R. (2012). the influence of silica nanoparticles on mechanical properties of polylactic acid as food packaging material. In Materials Science, Environmental Science (Vol. 39, Issue 2, pp. 97–102). University of Mohammed Premier Oujda

20. Stoica, M., & Stoica, D. (2021). Nanofillers for Food Packaging: Antimicrobial Potential of Metal-based Nanoparticles. In Current Nanotoxicity and Prevention (Vol. 1, Issue 1, pp. 44–66). Bentham Science Publishers Ltd. https://doi.org/10.2174/2665980801999200709172848

21. Wijesooriya, S. N., & Adassooriya, N. (2021). Water barrier properties of carboxymethyl cellulose/halloysite nanotubes nanocomposite to be used in food packaging applications. In Vidyodaya Journal of Science (Vol. 24, Issue 02). University of Sri Jayewardenepura. https://doi.org/10.31357/vjs.v24i02.5414

22. Tan, C., Han, F., Zhang, S., Li, P., & Shang, N. (2021). Novel Bio-Based Materials and Applications in Antimicrobial Food Packaging: Recent Advances and Future Trends. In International Journal of Molecular Sciences (Vol. 22, Issue 18, p. 9663). MDPI AG. https://doi.org/10.3390/ijms22189663

23. López de Dicastillo, C., Velásquez, E., Rojas, A., Guarda, A., & Galotto, M. J. (2020). The use of nanoadditives within recycled polymers for food packaging: Properties, recyclability, and safety. In Comprehensive Reviews in Food Science and Food Safety (Vol. 19, Issue 4, pp. 1760–1776). Wiley. https://doi.org/10.1111/1541-4337.12575

24. Zhang, W., Roy, S., & Rhim, J. (2023). Copper-based nanoparticles for biopolymer-based functional films in food packaging applications. In Comprehensive Reviews in Food Science and Food Safety (Vol. 22, Issue 3, pp. 1933–1952). Wiley. https://doi.org/10.1111/1541-4337.13136

25. Jorda-Beneyto, M., Ortuño, N., Devis, A., Aucejo, S., Puerto, M., Gutiérrez-Praena, D., Houtman, J., Pichardo, S., Maisanaba, S., & Jos, A. (2014). Use of nanoclay platelets in food packaging materials: technical and cytotoxicity approach. In Food Additives & Contaminants: Part A (Vol. 31, Issue 3, pp. 354–363). Informa UK Limited. https://doi.org/10.1080/19440049.2013.874045

26. Roy, S., Ezati, P., Priyadarshi, R., Biswas, D., & Rhim, J.-W. (2022). Recent advances in metal sulfide nanoparticle-added bionanocomposite films for food packaging applications. In Critical Reviews in Food Science and Nutrition (pp. 1–14). Informa UK Limited. https://doi.org/10.1080/10408398.2022.2144794

27. Jafarzadeh, S., & Jafari, S. M. (2020). Impact of metal nanoparticles on the mechanical, barrier, optical and thermal properties of biodegradable food packaging materials. In Critical Reviews in Food Science and Nutrition (Vol. 61, Issue 16, pp. 2640–2658). Informa UK Limited. https://doi.org/10.1080/10408398.2020.1783200

28. Abelti, A. L., Teka, T. A., Fikreyesus Forsido, S., Tamiru, M., Bultosa, G., Alkhtib, A., & Burton, E. (2022). Bio-based smart materials for fish product packaging: a review. In International Journal of Food Properties (Vol. 25, Issue 1, pp. 857–871). Informa UK Limited. https://doi.org/10.1080/10942912.2022.2066121

29. Kausar, A. (2019). A review of high performance polymer nanocomposites for packaging applications in electronics and food industries. In Journal of Plastic Film & Sheeting (Vol. 36, Issue 1, pp. 94–112). SAGE Publications. https://doi.org/10.1177/8756087919849459

30. Sarfraz, J., Gulin-Sarfraz, T., Nilsen-Nygaard, J., & Pettersen, M. K. (2020). Nanocomposites for Food Packaging Applications: An Overview. In Nanomaterials (Vol. 11, Issue 1, p. 10). MDPI AG. https://doi.org/10.3390/nano11010010

31. Kadhim, B. B., Salim, F. M., & Muhsen, H. O. (2011). Thermal Properties of Carbon Nano Tubes Reinforced Epoxy Resin Nano Composites. In Al-Mustansiriyah J. Sci. (Vol. 22, Issue 4, pp. 276–290). College of Science - Mustansiriyah University.

32. Jamróz, E., & Kopel, P. (2020). Polysaccharide and Protein Films with Antimicrobial/Antioxidant Activity in the Food Industry: A Review. In Polymers (Vol. 12, Issue 6, p. 1289). MDPI AG. https://doi.org/10.3390/polym12061289

33. Swaroop, C., & Shukla, M. (2017). Polylactic acid / magnesium oxide nanocomposite films for food packaging applications. In 21st International Conference on Composite Materials Xi'an (p. 9).

34. Olsson, R. T., Fogelström, L., Martínez-Sanz, M., & Henriksson, M. (2011). Cellulose nanofillers for food packaging. In Multifunctional and Nanoreinforced Polymers for Food Packaging (pp. 86–107). Elsevier. https://doi.org/10.1533/9780857092786.1.86
35. Marano, S., Laudadio, E., Minnelli, C., & Stipa, P. (2022). Tailoring the Barrier Properties of PLA: A State-of-the-Art Review for Food Packaging Applications. In Polymers (Vol. 14, Issue 8, p. 1626). MDPI AG. https://doi.org/10.3390/polym14081626
36. Gill, Y. Q., Jin, J., & Song, M. (2019). Comparative study of carbon-based nanofillers for improving the properties of HDPE for potential applications in food tray packaging. In Polymers and Polymer Composites (Vol. 28, Issues 8–9, pp. 562–571). SAGE Publications. https://doi.org/10.1177/0967391119892091
37. Cagnon, T., Guillaume, C., Guillard, V., & Gontard, N. (2012). Nanostructuring and Microstructuring of Materials from a Single Agropolymer for Sustainable MAP Preservation of Fresh Food. In Packaging Technology and Science (Vol. 26, Issue 3, pp. 137–148). Wiley. https://doi.org/10.1002/pts.1973
38. Czerwiński, K., Rydzkowski, T., Wróblewska-Krepsztul, J., & Thakur, V. K. (2021). Towards Impact of Modified Atmosphere Packaging (MAP) on Shelf-Life of Polymer-Film-Packed Food Products: Challenges and Sustainable Developments. In Coatings (Vol. 11, Issue 12, p. 1504). MDPI AG. https://doi.org/10.3390/coatings11121504
39. Kaur, S., Kaur, N., Aggarwal, P., & Grover, K. (2022). Sensory attributes, bioactive compounds, antioxidant activity and color values of jam and candy developed from Beetroot (Beta vulgaris L.). In Journal of Applied and Natural Science (Vol. 14, Issue 2, pp. 459–468). ANSF Publications. https://doi.org/10.31018/jans.v14i2.3407
40. Jaffe, T. R., Wang, H., & Chambers, E., IV. (2017). Determination of a lexicon for the sensory flavor attributes of smoked food products. In Journal of Sensory Studies (Vol. 32, Issue 3). Wiley. https://doi.org/10.1111/joss.12262
41. Bolha, A., Blaznik, U., & Korošec, M. (2020). Influence of intrinsic and extrinsic food attributes on consumers' acceptance of reformulated food products: A systematic review. In Slovenian Journal of Public Health (Vol. 60, Issue 1, pp. 72–78). Walter de Gruyter GmbH. https://doi.org/10.2478/sjph-2021-0011
42. Xie, F. (2021). Sustainable polymer composites: functionality and applications. In Functional Composite Materials (Vol. 2, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s42252-021-00027-z
43. Lydekaityte, J., & Tambo, T. (2020). Smart packaging: definitions, models and packaging as an intermediator between digital and physical product management. In The International Review of Retail, Distribution and Consumer Research (Vol. 30, Issue 4, pp. 377–410). Informa UK Limited. https://doi.org/10.1080/09593969.2020.1724555
44. Echegaray, N., Goksen, G., Kumar, M., Sharma, R., Hassoun, A., Lorenzo, J. M., & Dar, B. N. (2023). A critical review on protein-based smart packaging systems: Understanding the development, characteristics, innovations, and potential applications. In Critical Reviews in Food Science and Nutrition (pp. 1–16). Informa UK Limited. https://doi.org/10.1080/10408398.2023.2202256
45. Ullah, S., Hashmi, M., Lee, J., Youk, J. H., & Kim, I. S. (2022). Recent Advances in Pre-harvest, Post-harvest, Intelligent, Smart, Active, and Multifunctional Food Packaging. In Fibers and Polymers (Vol. 23, Issue 8, pp. 2063–2074). Springer Science and Business Media LLC. https://doi.org/10.1007/s12221-022-4192-2
46. Liu, Y., Li, L., Yu, Z., Ye, C., Pan, L., & Song, Y. (2023). Principle, development and application of time–temperature indicators for packaging. In Packaging Technology and Science (Vol. 36, Issue 10, pp. 833–853). Wiley. https://doi.org/10.1002/pts.2761
47. Stergiou, F. (2018). Effective management and control of the cold chain by application of Time Temperature Indicators (TTIs) in food packaging. In Journal of Food and Clinical Nutrition (Vol. 1, Issue 1, pp. 12–15). Medires PUBLISHING LLC.
48. Merkes, J. M., & Banala, S. (2021). Temperature-Controlled Conversion of Boc-Protected Methylene Blue: Advancing Solid-State Time-Temperature Indicators. In ChemistryOpen (Vol. 10, Issue 11, pp. 1129–1132). Wiley. https://doi.org/10.1002/open.202100142
49. Lanza, G. A., Perez-Taborda, J., & Avila, A. (2019). Time temperature indicators (TTIs) based on silver nanoparticles for monitoring of perishables products. In Journal of Physics: Conference Series (Vol. 1247, Issue 1, p. 012055). IOP Publishing. https://doi.org/10.1088/1742-6596/1247/1/012055

50. Silva, F. A. G. S., Dourado, F., Gama, M., & Poças, F. (2020). Nanocellulose Bio-Based Composites for Food Packaging. In Nanomaterials (Vol. 10, Issue 10, p. 2041). MDPI AG. https://doi.org/10.3390/nano10102041

51. Sun, X., Li, Q., Wu, H., Zhou, Z., Feng, S., Deng, P., Zou, H., Tian, D., & Lu, C. (2023). Sustainable Starch/Lignin Nanoparticle Composites Biofilms for Food Packaging Applications. In Polymers (Vol. 15, Issue 8, p. 1959). MDPI AG. https://doi.org/10.3390/polym15081959

52. Prabhakar, P. C. J., Sen, R., Mondal, D., & Dhand, C. (2021). PVA Based Biodegradable Antimicrobial Films for Food Packaging Application- Molecular Labeling for Sustainability. In Smart Green Connected Societies (Vol. 1, Issue 1, p. 3). Smart Green Connected Societies.

53. Trossaert, L., De Vel, M., Cardon, L., & Edeleva, M. (2022). Lifting the Sustainability of Modified Pet-Based Multilayer Packaging Material with Enhanced Mechanical Recycling Potential and Processing. In Polymers (Vol. 14, Issue 1, p. 196). MDPI AG. https://doi.org/10.3390/polym14010196

54. Shapi'i, R. R., Othman, S. H. (2016). Edible Nanofiller for Development of Edible Bio-Nanocomposite Film: A Review. In Journal of polymer and Technology (Vol. 1, Issue 1, pp. 37–45). EnPress Publisher.

55. Gvozdenko, A. A., Siddiqui, S. A., Blinov, A. V., Golik, A. B., Nagdalian, A. A., Maglakelidze, D. G., Statsenko, E. N., Pirogov, M. A., Blinova, A. A., Sizonenko, M. N., Simonov, A. N., Zhukov, R. B., Kolesnikov, R. O., & Ibrahim, S. A. (2022). Synthesis of CuO nanoparticles stabilized with gelatin for potential use in food packaging applications. In Scientific Reports (Vol. 12, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-022-16878-w

56. Siddiqui, S. A., Zannou, O., Bahmid, N. A., Fidan, H., Alamou, A.-F., Nagdalian, A. A., Hassoun, A., Fernando, I., Ibrahim, S. A., & Arsyad, M. (2022). Consumer behavior towards nanopackaging - A new trend in the food industry. In Future Foods (Vol. 6, p. 100191). Elsevier BV. https://doi.org/10.1016/j.fufo.2022.100191

57. Siddiqui, S. A., Khan, S., Mehdizadeh, M., Nirmal, N. P., Khanashyam, A. C., Fernando, I., Jatmiko, Y. D., Afiyanti, M., Bansal, S., Adli, D. N., Nagdalian, A. A., Blinov, A. V., Lodygin, A. D., Nugraha, W. S., Kasza, G., & Walker, T. R. (2023). Consumer Studies Focus on Prebiotics, Probiotics, and Synbiotics in Food Packaging: a Review. In Current Food Science and Technology Reports (Vol. 1, Issue 1, pp. 13–29). Springer Science and Business Media LLC. https://doi.org/10.1007/s43555-023-00003-7

58. Silva, G. D., Abeysundara, A. T., & Aponso, M. (2017). A review on nanocomposite materials for food packaging which comprising antimicrobial activity. In International Journal of Chemical Studies (Vol. 5, pp. 228–230). AkiNik Publications.

59. Garrido-Romero, M., Aguado, R., Moral, A., Brindley, C., & Ballesteros Martín, M. M. (2021). From Traditional Paper to Nanocomposite Films: Analysis of Global Research Into Cellulose for Food Packaging. In SSRN Electronic Journal. Elsevier BV. https://doi.org/10.2139/ssrn.3887405

60. Lakra, P. (2021). Significance of nanotechnology advances in food processing and packaging: consequences on human health. In Journal of Materials NanoScience (Vol. 2, Issue 1, pp. 8–14). ScienceIn Publishing.

61. Peidaei, F., Ahari, H., Anvar, S. A. A., & Ataee, M. (2021). Nanotechnology in Food Packaging and Storage: A Review. In Iranian Journal of Veterinary Medicine (Vol. 15, Issue 2, pp. 122–153). University of Tehran. https://doi.org/10.22059/ijvm.2021.310466.1005130

62. Mustafa, F., & Andreescu, S. (2020). Nanotechnology-based approaches for food sensing and packaging applications. In RSC Advances (Vol. 10, Issue 33, pp. 19309–19336). Royal Society of Chemistry (RSC). https://doi.org/10.1039/d0ra01084g

63. Yousefi, H., Su, H.-M., Imani, S. M., Alkhaldi, K., M. Filipe, C. D., & Didar, T. F. (2019). Intelligent Food Packaging: A Review of Smart Sensing Technologies for Monitoring Food Quality. In ACS Sensors (Vol. 4, Issue 4, pp. 808–821). American Chemical Society (ACS). https://doi.org/10.1021/acssensors.9b00440

64. Yuan, L., Gao, M., Xiang, H., Zhou, Z., Yu, D., & Yan, R. (2023). A Biomass-Based Colorimetric Sulfur Dioxide Gas Sensor for Smart Packaging. In ACS Nano (Vol. 17, Issue 7, pp. 6849–6856). American Chemical Society (ACS). https://doi.org/10.1021/acsnano.3c00530

65. Farousha, K., Tham, P. E., Chew, K. W., Amornraksa, S., & Show, P. L. (2023). The Future of Food Preservation: Active Packaging with Controlled Release Systems. In M. Sriariyanun, Y.-S. Cheng, D. Bhattacharyya, P. Venkatachalam, & M. P. Gundupalli (Eds.), E3S Web of Conferences (Vol. 428, p. 02009). EDP Sciences. https://doi.org/10.1051/e3sconf/202342802009

66. Carpena, M., Nuñez-Estevez, B., Soria-Lopez, A., Garcia-Oliveira, P., & Prieto, M. A. (2021). Essential Oils and Their Application on Active Packaging Systems: A Review. In Resources (Vol. 10, Issue 1, p. 7). MDPI AG. https://doi.org/10.3390/resources10010007

Funds:

This research received no external funding.

Acknowledgements:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Elena Pavlenko, Nevinnomyssk Technological Institute (branch) of North Caucasus Federal University, Department of Chemical Technology, Machines and Apparatuses of Chemical Production, Gagarina Srteet, 1, 357100, Nevinnomyssk, Russia,
Tel.: +79064675425
E-mail: pavlenko_ai60@mail.ru
ORCID: https://orcid.org/0000-0003-4612-3703

Evgenia Semkina, Nevinnomyssk Technological Institute (branch) of North Caucasus Federal University, Departments of Mathematical and Humanitarian Disciplines, Gagarina Srteet, 1, 357100, Nevinnomyssk, Russia,
Tel.: +79899737584
E-mail: semkina-e@bk.ru
ORCID: https://orcid.org/0000-0001-9903-1170

Alexander Pokhilko, Armavir State Pedagogical University, Scientific Department, Rose Lyuksemburg Srteet, 139, 352901, Armavir, Russia,
Tel.: +79633835378
E-mail: pokhilko-ad@mail.ru
ORCID: https://orcid.org/0000-0002-8945-322X

Ekaterina Sukhanova, North Caucasus Federal University, Faculty of Food Engineering and Biotechnology, Department of Food Technology and Engineering, Kulakova Street, 2, 355000, Stavropol, Russia,
Tel.: +79880989489
E-mail: lady.suha@yandex.ru
ORCID: https://orcid.org/0000-0003-2788-3897

Victor Fursov, North Caucasus Federal University, Institute of Economics and Management, Department of State and Municipal Administration and Labor Economics, Pushkina Street, 1, 355000, Stavropol, Russia,
Tel.: +78652354013
E-mail: fursov.va@mail.ru
ORCID: https://orcid.org/0000-0003-1095-0862

Natalia Lazareva, North Caucasus Federal University, Institute of Economics and Management, Department of Economic Security and Auditing, Pushkina Street, 1, 355000, Stavropol, Russia,
Tel.: +79624592272
E-mail: nlazareva@ncfu.ru
ORCID: https://orcid.org/0000-0002-5490-9936

Alexander Pyanov, North Caucasus Federal University, Institute of Economics and Management, Department of State and Municipal Administration and Labor Economics, Pushkina Street, 1, 355000, Stavropol, Russia,
Tel.: +79187549791
E-mail: archi4717@yandex.ru
ORCID: https://orcid.org/0000-0002-0925-7449

***Igor Baklanov**, North Caucasus Federal University, Humanitarian Institute, Department of Philosophy, Pushkina Street, 1, 355000, Stavropol, Russia,
Tel.: +79620027613
E-mail: baklanov72@mail.ru
ORCID: https://orcid.org/0000-0002-2781-3736

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 16.12.2023
Revised: 3.2.2024
Accepted: 15.2.2024
Published: 19.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 157-173
https://doi.org/10.5219/1948
ISSN: 1337-0960 online
www.potravinarstvo.com

Improving the citric acid production by mutant strains *Aspergillus niger* using carbohydrate-containing raw materials as a carbon source

Bakhyt Shaimenova, Gulnazym Ospankulova, Saule Saduakhasova, Linara Murat, Dana Toimbayeva

ABSTRACT

The demand for citric acid (CA) as a component of food products, pharmaceuticals, and cosmetics is increasing yearly. The use of adapted micro-organisms that convert naturally occurring carbohydrates into organic acids makes it possible to increase annual CA production significantly. The research aim was to study CA production by the *Aspergillus niger* strain in the medium based on carbohydrate-containing raw materials as a carbon source. We used a fermentation by *A. niger*. Starch hydrolysates were chosen as a nutrient medium. To improve the CA production of *A. niger*, multi-step mutagenesis was performed. This resulted in mutant strain *A. niger* R5/4, which had the highest acidogenic activity among the samples. The study evaluated the effect of temperature on the productivity of the mutant strains. The quantitative content of citric acid was analyzed at different incubation times (144, 168, and 192 h). The effect of the initial medium pH (4.5, 5.0, and 5.5) on acid formation was also investigated. The strain's optimum temperature, pH, and cultivation time parameters were determined. A three-factor, three-level Box-Benken design (BBD) was used to optimize CA production by *A. niger* strain R5/4 on a starch-containing medium. When assessing the impact of temperature on CA production, the ideal range was between 29 and 31 °C.

Keywords: citric acid, chemical mutagenesis, X-ray irradiation, *Aspergillus niger*, deep fermentation

INTRODUCTION

One of the limiting factors for food production development in the Republic of Kazakhstan is the low industrial processing of agricultural commodities, mainly due to insufficient technical and technological equipment in processing industries **[1]**.

Technologies for processing raw materials are sought after and advanced in global scientific practice. Deep processing is an established industry generating stable high revenues for many foreign countries **[2]**. Citric acid (CA) is the most valuable weak organic acid on the international market **[3]**. For example, the demand of the Customs Union countries is about 84 thousand tons of CA per year. Only one operating CA plant in the CIS region is located at the Skidel Sugar Refinery in the Republic of Belarus. The plant's output is 1,500 tonnes of acid per annum, with 800 tonnes for the domestic market and the remainder for export **[4]**. Thus, the production of CA by biotechnological approaches is an essential task all over the world **[3]**. Therefore, by developing CA production in the country, the enterprise producer can supply both the local market and export to neighboring countries.

The estimated market value of CA is projected to exceed $2 billion per kilo, indicating a global demand for CAs based on market trends **[4]**, **[5]**. As a result, it is essential to establish CA production in the Republic of Kazakhstan **[6]**. There are more than 100 patents in producing the CA globally **[3],** but it is essential to get the schema manufacturing given its abundance of raw materials that can be used in its production **[6]**.

The Republic of Kazakhstan has produced about 17 million tonnes of wheat grain annually for the past ten years. Deep processing of grain enables higher added value to be achieved. This is an innovative way to promote the biotechnological production development and the agro-industrial complex of the Republic of Kazakhstan. In this way, the range of valuable products obtained from grain expands, simultaneously increasing production in related industries: food, pharmaceuticals, engineering, metallurgy, construction, petrochemicals, and other sectors of the economy **[4]**, **[7]**.

There is a global trend towards using environmentally sustainable raw materials in CA production. Using various starches (potato, corn, wheat, rice) and their hydrolysates offers industrial potential **[8]**. The unique aspect of their use as a carbohydrate source for microbial CA producers is the simultaneous generation of enzymes catalyzing the hydrolysis of polysaccharides in acidic conditions during fermentation, accompanying the principal product **[9]**.

The CA accumulation process by *A. niger* in the sugar medium is directly related to substrate concentrations, requiring a high initial sugar concentration in the medium **[5]**, **[10]**, and also depends on the nature of the carbon source. Carbohydrates that are quickly metabolized are most efficient for production, with sucrose being the most optimal, followed by glucose and fructose. In contrast, galactose weakly promotes fungal growth and does not contribute to CA accumulation **[10]**.

The consumption of raw materials for CA production can show considerable variation **[11]** showed that the consumption coefficient of raw materials for producing one tonne of CA reduced from 2.7 (for beet molasses) to 1.2 (for corn and wheat starch hydrolysates).

The *A. niger* mold fungus is identified as the foremost microorganism for commercial CA production due to its higher CA production per time unit **[10]**, **[12]**.

Natural microbial strains need to produce by-products to be able to synthesize the desired product in optimal amounts easily. The most commonly used method to improve CA-producing strains is to induce mutations in parental strains using mutagens **[5]**, **[13]**. Gamma rays and ultraviolet radiation are the physical mutagens used **[14]**. UV treatment in combination with chemical mutagens like aziridine, N-nitroso-N-methylurea, ethyl methane sulfonate, and uracil is used to obtain hyperproductive strains. Fluorescent auxotrophic selection is also applied in the investigation of various promoters to obtain *A. niger* strains that are capable of synthesizing CA in higher yields **[15]** due to their ability to either inhibit or stimulate the enzymatic process **[16]**.

The study aims at improving the CA production by the wild strains of *A. niger* on medium-containing carbohydrate feedstocks as a carbon source. The emphasis has been placed on perfecting the fermentation conditions.

Scientific Hypothesis

Isolated wild local Aspergillus strains can be used in citric acid manufacturing. Mutant *A. niger* wild strain could be an efficiency producer in CA manufacturing. Carbohydrate-containing raw materials, because of the cereals processing, could be used as a base nutrition medium in CA production. This allows for introducing modern, resource-saving, and cost-effective technologies in the industrial production of organic acids.

MATERIAL AND METHODOLOGY

Samples

Wild strains of *A. niger* separated from the starch-rich mediums were checked for the possibility of CA production in native and after mutation.

Chemicals

Czapek Dox agar and Czapek Dox broth (HiMedia Laboratories LLC); indicators alizarin red and phenolphthalein (LLP Reaktivsnab, Shymkent, Kazakhstan); sucrose, methanol, ethanol, and isopropanol (LLP Reaktivsnab), chemical components as $(NH_4)_2SO_4$; K_2HPO_4; $MgSO_4 \cdot 7H_2O$, $CaCl_2$, NaOH, $MgSO_4 \cdot 7H_2O$, KH_2PO_4, $ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $NaNO_3$; *N*-nitroso-*N*-methylurea (NMU) (LLP Reaktivsnab).

Our experiment used a *composite medium* that concluded starch hydrolysate and sucrose as the carbon sources. The fermentation medium for CA manufacturing consisted of the following ingredients: corn starch maltodextrin with a DE of 18-20%, ammonium nitrate, magnesium sulfate heptahydrate, potassium dihydrophosphate, zinc sulfate heptahydrate, ferrous sulfate heptahydrate, and copper sulfate heptahydrate. The fermentable sugar content in the medium is 140 g/l.

Animals, Plants and Biological Materials

Wild strains (Almaty Region, Kazakhstan) of *Aspergillus niger* were able to consume carbohydrate-containing raw materials as a carbon source and produce CA.

Instruments

pH-meter Mettler Toledo Seven Compact S220-Basic (Mettler-Toledo Instruments Co., Ltd., Shanghai, China); Siemens medical X-ray machine at "Scientific-Research Institute for Radiation Medicine and Ecology" (Semey, the Republic of Kazakhstan); laboratory flasks shaker KJ-201BD (Mettler-Toledo Instruments Co., Ltd.); Erlenmeyer and other flasks types, tubes, paper filters (a filter ash weight was 0.54×10^{-3} g), microbiological needles, loops, and pipettes, Petri plates.

Laboratory Methods

Qualitative analysis of the CA secretion: in hydrolysis has been checked by the yellow zone formation due to adding an acid-base indicator, alizarin red, into the medium. Strains with the most significant CA secretion were selected for the subsequent trials - quantitative screening that was conducted in sterile Czapek Dox broth consisting of 50 ml in 250 ml Erlenmeyer flasks with additional ingredients: 30 g/l sucrose; 2.0 g/l $(NH_4)_2SO_4$; 1 g/l K_2HPO_4; 0.5 g/l $MgSO_4{\cdot}7H_2O$, 0.05 g/l $CaCl_2$. After incubation for 7 days at 30 °C, culture liquid from each flask was filtered through filter paper to separate the mycelium. Then, the filter was dried, and the yield of dry fungal biomass was recorded. The supernatant was used to determine CA, and the final pH values were recorded in a sample of 2 ml of culture liquid and 200 ml of distilled water: at the first stage – It was titrated with 0.1N NaOH solution using 1% phenolphthalein as an indicator. In the second stage, pH level was measured using a pH meter.

Mutations of fungal strains: NMU was used at concentrations of 0.005% and 0.015% to induce mutants in *A. niger* **[17]**. The material was washed twice with distilled water after incubation in the mutagenic solution at 30 °C for 45 min. The spores washed from the mutagen were resuspended in water and sown from serial tenfold dilutions on Petri plates containing agarified Czapek Dox medium with the addition of the acid-base indicator alizarin red.

At the physical stage, X-ray irradiation was used as a mutagenic agent to increase citric acid synthesis by *A. niger* **[18]**. Two irradiation regimes were used: the first test series included two irradiation periods of 1.8 s duration, irradiation dose – 3.6 μSv; the second test series had three irradiation periods with time – 1.8 s, irradiation dose – 5.4 μSv. After irradiation, the spores were resuspended in water and sown from serial tenfold dilutions on Petri plates with Czapek Dox agar. Survival rate was calculated as the ratio of colonies grown after cell treatment to those grown in the control (non-exposed strain).

Description of the Experiment

Sample preparation: *Aspergillus niger* pure cultures were isolated from the soil samples in Petri plates with sterilized Czapek Dox agar according to aseptic and antiseptic conditions: incubation at 30 ºC for 3-5 days and to 7 days for obtaining the highest sporulation level.

For quantitatively checking CA production, each inoculum (conidial suspension) was inoculated with 0.5 ml Czapek Dox broth and cultivated in a 150 rpm shaker at 30 °C for 7 days. Then, 2 ml of the cultivate liquid (with 200 ml distilled water) was titred by NaOH and tested at pH level by the pH meter.

For the mutation experimental trials, seven-day *A. niger* cultures grown on Petri plates with Czapek Dox medium were used.

Number of samples analyzed: At the first stage, 17 fungi isolates were studied; after mutagenesis, 383 mutant strains and 18 strains with high acidogenic activity were tested after the mutation factors acting.

Number of repeated analyses: to get significant results, each analysis has been repeated in three times.

Number of experiment replication: 3 parallel replication measurements have been conducted for each tested parameter.

Design of the experiment: We studied the CA production process and the modification of this process by using mutations in CA-producing strains. First of all, all isolated fungi strains were checked in CA production. The best product strains were conducted under chemical and physical factors.

In the next stage, the CA-producing ability of the mutant A. niger strain has been tested as well as checking the best conditions for it.

Seed mycelium cultivation was conducted in a 750 ml flask filled with 50 ml of nutrient medium, followed by 1% conidia suspension on an orbital shaker (160 min^{-1}) and incubated at 30 °C for 36 h.

A liter of the nutrient medium was placed in a 3-liter fermenter and sown with 10 ml of growing mycelium. The flask was placed on a rocker (160 min^{-1}) and incubated for 6-7 days at 30°C. After fermentation, the fungal biomass was separated on a Buechner funnel, and the CA amount and the conversion of sugars to CA in the culture solution were determined.

Determination of fermentation factors was tested by adding different concentrations of nutrients such as $MgSO_4{\cdot}7H_2O$, KH_2PO_4, $ZnSO_4{\cdot}7H_2O$, $FeSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$, $NaNO_3$, and ethanol to the growth medium during fermentation. Therefore, the nutrient factors affecting CA production by *A. niger* R5/4 were investigated to optimize the composition of the nutrient medium.

The effect of different inoculum amounts on CA production has been checked. The fermentation medium was inoculated with varying amounts of inoculum – 1, 3, 5, and 7% and incubated for 7 days at 30 °C.

A Box-Benken three-factor three-level design (BBD) was used to optimize CA production by *A. niger* R5/4 strain on a starch-containing medium (14%). Temperatures of 29, 30, and 31 °C were chosen to evaluate the effect of temperature on CA yield. The CA quantitative content was analyzed at different incubation times of 144, 168, and 192 h, and the impact of initial pH on acid formation was also investigated at 4.5, 5.0, and 5.5.

Fermentation efficiency was measured by mycelial dry weight, substrate concentration, and CA concentration. Citric acid mass yield coefficient (Y_{CA}), expressed in grams of CA per gram of substrate consumed, was calculated from formula (1):

$$Y_{CA} = \frac{P}{S} \quad (1)$$

The specific culture productivity, expressed in grams of CA per gram of biomass per hour, was calculated according to formula (2):

$$q_{CA} = \frac{P}{X \cdot t} \quad (2)$$

Where:

P – the total CA amount in the culture liquid at the end of cultivation, g; S – the total amount of substrate consumed, g; t – fermentation time, h; X – biomass in the fermenter, g.

The determination of acid concentration in the fermentation medium was evaluated by titrimetric method **[19]**.

Statistical Analysis

Statistical analysis was performed using SPSS version 22 statistical software for Microsoft Excel spreadsheets. Results were presented as standard deviation (SD) of the mean values from 3 parallel measurements. Data with p <0.05 with a 95% confidence interval were considered statistically significant. The mathematical processing of the measurements was carried out using SigmaPlot 12.5 with a non-linear regression method. Mathematica 12 software was used to visualize the data obtained and calculate the optimum. Statistical testing of the model using Box-Benken design was performed using Student's t-test to analyze variance (ANOVA).

RESULTS AND DISCUSSION

Study corn and wheat starch's physicochemical properties and quality parameters

The physicochemical properties of the raw materials, especially native starches, significantly influence the final properties of maltodextrins used in CA production **[9]**, **[11]**.

The phosphates and lipids influence the functional properties of starch in its composition. The phospholipid content in starch granules is proportionally related to the amylose content, as phospholipids tend to be complex with amylose and long branches of amylopectin and affect starch solubility **[20]**. The study revealed (Table 1) that the amylose amount was higher in wheat starch than in corn starch. The amount of phosphate (0.047%), lipid (0.261%), and amylose (28.3%) prevailed in wheat starch; on the contrary, the amount of phosphate was 0.037%, lipid 0.207%, and amylose 24.7% in corn starch.

Table 1 Physicochemical properties of native starches.

Indicator	Wheat starch	Corn starch
Moisture content, %	13.2 ±0.18	13.4 ±0.17
Phosphates, %	0.047 ±0.003	0.037 ±0.002
Lipids, %	0.261 ±0.001	0.207 ±0.005
Proteins, %	0.231 ±0.04	0.253 ±0.02
Minerals, %	0.237 ±0.01	0.220 ±0.02
Amylose, %	28.3 ±0.8	24.7 ±0.7
Amylopectin, %	71.75 ±0.8	75.3 ±0.7

Note: p <0,05; Mean value ± SD from three repetitions.

Starch hydrolysates – maltodextrins with DE 18-20% obtained by enzymatic corn and wheat starch were used for the cultivation of selected *A. niger* strains (Table 2).

Table 2 Physicochemical properties of starch hydrolysates used for CA production.

Indicators	Maltodextrins	
	wheat starch	corn starch
Mass fraction of dry substances, not less %	97.3	96.2
Mass fraction of reducing agents per dry substance, %	17.8	17.7
pH	6.5	6.5
Mass fraction of total ash per dry substance, %, not less	0.16	0.11
Proteins, %	0.063	0.070
Phosphorus, per P2O5, %	0.031	0.050

Maltodextrins have been sourced, considering that the presence of high protein in starches is particularly undesirable. It causes foaming of syrups during cooking and impedes filtration, resulting in increased syrup losses **[9]**, **[11]**, **[21]**. Protein content between 0.20 and 0.25% and ash content between 0.220 and 0.262% indicate the high quality of the starch samples obtained **[11]**. We have designed a two-stage starch dextrinisation process, which is considered optimal for complete starch liquefaction and the production of high-quality maltodextrin: first stage to DE 8-11%, then attaining DE 12-19% in the second phase **[11]**.

Note that acid formation tests cannot definitively determine which acid accumulates in the medium **[9]**, **[16]**. Strains were grown on mineral broth to identify acids and to verify the results obtained by express methods. The *A. niger* A1 and *A. niger* A6 strains with the highest CA production were then tested for quantitative CA formation (Table 3). The determination was performed in culture liquid at 30±1 °C for 168 h with pH changes according to **[22]** recommendations. CA concentration in the fermentation medium was estimated titrimetrically **[23]**.

Table 3 Quantitative determination of CA in culture liquid.

No.	Strain	CA quantity, g/100ml					
		Wheat maltodextrin			Corn maltodextrin		
		72 h	120 h	168 h	72 h	120 h	168 h
1	*A. niger* A1	1.412 ±0.12	7.64 ±0.04	10.4 ±0.05	1.345 ±0.13	8.75 ±0.07	11.21 ±0.2
2	*A. niger* A6	0.815 ±0.11	5.51 ±0.26	8.05 ±0.03	0.748 ±0.10	5.6 ±0.25	8.9 ±0.05

Note: $p < 0.05$; Mean value ± SD from three repetitions.

Table 3 shows that the CA levels after 168 hours of cultivation were between 8.05 and 10.4 g/100 ml on wheat maltodextrin medium and between 8.9 and 11.21 g/100 ml on corn maltodextrin medium. This indicates that products resulting from cereal processing can be used in CA manufacturing. Therefore, a closed cycle for cereal processing can be implemented to improve the economic efficiency of CA production. As noted **[3]**, this approach can efficiently produce organic acids and meet the demands of a market that is increasingly focused on a circular economy. A schema for the production of CA using sweet potato peels was published by **[24]** and found it to be a viable and sustainable option for manufacturing. proposed using cocoa pod husks to produce CA and demonstrated its potential for the future **[5]**.

As fermentation progressed, a gradual decrease in pH (Figure 1) was observed in all experiments, indicating that CA accumulated in the medium. These data agree with those of **[25]**, who found that the initial pH of 6.5 was gradually reduced to 1.5 during fermentation in a control production medium **[22]** suggests the maintenance of a low pH (less than 2.0), as a higher pH (around 4.0) may result in gluconic acid accumulation. In addition, an intermediate acidity between 1.8 and 2.5 is recommended by **[25]**.

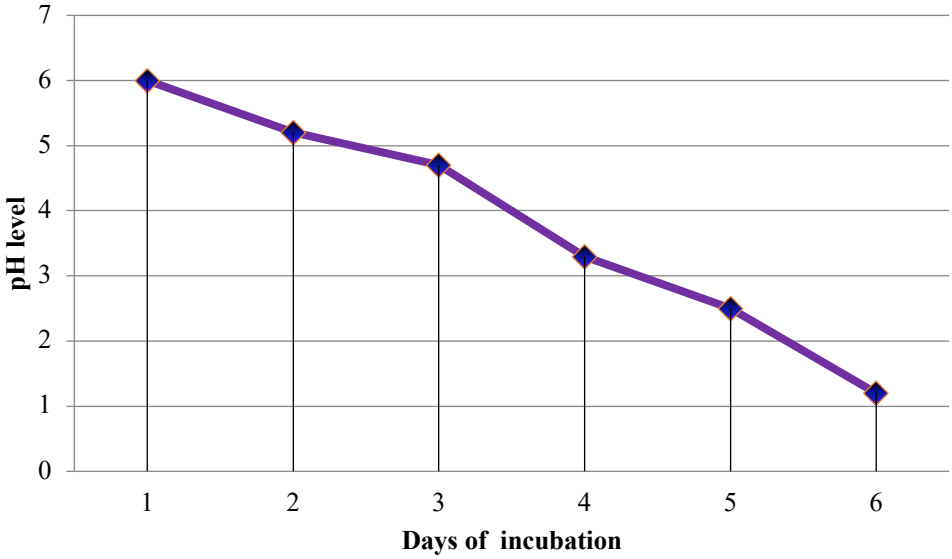


Figure 1 pH change during fermentation of *A. niger* A1.

Therefore, the *A. niger* A1 strain was selected (from now on, *A. niger* CA20).

Mutant strain production

Following one step of NMU 0.005% mutagenesis, 302 mutant lines were isolated, and following 0.015%, 81 lines were isolated. As shown, the survival rates for the strains decrease as the NMU concentration increases. A qualitative assay method based on pH change was used to select mutants with improved acid production capacity **[14]**, **[26]**

From 383 mutant strains, 18 strains with high acidogenic activity (diameter of yellow zone around colony more than 15 mm) were selected. In 211 cultures, the indices were between 8 mm and 15 mm, and 172 were less than 8 mm.

Mutant strains with the highest acidogenic rates were selected. The acidogenic activity was quantified by measuring the volume of titratable acid using 1% phenolphthalein as an indicator. Acid synthesis rates were calculated after 168 h. Analysis of the results showed that acid production increased in the mutant strains. After 168 h of cultivation, acid production ranged from 12.05 to 20.30 g/l (Table 4).

Table 4 CA yield in mutant strains after the first chemical mutagenesis step.

No.	Mutant strains	CA quantity, g/l
1	*Aspergillus niger* CA20-1(0.005)	15.80 ±0.9
2	*Aspergillus niger* CA20-2(0.005)	18.10 ±1.5
3	*Aspergillus niger* CA20-3(0.015)	15.30 ±0.2
4	*Aspergillus niger* CA20-4(0.005)	16.09 ±0.3
5	*Aspergillus niger* CA20-5(0.015)	12.80 ±2.1
6	*Aspergillus niger* CA20-6(0.015)	15.05 ±1.9
7	*Aspergillus niger* CA20-7(0.015)	12.05 ±1.5
8	*Aspergillus niger* CA20-8(0.005)	19.56 ±0.2
9	*Aspergillus niger* CA20-9(0.015)	13.40 ±0.8
10	*Aspergillus niger* CA20-10(0.015)	12.63 ±1.7
11	*Aspergillus niger* CA20-11 (0.005)	20.30 ±0.5
12	*Aspergillus niger* CA20-12(0.005)	18.90 ±2.1
13	*Aspergillus niger* CA20-13(0.005)	18.24 ±0.5
14	*Aspergillus niger* CA20-14(0.005)	15.03 ±0.9
15	*Aspergillus niger* CA20-15(0.005)	16.80 ±0.8
16	*Aspergillus niger* CA20-16(0.005)	18.70 ±0.3
17	*Aspergillus niger* CA20-17(0.005)	13.11 ±1.5
18	*Aspergillus niger* CA20-18(0.005)	15.71 ±0.4
19	Control *Aspergillus niger* CA20	11.21 ±0.2

The most excellent acid-forming ability of *A. niger* CA20-11(0.005) was reached after 7 days, resulting in 20.3 g/l CA, almost twice as high as the control wild strain. Since the spore survival rate was maximal and the mutant strains obtained had a high acid-forming ability, the recommended dosage of NMU application for further studies was 0.005%.

For the second mutagenesis step, the strains with the highest acid production rate, *A. niger* CA20-8 (0.005) and *A. niger* CA20-11 (0.005), were used. After 0.005% NMU action, 57 strains of *A. niger* CA20-8 (0.005) and 118 strains of *A. niger* CA20-11 (0.005) were isolated. Similarly, mutants were selected using a qualitative analysis method based on pH change and a quantitative analysis method. This resulted in 11 strains with 20.30 g/l activity (Table 5).

Table 5 CA yield in mutant strains after the second chemical mutagenesis step.

No.	Mutant strains	CA quantity, g/l
1	*Aspergillus niger* CA20-8(0.005)-1	22.80 ±1.2
2	*Aspergillus niger* CA20-8(0.005)-2	21.10 ±1.3
3	*Aspergillus niger* CA20-8(0.005)-3	22.30 ±0.7
4	*Aspergillus niger* CA20-11 (0.005)-4	25.09 ±0.5
5	*Aspergillus niger* CA20-11 (0.005)-5	22.80 ±1.1
6	*Aspergillus niger* CA20-11 (0.005)-6	25.05 ±0.9
7	*Aspergillus niger* CA20-11 (0.005)-7	23.05 ±0.2
8	*Aspergillus niger* CA20-11 (0.005)-8	20.56 ±0.2
9	*Aspergillus niger* CA20-11 (0.005)-9	23.40 ±0.7
10	*Aspergillus niger* CA20-11 (0.005)-10	26.63 ±0.4
11	*Aspergillus niger* CA20-11 (0.005)-11	21.30 ±0.5
12	Control *Aspergillus niger* CA20	11.21 ±0.2

As a result of these studies, a mutant strain of *A. niger* CA20-11 (0.005)-10 was obtained, which has 2.4 times the acidification capacity of the original strain.

The mutants produced by γ-irradiation are not considered genetically modified organisms, so their release into the environment after use is permitted, making biotechnological products cost-effective **[5]**, **[27]**. Using radiation achieves a high mutation probability. By exposing spores rather than mycelium to X-rays, micromycetes develop higher stress tolerance levels, minimizing unwanted side effects **[28]**.

Following radio irradiation of the *A. niger* strain CA20-11 (0.005)-10 at a dose of 3.6 μSv, 37 strains were isolated. Some of these colonies showed changes in spore color, colony growth rate, and pigment formation (Figure 2).

Figure 2 Cultural, morphological, and physiological characteristics of the strain CA20-11 after irradiation (3.6 μSv): A. Single colony at the Czapek Dox agar; B. Morphological study CA20-11 strain; magnification x40; C, D. – quality testing of the CA production – at the third (C) and seventh (D) day.

The mutation process affects the enzyme system of the mutant fungi, potentially increasing the activity of the enyzmes and improving the fungi's conforming activity or stability **[29]**. For instance, mutagen action can enhance the production of CA, which is useful in manufacturing. So, all mutant strains were tested for acidogenic activity in this way. Seven strains showed higher rates of CA formation than the parental strain (refer to Table 6). Some authors **[5]**, **[30]** found increased levels of protein engineering and CA production, respectively, in mutant strains of fungi and A. niger B6-CCT 7717.

Table 6 CA yield in mutant strains after radio irradiation (3.6 µSv) of *A. niger* CA 20-11 (0.005)-10.

No.	Mutant strains	CA quantity, g/l
1	*Aspergillus niger R3/6-1*	32.2 ±1.9
2	*Aspergillus niger R3/6-2*	26.8 ±1.1
3	*Aspergillus niger rR3/6-3*	28.1 ±0.5
4	*Aspergillus niger R3/6-4*	26.7 ±1.3
5	*Aspergillus niger R3/6-5*	33.5 ±0.2
6	*Aspergillus niger R3/6-6*	27.9 ±0.7
7	*Aspergillus niger R3/6-7*	27.3 ±0.9
8	Control *Aspergillus niger CA20-11 (0,005)-10*	26.6 ±0.4

In a second set of experiments, *A. niger* CA20-11(0.005)-10 received 5.4 µSv, which significantly reduced survival. A total of 21 mutant strains were isolated, and their CA productivity was analysed quantitatively. All strains tested showed a wide variation in CA synthesis parameters (Table 7). The highest CA yield was found in *A. niger* R5/4-8 (37.5 g/l) and the lowest in *A. niger* R5/4-11 (15.7 g/l).

Table 7 CA yield in mutant strains after radio irradiation (5.4 µSv) of *A. niger* CA 20-11 (0.005)-10.

No.	Mutant strains	CA quantity, g/l
1	*Aspergillus niger* R5/4-1	34.4 ±0.5
2	*Aspergillus niger* R5/4-2	28.7 ±1.8
3	*Aspergillus niger* R5/4-3	31.1 ±0.4
4	*Aspergillus niger* R5/4-4	33.5 ±0.2
5	*Aspergillus niger* R5/4-5	17.1 ±1.7
6	*Aspergillus niger* R5/4-6	25.5 ±1.1
7	*Aspergillus niger* R5/4-7	29.9 ±0.4
8	*Aspergillus niger* R5/4-8	37.5 ±0.7
9	*Aspergillus niger* R5/4-9	29.4 ±0.9
10	*Aspergillus niger* R5/4-10	27.5 ±1.1
11	*Aspergillus niger* R5/4-11	15.7 ±1.8
12	*Aspergillus niger* R5/4-12	22.1 ±1.3
13	*Aspergillus niger* R5/4-13	21.4 ±0.5
14	*Aspergillus niger* R5/4-14	36.1 ±0.6
15	*Aspergillus niger* R5/4-15	23.9 ±0.5
16	*Aspergillus niger* R5/4-16	19.8 ±1.1
17	*Aspergillus niger* R5/4-17	27.0 ±0.9
18	*Aspergillus niger* R5/4-18	35.1 ±0.4
19	*Aspergillus niger* R5/4-19	29.1 ±1.8
20	*Aspergillus niger* R5/4-20	34.2 ±0.5
21	*Aspergillus niger* R5/4-21	19.6 ±1.8

Thus, radio irradiation of the parental strain *A. niger* CA20-11 (0.005)-10 at a dose of 5.4 µSv resulted in *A. niger* R5/4-8 (hereafter referred to as *A. niger* R5/4) with the highest acidogenic activity (37.5 g/l).

Optimization of culture medium composition for *Aspergillus niger* R5/4

Aspergillus spp. is used in industry to produce citric acid, grow aerobically, and are heterotrophic **[16]**. The most important factors for citric acid production are fermentation time, medium pH, carbon sources, and nitrogen concentration **[24]**. These factors have a stimulating effect on the process. Therefore, we experimented to select the optimal conditions. The optimal maltodextrin-based nutrient medium composition for A. niger R5/4 with DE 18-20% was performed on a semi-pilot scale in a Labfors 5 Infors-HT fermenter (Switzerland), 3.6 l capacity with 1-2 l working volume but were took care not only of these four points and enlarged tested parameters.

Various concentrations of essential nutrients such as NH_4NO_3, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $FeSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$ were included in the fermentation medium to boost product yield (Table 8).

Table 8 Effect of some micronutrients on CA biosynthesis by *A. niger* R5/4.

Ingredients	Concentration	CA yield (g/l)			Biomass (g/l)		
		96 h	120 h	144 h	96 h	120 h	144 h
NH_4NO_3	1 g/l	6.46	7.52	8.75	0.75	0.95	1.30
	2 g/l	7.50	8.00	9.00	0.72	1.05	1.25
	3 g/l	7.51	8.28	9.80	0.82	1.25	1.62
	4 g/l	7.50	8.08	9.05	0.92	1.30	1.72
KH_2PO_4	2 g/l	6.32	7.01	8.62	0.68	0.92	1.20
	3 g/l	5.66	6.84	7.95	0.98	1.35	1.96
	4 g/l	6.03	7.01	8.32	0.95	1.20	1.80
	5 g/l	6.02	7.00	7.31	1.21	1.45	2.05
$MgSO_4 \cdot 7H_2O$	0.1 g/l	5.82	7.00	7.11	1.16	1.62	2.00
	0.2 g/l	4.96	6.04	6.55	1.35	1.85	2.56
	0.3 g/l	6.47	7.16	7.67	1.21	1.35	1.98
	0.4 g/l	5.27	6.14	6.79	0.99	1.30	1.91
$ZnSO_4 \cdot 7H_2O$	0.5 mg/l	6.37	7.23	8.08	1.36	1.52	2.20
	1 mg/l	6.16	7.06	8.17	0.94	1.16	1.80
	2 mg/l	5.28	6.47	7.58	1.50	2.15	2.95
	2.5 mg/l	5.24	6.45	6.56	1.34	1.75	2.28
$FeSO_4 \cdot 7H_2O$	2 mg/l	5.36	6.56	6.67	1.92	2.80	3.55
	7 mg/l	5.66	6.80	6.98	1.68	2.10	2.85
	12 mg/l	5.79	7.00	7.92	1.34	1.85	2.30
	17 mg/l	5.78	6.96	7.11	1.19	1.62	2.10
$CuSO_4 \cdot 5H_2O$	50 mg/l	5.62	5.87	6.31	1.49	1.68	1.87
	60 mg/l	6.02	6.26	6.97	1.69	1.76	1.92
	70 mg/l	6.94	7.12	7.53	1.85	2.11	2.25
	80 mg/l	6.23	6.77	7.14	1.79	2.01	2.19

Nitrogen (N_2) is a crucial factor for fungal growth as it helps to maintain the pH in the fermentation medium. **[22]** reported that high N_2 concentrations increase mycelium growth and sugar concentration, but they also note that it decreases CA production. Therefore, it is essential to maintain a balanced N_2 concentration in the medium to ensure an efficient process.

The addition of ammonium nitrate to the medium resulted in an increase in CA production in all experimental variants. The maximum bioproduct level was observed at the addition of 3 g/l after 144 h fermentation. This is explained by the fact that nitrogen is crucial for cell metabolism and a significant component of cellular proteins **[31]**. Ammonium nitrate contributed to reduced vegetative growth, while ammonium sulfate resulted in a longer vegetative growth period; the salt consumption caused a decrease in pH within the medium without producing oxalic acid. The maximum CA yield in the laboratory-stirred fermenter was obtained with an ammonium nitrate concentration of 0.2%, which correlates with the data **[31]**.

The accumulation of CA under phosphate-deficient conditions was reported by Perquin in 1938. This concept was patented by Szucs in 1944. The authors concluded that although phosphate concentration should be low, it should not be a limiting factor for CA accumulation **[32]**, **[33]** researched CA production in a chemostat culture, specifically studying the impact of phosphate-limited growth. According to their report, the citric acid output was lower in phosphate-limited steady-state conditions than in nitrogen-limited steady-state conditions. Their findings suggest that nitrogen excess had a more significant effect than phosphate deficiency.

The work by **[34]** showed that CA production by *A. niger* under phosphate-limited growth conditions is possibly suppressed by nitrogen catabolism. Fed-batch fermentation with double nitrogen and phosphorus limitation is an efficient and cost-effective method of CA production.

So, we have indicated a higher production of citric acid (8.62 g/l) when the phosphate concentration in the medium is reduced to 2 g/l, as depicted in Table 8. They are further increasing the concentration of potassium dihydrophosphate, resulting in a decrease in CA production. Adding phosphate at the maximum experimental concentration (5 g/l) reduced citric acid yield to 7.31 g/l after 144 h of cultivation using *A. niger* R5/4.

The necessity of micromycetes for metallic zinc as a micronutrient cannot be denied. Nevertheless, elevated concentrations of this metallic element show toxicity and can cause mutagenic changes in micromycetes **[35]**. The study suggests that zinc sulfate enhanced the fermentation process for the production of citric acid by *A. niger* R5/4. The highest CA production (8.17 g/l) occurred when the concentration of 1 mg/l $ZnSO_4 \cdot 7H_2O$ was

maintained for 144 h. As shown in Table 8, the use of elevated salt concentrations resulted in a decrease in CA yield during the corresponding incubation period.

Minor amounts or even small traces of copper are crucial for many microbes, such as microfungi. The copper concentration is the determinant factor in its impact on fungal growth, whether it be toxic or stimulating **[36]**. The inclusion of $CuSO_4 \cdot 5H_2O$ positively affected CA production during the 144 h synthesis based on the results obtained. The maximum amount of CA produced (7.53 g/l) was obtained with 70 mg/l CuSO4·5H2O in the fermentation medium. Conversely, a higher copper concentration (80 mg/l) was detrimental to A. niger activity, resulting in a lower CA content of 7.14 g/l.

Magnesium is essential for growth and CA production. The optimum amount of magnesium sulfate falls within the range of 0.02-0.025% **[10]**, **[22]**. According to the results obtained, the most favorable condition for the synthesis of citric acid by A. niger R5/4 is the introduction of 0.3g/l $MgSO_4 \cdot 7H_2O$ (7.67 g/l) into the medium.

One of the less well-understood requirements for cultivating A. niger to overproduce citrate is the limitation of iron concentration growth **[9]**. The direct relationship between citrate-metabolizing iron-dependent enzymes has been explained. Nonetheless, this may not always hold.

In aerobic media, bivalent iron gets converted into trivalent iron oxyhydroxide polymers (FeOOH), which are stable and exhibit low solubility in a medium, particularly at a neutral pH. Thus, despite the ubiquitous presence of iron, it is often biologically unavailable. Excess iron can also have detrimental effects, as Fe (II) can catalyze the formation of reactive oxygen species that impair cells. Consequently, microbes have implemented elaborate mechanisms to regulate iron uptake and storage within cells **[37]**. There are four recognized systems for acquiring iron in fungi: divalent iron uptake, heme decomposition, reduced iron assimilation, and trivalent iron uptake, which is mediated by siderophores. Low bivalent iron uptake is of less importance in iron-deficient media, while recent studies have investigated the haem uptake system of *A. niger*. Siderophores are small molecules microorganisms produce to sequester trivalent iron from the surrounding environment and facilitate its uptake by microbes. Their synthesis and function are crucial in microbial iron metabolism and growth **[10]**, **[37]**, **[38]**, indicating that A. niger's role as a siderophore is supported by the physiological response of increased citrate secretion under iron-limited conditions.

Our results indicate that the iron (II) sulfate addition to the culture medium at a concentration of 12 mg/l leads to a significant increase in CA secretion by *A. niger* R5/4, reaching a concentration of 7.92 g/l. At $FeSO_4$ levels above 12mg/l, CA decreases to 7.11g/l (Table 8).

The fermentation medium for *A. niger* R5/4 has been optimized using corn starch maltodextrins (DE 18-20%) as the basis, with a concentration of 140 g/l of fermentable sugars in the medium. The medium also contains NH_4NO_3 – 3 g/l, KH_2PO_4 – 2 g/l, $MgSO_4 \cdot 7H2O$ – 0.3 g/l, $ZnSO_4 \cdot 7H_2O$ – 1 mg/l, $FeSO_4 \cdot 7H_2O$ – 12 mg/l, and $CuSO_4 \cdot 5H_2O$ – 70 mg/l.

Effect of different alcohol concentrations on CA production

Among the three alcohols studied, CA production was optimized under pilot-scale conditions using maize starch hydrolysate medium with 3% ethanol after 120 h fermentation (Table 9).

Table 9 Effect of alcohol addition on CA production by *Aspergillus niger* R5/4.

Alcohol		Final pH	CA yield (g/l)	Conversion rate, (%)
Name	Concentration, %			
Control	0	3.1	3.4	4.5
Methanol	1.0	3.4	0.4	0.5
Methanol	2.0	3.8	7.1	9.8
Methanol	3.0	3.7	2.4	2.0
Ethanol	1.0	3.9	7.9	11.4
Ethanol	2.0	3.3	13.7	20.3
Ethanol	3.0	3.1	19.6	27.9
Ethanol (6 h)[2]	3.0	3.0	20.9	31.2
Ethanol (18 h)[2]	3.0	3.3	8.4	10.5
Ethanol (24 h)[2]	3.0	3.3	8.7	11.2
Ethanol	4.0	3.2	7.6	10.1
Ethanol	5.0	3.6	4.2	6.1
Isopropanol	1.0	3.9	2.1	3.1
Isopropanol	2.0	3.7	1.4	1.6
Isopropanol	3.0	3.8	0.2	0.0

A concentration of 2% of methanol led to a minor increase in CA yield, but at higher levels, both CA production and growth were significantly reduced. There was a notable reduction in the growth and productivity of citric acid upon the addition of isopropanol. The higher the concentration of alcohol, the less product was synthesized. At a concentration of 3% isopropanol, the synthesis of citric acid was greatly inhibited.

Adding ethanol has been found to enhance the excretion of citric acid, with the maximum level of formation reached at 3%. However, higher doses resulted in a decrease in product yield. Upon addition of ethanol 6 h after inoculation, the sugar conversion to citric acid was 31%. No increase in CA productivity was observed when ethanol was added after 18 and 24 hours of culture growth. Ethanol increased citrate synthase activity twofold but decreased aconitase activity by 75%, according to **[39]**. It can form acetyl-CoA, which is a precursor of citric acid. Additionally, micro-fungi utilize ethanol as a carbon source. Reduced growth and CA production at higher ethanol concentrations may be attributed to its toxic impact. So, contrary to the findings of other researchers who identified methanol as the most potent simulator, our research indicates that ethanol is the most potent inducer of citric acid biosynthesis by *A. niger* R5/4.

Effect of different *Aspergillus niger* R5/4 inoculum concentrations on CA production

Based on the results depicted in Figure 3, it is evident that the highest quantity of citric acid was obtained with a 3% inoculum.

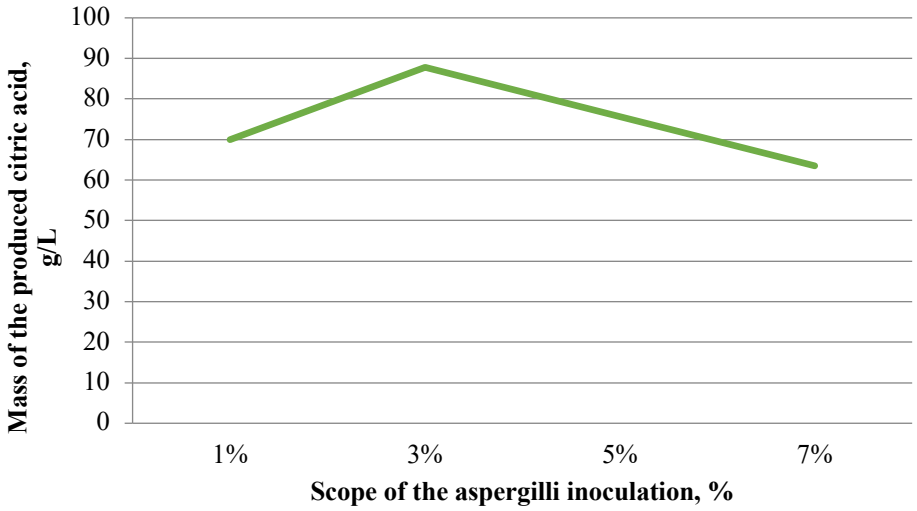


Figure 3 Effect of different inoculum concentrations on CA production.

According to the data presented in Figure 3, it is evident that the optimal CA yield was attained using a 3% inoculum concentration. The CA production peaked at a seed size of 5%, resulting in a maximum output of 83.24 g/l **[40]**. The highest CA amount was obtained with a 1% inoculum (96.86 g/l), as reported by **[41]**.

In our research on enhancing citric acid biosynthesis through *A. niger* R5/4 in a 3.6 l Labfors 5 Infors-HT bioreactor located in Switzerland, the Box-Behnken design of the experiment was used. The effect of medium acidity, incubation time, and fermentation temperature on CA production was optimized in a fermenter using a Box-Behnken design. Critical values of the medium components previously obtained in optimizing the culture medium were adopted. The optimized fermentation medium consists of corn starch maltodextrins with a DE of 18-20, with a fermentable sugar concentration of 140 g/l medium, containing the following components NH_4NO_3 – 3 g/l, KH_2PO_4 – 2 g/l, $MgSO_4 \cdot 7H_2O$ – 0,3 g/l, $ZnSO_4 \cdot 7H_2O$ – 1 mg/l, $FeSO_4 \cdot 7H_2O$ – 12 mg/l, $CuSO_4 \cdot 5H_2O$ – 70 mg/l. Previous studies have also identified the optimum 3% ethanol and 3% inoculum concentrations.

Optimization studies using the Box-Behnken design (Eq. 3) under in vitro conditions in flasks showed that the maximum CA yield occurred at a temperature regime of 30 ±1 °C, a medium acidity of pH 5.0 and an incubation time of 168 h. These physical and chemical parameters provided sufficient reference points. Citric acid yield values were obtained after 168 h of fermentation in 17 experiments (Table 10). Similar findings were published by other authors. They noted that CA production increased proportionally with incubation time until the seventh day, after which the production rate decreased. This tendency can be explained by several parameters, including decreased sugar content, the age and increased biomass of Aspergillus spp., the concentration of dissolved oxygen (important because fungi are aerobes), and the cation level in the cultivation medium **[14]**, **[17]**, **[24]**.

Table 10 Experimental *A. niger* R5/4 sowning for CA production by using a Box-Behnken design.

Experiment number	Factors					
	Coded variables			Real variables		
	X1	X2	X3	X1	X2	X3
1	-1	-1	0	29	4.5	168
2	+1	-1	0	31	4.5	168
3	-1	+1	0	29	5.5	168
4	+1	+1	0	31	5.5	168
5	-1	0	-1	29	5.0	144
6	+1	0	-1	31	5.0	144
7	-1	0	+1	29	5.0	192
8	+1	0	+1	31	5.0	192
9	0	-1	-1	30	4.5	144
10	0	+1	-1	30	5.5	168
11	0	-1	+1	30	4.5	192
12	0	+1	+1	30	5.0	192
13	0	0	0	30	5.0	168
14	0	0	0	30	5.0	168
15	0	0	0	30	5.0	168
16	0	0	0	30	5.0	168
17	0	0	0	30	5.0	168

$$Y = -1529.9 + 1615.1X31 + 70.19X2 + 58.7X3 - 1.25X1X2 - 8.62X1X3 - 0.41X2X3 - 1501.3X2.1 - 1.68X2.2 - 0.87X2.3 \quad (3)$$

Where:

Y – CA yield; *X1*, *X2* and *X3* - coded variables of temperature, medium acidity, *pH* and incubation period, respectively (Table 11).

Table 11 Box-Behnken design: evaluating the effects of medium parameters on CA production in a fermenter.

Medium parameters	Regression	*t*	*p*
Mean value/interaction	−1529.9	−5.46	0.003
(1) Temperature (°C) (L)	1615.1	4.24	0.006
Temperature (°C) (Q)	−1501.3	−5.37	0.003
(2) Medium acidity, pH (L)	70.19	5.396	0.003
Medium acidity, pH (Q)	−1.68	−5.561	0.002
Incubation period (h) (L)	58.7	3.516	0.018
Incubation period (h) (Q)	−0.87	−2.756	0.041
1 L k 2 L	−1.25	−0.133	0.878
1 L k 3 L	−8.6	−0.988	0.375
2 L k 3	−0.41	−1.267	0.259

The regression analysis calculation results in a coefficient of determination (R2 = 0.959), demonstrating that only 3.35% of the total variation remains unexplained by the model. The adjusted coefficient of determination (adj. R2 = 0.899) is similarly high, signifying strong significance of the model.

The statistical design analysis shows high accuracy of the polynomial model, reflecting a high degree of agreement between predicted and experimental data. Response surface plots as a function of two factors simultaneously, with all other factors held at fixed levels (e.g. zero), can be easily obtained by calculating from the model and taking variables for one factor as the other is varied (from -1.0 to +1.0, e.g. a step of 0.5), with this value of Y constrained. The corresponding response surface plots can also predict the productivity values for different variable concentrations (Figure 4).

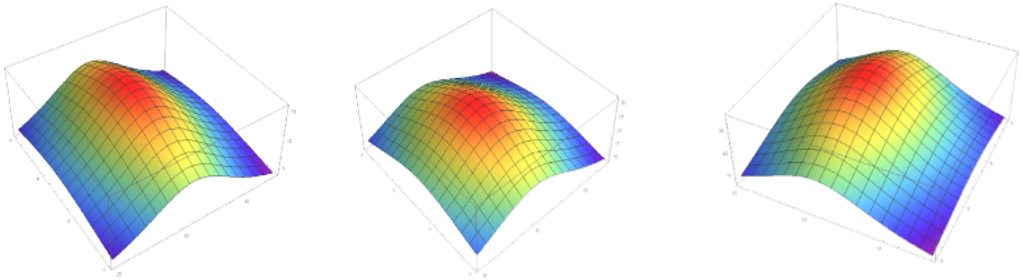


Figure 4 Response surface curve for CA production in the fermenter: A. – represents the interaction between temperature and medium pH; B – represents the interaction between temperature and fermentation period; C – represents the interaction between medium pH and fermentation period.

The surface plot illustrates the separate impact of the variables and their combined effect on the response, stemming from the two test variables in question. Note that the preferred parameters for the optimal process of CA production by *A. niger* R5/4 by deep fermentation for 168 h are: temperature 30.4 °C and initial medium pH 5.2. This information is consistent with previous research but provides a clearer range. Previous studies have shown that a medium pH above 6.0 is not ideal for CA production **[24]**, which is supported by the findings **[22]**. Our experiment provides more detailed data and confirms that a pH of 5.2 is the best for synthesizing CA.

The fermentation process's productivity was assessed by computing fermentation efficiency ratios after 168 h (Table 12).

Table 12 Fermentation process productivity indicators.

Experiment number	Fermentation efficiency coefficients		CA content, g/l	Conversion rate, %
	Product yield from the substrate, $Y_{P/S}$	Specific product formation rate, q_P		
1	0.52 ±0.20	0.07 ±0.5	88.1 ±1.3	62.9
2	0.68 ±0.12	0.08 ±0.2	99.5 ±0.4	71.1
3	0.68 ±1.3	0.04 ±0.5	84.14 ±1.2	60.1
4	0.70 ±0.58	0.06 ±0.5	97.7 ±1.0	69.8
5	0.62 ±0.3	0.07 ±0.10	93.0 ±0.2	66.4
6	0.55 ±0.05	0.05 ±0.26	64.12 ±2.1	45.8
7	0.69 ±0.02	0.09 ±0.04	99.7 ±0.3	71.2
8	0.59 ±0.68	0.08 ±0.61	97.6 ±1.9	69.7
9	0.66 ±0.72	0.05 ±0.02	68.3 ±0.5	48.6
10	0.64 ±0.48	0.07 ±0.98	81.5 ±0.8	58.2
11	0.70 ±0.21	0.08 ±0.16	85.8 ±0.9	61.3
12	0.73 ±0.13	0.09 ±0.07	96.3 ±1.2	68.8
13	0.92 ±0.29	0.11 ±0.03	117.3 ±0.5	83.8
14	1.01 ±0.04	0.12 ±0.05	117.2 ±0.8	83.73
15	0.95 ±0.1	0.11 ±0.1	117.2 ±0.8	83.7
16	1.01 ±0.01	0.12 ±0.02	120.6 ±1.2	86.15
17	1.1 ±0.99	0.11 ±0.01	119.56 ±0.8	85.4

Therefore, a Box-Behnken design with three variables - temperature, pH, and fermentation time - was used to optimize the production of citric acid in a fermenter using an optimized corn hydrolysate culture medium with the following composition: sugars - 140 g/l, NH_4NO_3 – 3 g/l, KH_2PO_4 – 2 g/l, $MgSO_4 \cdot 7H_2O$ – 0.3 g/l, $ZnSO_4 \cdot 7H_2O$ – 1 mg/l, $FeSO_4 \cdot 7H_2O$ – 12 mg/l, $CuSO_4 \cdot 5H_2O$ – 70 mg/l. The micro-mycete *A. niger* R5/4 produces the highest amount of citric acid by deep fermentation at a temperature of 30.4 °C, a pH of 5.2, and an incubation period of 168 h. Accordingly, the specific product formation rate (CA) was 0.12 Qp, and the conversion rate reached 86.15%.

CONCLUSION

The study demonstrated that multi-step mutagenesis yields *A. niger* strains with high levels of CA production. The fermentation medium for *A. niger* R5/4 has been optimized. The medium base consisted of corn starch maltodextrins with DE 18-20 and a fermentable sugars concentration of 140 g/l. The medium contained the following components: NH_4NO_3 – 3 g/l, KH_2PO_4 – 2 g/l, $MgSO_4 \cdot 7H_2O$ – 0.3 g/l, $ZnSO_4 \cdot 7H_2O$ – 1 mg/l, $FeSO_4 \cdot 7H_2O$ – 12 mg/l, $CuSO_4 \cdot 5H_2O$ – 70 mg/l. Adding ethanol increased citric acid excretion, with the highest bioproduct obtained at a concentration of 3%. The sugars were converted into CA at a rate of 31% when ethanol was added 6 h after the inoculation. Additional ethanol supplementation at 18 and 24 h of culture growth did not increase CA production. The conclusion from our study is that the inoculum concentration has a significant effect on the level of external citrate accumulation. The highest amount of citric acid was achieved with a 3% inoculum. A Box-Benken design was used to optimize CA production in a fermenter using an optimized culture medium involving three variables: temperature, pH, and fermentation time. At a temperature of 30.4 °C, with a medium pH level of 5.2 and an incubation period of 168 h, *A. niger* R5/4, a micromycete, produces the highest CA concentration through deep fermentation procedures. These findings also demonstrated that the specific rate of product formation was 0.12 qP, and conversion rates reached 86.15%.

REFERENCES

1. Ibyzhanova, A., Rustenova, E., Jakupova, A., & Talapbayeva, G. (2022). Food market of the Republic of Kazakhstan: Export opportunities. In Eurasian Journal of Economic and Business Studies (Vol. 65, Issue 3, pp. 60–6). Kenzhegali Sagadiyev University of International Business.. https://doi.org/10.47703/ejebs.v3i65.106
2. Lu, L. C., Chiu, S. Y., Chiu, Y. H., & Chang, T. H. (2022). Three-stage circular efficiency evaluation of agricultural food production, food consumption, and food waste recycling in EU countries. In Journal of Cleaner Production (Vol. 343, 130870). Elsevier Ltd. https://doi.org/10.1016/j.jclepro.2022.130870
3. Amato, A., Becci, A., & Beolchini, F. (2020). Citric acid bioproduction: The technological innovation change. In Critical Reviews in Biotechnology (Vol. 40, Issue 2, pp. 199–212). Taylor & Francis. https://doi.org/10.1080/07388551.2019.1709799
4. Kulev, D. H., Osintseva, E. V., Nikolaev, A. G., Kremleva, O. N., & Tabatchikova, T. N. (2014). Development of a certified reference materials for composition of citric acid. In Measurement Standards. Reference Materials (Issue 3, pp. 100–108). Ural Research Institute of Metrology.
5. de Oliveira, P. Z., de Souza Vandenberghe, L. P., Rodrigues, C., de Melo Pereira, G. V., & Soccol, C. R. (2022). Exploring cocoa pod husks as a potential substrate for citric acid production by solid-state fermentation using Aspergillus niger mutant strain. In Process Biochemistry (Vol. 113, pp. 107–112). Elsevier Ltd. https://doi.org/10.1016/j.procbio.2021.12.020
6. Kudasov, K. K., Berstenev, S. V., Volkov, D., & Zhambakin, K. J. (2015). Citric acid. Review. In News of Kazakhstan science (Vol. 3, Issue 125, pp. 121–153). JSC "National Center for State Scientific and Technical Evaluation".
7. Dzholdasbaeva, G., & Yesakhmetova, L. (2020). Export of grain processing enterprises' products. In Problems of Agrimarket (Issue 2, pp. 126–133). Almaty: Kazahskij naučno issledovatel′skij institut èkonomiki agropromyšlennogo kompleksa i razvitiâ sel′skih territorij.
8. Sugimoto, T., Murashima, K., & Ito, K. (2001). Characterization of alpha-amylase genes of industrial fungus Aspergillus kawachii. In XXI Fungal Genetics Conference (Vol. 48, p. 80). Fungal Genetics Reports.
9. Sharova, N. Y., & Nikiforova, T. A. (2005). Sovremennaya biotekhnologiya limonnoy kisloty i kislotostabil'nykh fermentov. In Modern high technologies (Issue 8, p. 79). Russian academy of natural history.
10. Behera, B. C., Mishra, R., & Mohapatra, S. (2021). Microbial citric acid: Production, properties, application, and future perspectives. In Food Frontiers (Vol. 2, Issue 1, pp. 62–76). Fujian Agriculture and Forestry University. https://doi.org/10.1002/fft2.66
11. Kulev, D H., & Sharova, N. Y. (2013). Primenenie gidrolizatov krakhmala v proizvodstve pishchevykh kislot. In Glubokaya pererabotka zerna dlya proizvodstva krakhmala, yego modifikatsiy i sakharistykh produktov. Tendentsii razvitiya proizvodstva i potrebleniy (pp. 159–163). Russian Academy of Agricultural Sciences.
12. Aghera, P. R., & Bhatt, N. S. (2019). Citric acid: Biosynthesis, properties and application. Lambert Academic Publishing.
13. Jiang, C., Wang, H., Liu, M., Wang, L., Yang, R., Wang, P., Lu, Z., Zhou, Y., Zheng, Z., & Zhao, G. (2022). Identification of chitin synthase activator in Aspergillus niger and its application in citric acid fermentation.

In Applied Microbiology and Biotechnology (Vol. 106, Issue 21, pp. 6993–7011). Springer. https://doi.org/10.1007/s00253-022-12174-9

14. Munshi, M. K., Hossain, F., Huque, R., Rahman, M. M., Khatun, A., Islam, M., & Khalil, I. (2013). Effect of biomass and sugar in citric acid production by Aspergillus niger using molasses and jackfruit as substrates. In American Journal of Food and Nutrition (Vol. 1, Issue 1, pp. 1–6). Science and Education Publishing Co. Ltd.
15. Lu, Y., Zheng, X., Wang, Y., Zhang, L., Wang, L., Lei, Y., Zhang, T., Zheng, P, & Sun, J. (2022). Evaluation of Aspergillus niger six constitutive strong promoters by fluorescent-auxotrophic selection coupled with flow cytometry: A case for citric acid production. In Journal of Fungi (Vol. 8, Issue 6, p. 568). MDPI. https://doi.org/10.3390/jof8060568
16. Sawant, O., Mahale, S., Ramchandran, V., Nagaraj, G., & Bankar, A. (2018). Fungal citric acid production using waste materials: A mini-review. In Journal of Microbiology, Biotechnology and Food Sciences (Vol. 8, Issue 2, pp. 821–828). Faculty of Biotechnology and Food Sciences Nitra. https://doi.org/10.15414/jmbfs.2018.8.2.821-828
17. Dezam, A. P. G., Vasconcellos, V. M., Lacava, P. T., & Farinas, C. S. (2017). Microbial production of organic acids by endophytic fungi. In Biocatalysis and Agricultural Biotechnology (Vol. 11, pp 282–287). Elsevier Ltd. https://doi.org/10.1016/j.bcab.2017.08.001
18. Kirimura, K., Hirowatari, Y., & Usami, S. (1987). Alterations of respiratory systems in Aspergillus niger under the conditions of citric acid fermentation. In Agricultural and biological chemistry (Vol. 51, Issue 5, pp. 1299–1303). Japan Society for Bioscience, Biotechnology, and Agrochemistry. https://doi.org/10.1271/bbb1961.51.1299
19. Wyatt, T. T., Wosten, H. A., & Dijksterhuis, J. (2013). Fungal spores for dispersion in space and time. In Advances in Applied Microbiology (Vol. 85, pp. 43–91). Elsevier Inc. https://doi.org/10.1016/B978-0-12-407672-3.00002-2
20. Estivi, L., Brandolini, A., Condezo-Hoyos, L., & Hidalgo, A. (2022). Impact of low-frequency ultrasound technology on physical, chemical and technological properties of cereals and pseudocereals. In Ultrasonics Sonochemistry (Vol. 86, 106044). Elsevier BV. https://doi.org/10.1016/j.ultsonch.2022.106044
21. Bottenmuller, A., Théodon, L., Debayle, J., Vélez, D. T., Tourbin, M., Frances, C., & Gavet, Y. (2023). Granulometric analysis of maltodextrin particles observed by scanning electron microscopy. In 2023 IEEE 13th International Conference on Pattern Recognition Systems (ICPRS) (pp. 1–7). IEEE. https://doi.org/10.1109/ICPRS58416.2023.10179067
22. Bhattacharjee, I., & Baruah, P.K. (2015). Isolation and screening of citric acid producing Aspergillus spp and optimisation of citric acid production by Aspergillus niger S-6. In Journal of Environmental Science, Toxicology and Food Technology (Vol. 9, Issue 3, pp. 19–23). Elsevier and Science Press.
23. Cunniff, P., & Association of Official Analytical Chemists. (1995). Official methods of analysis of AOAC International (16th ed.). Association of Official Analytical Chemists.
24. Aboyeji, O. O., Oloke, J. K., Arinkoola, A. O., Oke, M. A., & Ishola, M. M. (2020). Optimization of media components and fermentation conditions for citric acid production from sweet potato peel starch hydrolysate by Aspergillus niger. In Scientific African (Vol. 10, e00554). Elsevier. https://doi.org/10.1016/j.sciaf.2020.e00554
25. Angumeenal, A., Kamalakannan, P., Prabhu, H. J., & Venkappayya, D. (2003). Effect of transition metal cations on the production of citric acid using mixed cultures of Aspergillus niger and Candida gulliermondii. In Journal of the Indian Chemical Society (Vol. 80, Issue 10, pp. 903–906). Elsevier BV.
26. Behera, B. C. (2020). Citric acid from Aspergillus niger: A comprehensive overview. In Critical Reviews in Microbiology (Vol. 46, Issue 6, pp. 727-749). Informa UK Limited. https://doi.org/10.1080/1040841X.2020.1828815
27. Zheng, X., Cairns, T. C., Ni, X., Zhang, L., Zhai, H., Meyer, V., ... & Sun, J. (2022). Comprehensively dissecting the hub regulation of PkaC on high-productivity and pellet macromorphology in citric acid producing Aspergillus niger. In Microbial Biotechnology (Vol. 15, Issue 6, pp. 1867–1882). John Wiley & Sons, Inc. https://doi.org/10.1111/1751-7915.14020
28. Vinarov, A. J., Sidorenko, T. E., Smetanina, S. E., & Jashina, V. N. (1998). Moskow: Russian Agency for Patents and Trademarks. (Russian Patent No. RU 2103346C1). IP Russia.
29. Cao, J.-R., Fan, F.-F., Lv, C.-J., Wang, H.-P., Li, Y., Hu, S., Zhao, W.-R., Chen, H.-B., Huang, J., & Mei, L.-H. (2021). Improving the Thermostability and Activity of Transaminase From Aspergillus terreus by Charge-Charge Interaction. In Frontiers in Chemistry (Vol. 9). Frontiers Media SA. https://doi.org/10.3389/fchem.2021.664156

30. Jiang, X., Wang, Y., Wang, Y., Huang, H., Bai, Y., Su, X., Zhang, J., Yao, B., Tu, T., & Luo, H. (2021). Exploiting the activity–stability trade-off of glucose oxidase from Aspergillus niger using a simple approach to calculate thermostability of mutants. In Food Chemistry (Vol. 342, p. 128270). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.128270
31. Kareem, S. O., & Rahman, R. A. (2013). Utilization of banana peels for citric acid production by Aspergillus niger. In Agriculture and Biology Journal of North America (Vol. 4, Issue 4, pp. 384–387). Science Hub. http://doi.org/10.5251/abjna.2013.4.4.384.387
32. Aguirre, S. N., Majolli, M. V. I., de Figueroa, L. I., & Callieri, D. A. (2004). Effect of Phosphorus concentration on the citric acid production by Yarrowia lipolytica s26, in a medium containing yeast extract and sugarcane molasses. In R. Jonas, A. Pandey, & G. Tharun (Eds.), Biotechnological advances and applications in bioconversion on renewable raw material (pp. 113–116). Gesellschaft für Biotechnologische Forschung mbH Bibliothek.
33. Davati, N., & Najafi, M. B. H. (2013). Overproduction strategies for microbial secondary metabolites: A review. In International Journal of Life Sciences & Pharma Research (Vol. 3, Issue 1, pp. 23–27). UbiJournal Pvt Ltd.
34. Rajpurohit, H., & Eiteman, M. A. (2022). Nutrient-limited operational strategies for the microbial production of biochemicals. In Microorganisms (Vol. 10, Issue 11, p. 2226.). MDPI. https://doi.org/10.3390/microorganisms10112226
35. Li, J. F., Rupa, E. J., Hurh, J., Huo, Y., Chen, L., Han, Y., Ahn, J. C., Park, J. K., Lee, H. A., Mathiyalagan, R., & Yang, D.-C. (2019). Cordyceps militaris fungus mediated Zinc Oxide nanoparticles for the photocatalytic degradation of Methylene blue dye. In Optik (Vol. 183, pp. 691–697). Elsevier GmbH. https://doi.org/10.1016/j.ijleo.2019.02.081
36. Ali, S., & UI Haq, I. (2001). Submerged fermentation of citric acid: Counter-act effect of Cu^{2+} on the deleterious effect of Fe^{2+} in blackstrap molasses. In Journal of Biological Sciences (Vol. 1, Issue 9, pp. 852–-853). Science Alert.
37. Haas, H. (2014). Fungal siderophore metabolism with a focus on Aspergillus fumigatus. In Natural Product Reports (Vol. 31, Issue 10, pp. 1266–1276). Royal Society of Chemistry. https://doi.org/10.1039/C4NP00071D
38. Odoni, D. I., van Gaal, M. P., Schonewille, T., Tamayo-Ramos, J. A., Martins dos Santos, V. A. P., Suarez-Diez, M., & Schaap, P. J. (2017). Aspergillus niger secretes citrate to increase iron bioavailability. In Frontiers in Microbiology (Vol. 8, 1424). Frontiers Media SA. https://doi.org/10.3389/fmicb.2017.01424
39. Kundu, P., & Ghosh, A. (2023). Genome-scale community modeling for deciphering the inter-microbial metabolic interactions in fungus-farming termite gut microbiome. In Computers in Biology and Medicine (Vol. 154, 106600). Elsevier Ltd. https://doi.org/10.1016/j.compbiomed.2023.106600
40. Maharani, V., Reeta, D., Sundaramanickam, A., Vijayalakshmi, S., & Balasubramanian, T. (2014). Isolation and characterization of citric acid producing Aspergillus niger from spoiled coconut. In International Journal of Current Microbiology and Applied Science (Vol. 3, Issue 3, pp. 700–705). IJCMAS.
41. Ali, S., UI Haq, I., Qadeer, M., & Iqbal, J. (2002). Production of citric acid by Aspergillus niger using cane molasses in a stirred fermentor. In Electronic Journal of Biotechnology (Vol. 5, Issue 3, pp. 19–20). https://doi.org/10.2225/vol5-issue3-fulltext-3

Funds:

This research was funded by the Ministry of Agriculture of the Republic of Kazakhstan, grant number BR06249419.

Acknowledgments:-

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

*Bakhyt Shaimenova, NCJSC, S. Seifullin Kazakh Agro Technical Research University, Technical Faculty, Department of Food Technology and Processing Products, Zhenis Avenue 62, Astana 010011, the Republic of Kazakhstan,
Tel.: + 77071372147
E-mail: shaimenovabakhyt@gmail.com
ORCID: https://orcid.org/0000-0001-5472-036X

Gulnazym Ospankulova, NCJSC S. Seifullin Kazakh Agro Technical Research University, Technical Faculty, Department of Food Technology and Processing Products, Zhenis Avenue 62, Astana 010011, the Republic of Kazakhstan,
Tel.: + 77076413060
E-mail: bulashevag@mail.ru
ORCID: https://orcid.org/0000-0002-6043-4658

Saule Saduakhasova, NCJSC S. Seifullin Kazakh Agro Technical Research University, Technical Faculty, Department of Food Technology and Processing Products, Zhenis Avenue 62, Astana 010011, the Republic of Kazakhstan,
Tel.: + 77052449085
E-mail: saule_aru@list.ru
ORCID: https://orcid.org/0000-0002-9483-5732

Linara Murat, NCJSC S. Seifullin Kazakh Agro Technical Research University» Technical Faculty, Department of Food Technology and Processing Products, Zhenis Avenue 62, Astana 010011, the Republic of Kazakhstan,
Tel.: + 77753459061
E-mail: linaraazamatkyzy@mail.ru
ORCID: https://orcid.org/0000-0001-5684-0621

Dana Toimbayeva, NCJSC S. Seifullin Kazakh Agro Technical Research University, Technical Faculty, Department of Food Technology and Processing Products, Zhenis Avenue 62, Astana 010011, the Republic of Kazakhstan,
Tel.: +77013563619
E-mail: bio.dana@mail.ru
ORCID: https://orcid.org/0000-0001-9595-0559

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 1.11.2023
Revised: 10.1.2024
Accepted: 22.1.2024
Published: 28.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 174-184
https://doi.org/10.5219/1934
ISSN: 1337-0960 online
www.potravinarstvo.com

Probiotics in the creation of fish-based herodietic half-finished products

Dinara Moldagaliyeva, Nurzhan Sarsembaeva, Yasin Uzakov, Bozhena Lozovicka

ABSTRACT

Feeding is a basic need in human life. The current manuscript presents the first stage in developing of a fish semi-finished product for functional purposes in the production of commercial tilapia fish in high-tech industrial aquaculture. So, the clinical rationale for the probiotic is based on the Escherichia coli 64G strain used in the process of fish breeding for the hero dietic half-finished products manufacturing. So, a has been created to get a functional product (smoked sausage) from fresh tilapia fish grown on specialised feeds at the stage of biotechnological processing. The possibility of using the drug Enterocol as a probiotic strain of Kazakh production is being considered. Probiotics in aquaculture have been used to reduce the level of conditionally pathogenic microbiota in the organs and tissues of fish. This was achieved by Enterocol's action in reducing the organic pollution of water with fish metabolism products at a high stocking density. Probiotics used in industrial aquaculture are an excellent alternative to antibiotics. Moreover, we can get an environmentally friendly product due to probiotics, which is important in creating a healthy food strategy. In an in vivo experiment, authors proved the safety, antagonistic activity, and probiotic proprieties of the *E.coli* 64G strain.

Keywords: functional product, *E.coli* 64G strain, Enterocol, probiotic, herodietic

INTRODUCTION

Modern civilization is focused on a healthy lifestyle. A healthy lifestyle is a healthy diet in which functional products are leading **[1]**, **[2]**. The concept of functional products was developed in Japan. In the conditions of modern civilization, a person eats industrially processed products, and the sedentary lifestyle that has become the norm requires much less energy consumption than physical labor. Under these conditions, food consumption is reduced. Food must be enriched with them to replenish the nutrients necessary for the body **[3]**. The rapid growth in consumption of functional products worldwide indicates that they are the future. Functional products in the modern world are considered an alternative to drug therapy. Functional foods are designed to reduce medication intake. Functional products are not positioned as medicines, but their purpose is disease prevention **[4]**.

The dysfunction of the immune system with age is manifested by an increased susceptibility to infections and a decrease in the ability to respond to vaccinations. In persons over 60 years old, a decrease in the activity of the reaction of secretory intestinal and serum IgA-specific antibodies was found. Changes in the microbiota of the elderly are associated with impaired immune status, characterized by a higher production of pro-inflammatory cytokines (IL-6 and IL-8) in blood plasma. Despite the increased levels of pro-inflammatory cytokines, the reactivity of the innate and adaptive immune systems in the elderly is reduced. In aging, there is a decrease in the activity of the humoral response after vaccination or infection and the formation of T and B cells, natural killer (NK) cells. These changes increase the frequency and severity of infectious, chronic inflammatory,

autoimmune, and oncological diseases **[5]**, **[6]**. The proven efficacy of using probiotics to improve intestines' immune function makes older adults an important target group for probiotic therapy **[7]**.
The most commonly used probiotic carrier is dairy products (cheese, milk, or yogurt) **[8]**. Consumption of the probiotic cheese contributed to the improvement of innate immunity indicators in elderly volunteers: it increased the activity of phagocytosis and the number of NK cells **[9]**. Functional products can improve the gut microflora and, in this way, reduce symptoms of gastrointestinal functional disorders such as irritable bowel syndrome (IBS) **[10]**, which are characterized by impaired intestinal motility and visceral hypersensitivity. Effective treatments for IBS are limited; they provide only partial or short-term relief of symptoms and are often associated with significant side effects. Probiotics can prevent or reduce abdominal symptoms of IBS, such as abdominal pain and flatulence **[11]**.

Abdominal pain in an elderly person is often found in various gastrointestinal tract diseases (including IBS patients) and is associated with visceral hypersensitivity. Probiotics can induce the expression of receptors on epithelial cells that control the transmission of information from the nervous system to the intestine, including opioid (M-, D- and K-) and cannabinoid 2 (CB2) receptors, which can provide analgesic and anti-inflammatory effects **[12]**. World Health Organization shows that 20% of the Kazakh population are elderly people (over 60 years old). According to forecasts 2030, this figure will increase to 25%. In the diet of the population of developed European countries, the proportion of functional products is at least a quarter of their diet. The Kazakh consumer receives, on average, less than 3 kg of functional products per year **[13]**. Developing the local functional products can resolve this case **[14]**.

Scientific Hypothesis

We aimed to study the probiotic strain *E. coli* 64G, which will be a base for the Enterocol supplement drug (probiotic) that will be a component of the functional gerontology dietic semi-finished product. We had to check the hypothesis that the created functional product (semi-finished sausage) could be used in a gerontology diet with the following requirements:

- The functional product has to be a food, not a drug;
- It should be prepared from natural ingredients.
- The content of the component that determines the product belongs to the functional and health-improving group should be at least 1/5 of the body's needs in this food.

So, we wanted to check the ability of the *E. coli* 64G strain to pass the aggressive gastric and intestinal conditions, colonize the intestine, have antagonistic to harmful bacteria action, and be safe for the host organism (laboratory mice). If all these conditions are real, we can make the next trials in creating a safe, efficient, and functional product.

MATERIAL AND METHODOLOGY

The controlled prospective experiment was conducted at the Veterinary Sanitary Expertise Department at the Kazakh National Research Agrarian University, Almaty Technological University, at the Kazakh-Japanese Innovation Center laboratory and the AsylTasEngineering LLP research base.

Samples

The E. coli 64G strain was used to create the Enterocol supplement drug. For testing the proprieties of the probiotic strain, pure microbe cultures (*Salmonella spp*., *Klebsiella spp*., and *Streptococcus spp.*) were isolated from the sick animals in the farms of the Almaty region. *Escherichia coli* 25, *Salmonella typhimurium* 371, and *Klebsiella pneumoniae* 30 were used to test the antagonistic action of the E. coli 64G strain.

Chemicals

In our study, we used nutrient mediums such as meat-peptone agar (MPA) and meat-peptone broth (MPB) produced by the Federal Budgetary Institution of Science "State Scientific Center for Applied Microbiology and Biotechnology" (Obolensk – 142279, Russia). For the *Enterobacteriaceae* family, it has been used Endo agar produced by HiMedia Laboratories, LLC (Mumbai – 400086, India), Ploskirev agar, and HIS-selective medium (Obolensk – 142279, Russia). The Bacillus family has used M-Enterococcus Agar Base (Mumbai – 400086, India) and Blauroccus nutrient medium (Obolensk – 142279, Russia).

For testing proprieties of the *E. coli* 64G strain, hydrochloric acid, bile, and sodium chloride solution (NaCl) were used.

Animals, Plants and Biological Materials

Probiotic Enterocol was experimentally created in the base of the *E. coli* 64G strain *E.coli* was isolated from the healthy lamb intestine and is a normal inhabitant of the gastrointestinal tract. Its identification has been made by the automatic bacterial analyzer in the laboratory of the Kazakh-Japanese Innovation Center in the previous research.

To test the proprietie of the Enterocol we have used laboratory mice. For the study, the preventive efficacy of the Enterokol drug has been used. A total of 55 unbreaded weight mice (14-16 g) were used: 50 of them formed experimental groups, and 5 mice were included in a control one. The white mice from the control group got per os for 1 mL of saline instead of the tested suspension.

Instruments

Autoclav, thermostat, heating cabinet, automatic cell counter Countess® (California, USA), Microbiological analyzer VITEK^{MS} (bioMerieux, USA), and Photocalorimeter KPK-3 (LLP Reaktyvsnab, Shymkent, Kazakhstan); flasks, test tubes, and Petri plates; syringes and pipettes were used in research flowing.

Laboratory Methods

ISO 7218 **[15]** was used as a manual in our research in CFU counting and Bergey's manual of determinative **[16]** was the base for bacteria identification in our experiment. Biological material has been inoculated at the Petri plates on the MPB and Endo mediums and cultivated in a thermostat (t=37°C) for 18 hours.

Morphological: (and tictoral) isolated bacteria features were studied using the Gram method. In case the morphological properties of the enterobacteria were similar, cultures were subcultured onto slanted MPA and Hiss-medium.

Cultural features: were studied in transmitted and reflected light. The crushed drop method was used to check the studied isolates' mobility properties.

Physiological features: were studied at MPB with 0.5% of the corresponding carbohydrate and Andrade indicator. Checking the serogroup E. coli belonging was conducted by using O-coli agglutinating sera. The polyvalent serum and monovalent O- and H-seras were taken to identify the *Salmonella* bacteria group.

Determination of the virulence: of the isolated pure cultures was conducted at the following schema: isolated bacteria were grown on the MPA (t=37-38 °C for 18-20 hours), washed off with the sterile saline, and infested intraperitoneally to the experimental animals in various doses.

The antagonistic activity: of isolated strains was studied on solid nutrient media. The degree of antagonistic activity to each test microbe was checked by the width of the growth inhibition zone of the latter: up to 10 mm - medium, more than 20 mm – high; in the absence of the growth inhibition zone - , the antagonistic activity is missing.

The resistance in bile and hydrochloric acid: was tested in growing *E. coli* 64G on the media with different bile and hydrochloric acid concentrations: meat-peptone agar (MPA) for bile and meat-peptone broth (MPB) for hydrochloric acid with involving photo colorimetric method. Morphological, tinctorial, enzymatic, cultural, pathogenic, and adhesive properties of the isolated bacteria were studied by us.

All bacterial and biochemical studies were conducted under the norms of aseptic and antiseptic.

Description of the Experiment

Sample preparation: The probiotic preparation developed from the selected strain is expected to be used orally. To establish the pathogenicity of *E. coli* 64G strain, 24 h broth and agar cultures were used. Broth cultures *of E. coli* 64G strain in the dosage of 0.5, 0.7, 1.0, 1.2, and 1.5 mL were given orally to white mice.

To determine the harmlessness of the probiotic strain, capturing part of the intestinal chyme and preparing serial 10-fold dilutions in 0.89% NaCl were conducted. One drop of the suspension was taken from each dilution and applied to various nutrient mediums, and the results were carried out in 24 h.

Number of samples analyzed: 21 samples were analyzed in our experiment.

Number of repeated analyses: Biochemical studies were repeated twice. The experimental analyses were repeated three times.

Number of experiment replication: Each experiment has been conducted three times.

Design of the experiment: Morphological, cultural, and biochemical properties of the tested strain were conducted according to common approaches at the different nutrition media: Endo agar, MPA, and MPB (in 24 h and 18 h for the liquid media). The growth temperature range was fixed at 37-39 °C with the optimum at 37 °C. The optimum pH was 6.8-7.5. Glucose, lactose, maltose, arabinose, sorbitol, sucrose, dulcite, and salicin can be used as a carbon source. It forms indole and does not form hydrogen sulfide. It possesses lysine and ornithine decarboxylase activity but does not possess urease activity.

The resistance of the *E. coli* 64G strain to hydrochloric acid and bile was tested according to the biomass accumulation level, changes in the CFU (colony-forming units) number, and pH medium in 18-20 h (for bile medium) and 18 and 24 h for hydrochloric acid medium.

The antagonistic activity: of the *E. coli* 64G strain to the test microbe (*Salmonella spp*., *Klebsiella spp*., and *Streptococcus spp.*) was studied at MNA by the width of the zone of growth retardation of the latter: up to 10 mm - medium, more than 20 mm – high; absence of growth retardation zone - zero antagonistic activity.

The determination of the harmlessness of the probiotic E. coli 64G strain: was studied. In this aim, white mice weighing 16-18 g were infected by the *E. coli* 64G strain. At the pretrial stage, from the mice's

intestinal chyme, 14-18 cultures of Escherichia were isolated with typical cultural and biochemical properties and without hemolytic ability in the bacteriological study. Animals of about the same age were used to obtain comparable results. The results were taken in observing the 50% lethal dose (LD50) for white mice.
The preventive properties of the E. coli 64G strain were studied on mice no later than 30 min after giving oral a geodetic semi-finished product from tilapia fish with the addition of *E. coli* 64G strain (sausages). In the experiment, virulent cultures of *Escherichia coli* 25, *Salmonella typhimurium* 371, *Klebsiella pneumoniae* 30 were used to infect the experimental animals. The experimental animals were infected on the third day by being given the virulent culture orally.

To determine the lethal dose (LD), virulent cultures were titrated on 1.5-year-old mice. 4 experimental animal groups were formed, and the last one was a control. The 20 mice were in each one. To establish the pathogenicity of *E. coli* 64G strain, 24 h broth and agar cultures were used. Each group was introduced to cereal dose of the Enterocol drug: the first – $3x10^9$, 20 mL; the second – $5x10^9$, 20 mL; the third – 10^{10}, 20 mL; the fourth – $2x10^{10}$, 20 mL; and the last (control) group was getting 20 mL of the NaCl (0.89 %). Observation was conducted for 10 days.

Statistical Analysis

Microsoft Excel 2010 and Statistica 15 (USA) were used to test statistically significant differences in our experiment. The presented results are the outcomes of the number of replications experimental results. The differences between groups were taken as significant if $p < 0.05$.

RESULTS AND DISCUSSION

Several hundred thousand functional products are currently recommended to prevent and treat cardiovascular, gastrointestinal tract, and endocrine diseases **[17]**. In looking for future benefits of functional food development, we need to note that it is essential to create functional products for people from certain regions with nutrients most lacking in the diet **[14]**. Elderly people have their need for well-consumed protein and fat components. In this, fish products are one of the best **[18]**. Fish meat (and fish-based products) contains many valuable nutrients like protein, easily digestible fat, vitamins, and minerals **[19]**. The gerontology and heretics development has presented the data that fish from the Cichlidae family, primarily tilapia, contain unique components with neuroprotective properties that are recommended for increased use by elderly people (glycine, chondroitin sulfate, hyaluronic acid, phosphoric calcium salts, phospholipids). That served as the basis for the technology of semi-finished products developed from tilapia fish intended for nutrition in gerontology **[18]**. For example, the recommended daily protein intake of 60-74-year-old men is 85 g, and for women, this rate is 78 g. The daily intake of ascorbic acid is 70-80 mg for both groups. Recently, technologies of canned meat and vegetables, fermented milk drinks, curds, and fish molded semi-finished products have been proposed. However, the list of available specialized products, especially domestically produced ones, is limited **[20]**. It seems relevant and practical to design fish hero-dietary products (and tilapia as a part). So, we had the future task to create a semi-finished fish sausage for introduction in gerontology feeding. In this, we took care that antibiotics used to prevent and treat gastrointestinal diseases are unsafe for the elderly and are becoming less effective.

It is no coincidence that diseases of people over 65, accompanied by diarrheal syndrome, remain the most difficult problem in medicine. Therefore, an important role in preventing intestinal disorders is maintaining an optimal ratio between lactic acid and conditionally pathogenic microorganisms, fungi, and protozoa in the gastrointestinal tract in humans **[21]**. Moreover, probiotic using can reduce the neediness for the antibiotic cure because of the normalized intestine microbiome, so probiotic use makes its income in antibiotic resistance problem **[22]**. Practical experience shows that substitution therapy, aimed at restoring the intestinal microbiome through the regulatory introduction of live bacteria - representatives of normal intestinal microflora, i.e., probiotics, is of great importance in preventing gastrointestinal diseases in people of different ages A probiotic can contain one or more strains of one type of bacteria or several different types **[21]**. The possibility of using multi-species compositions of probiotics assumed that their complex species composition most closely corresponds to the natural composition of normal intestinal microflora. The mechanism of action of probiotics, in contrast to antibiotics, is aimed not at the destruction but at the competitive exclusion of conditionally pathogenic bacteria from the intestinal microbiome to prevent the intensification and transmission of virulence factors in the population of conditionally pathogenic bacteria **[23]**. We were looking for a common spreeded microbe. Kryvda and Rybachuk confirmed our choice of *E. coli* as a common spreeded bacteria **[24]**. The first task was to test the proprieties of *E.coli* 64G strain as a probiotic strain: its bile and hydrochloric acid stability.

Detection of the *E.coli* 64G strain features

At the Endo and meat-peptone agar in 24 h were fixed Escherichia with typical cultural (Figure 1), biochemical, morphological, and tinctorial properties: short rods, motile, gram-negative, do not form spores and capsules.

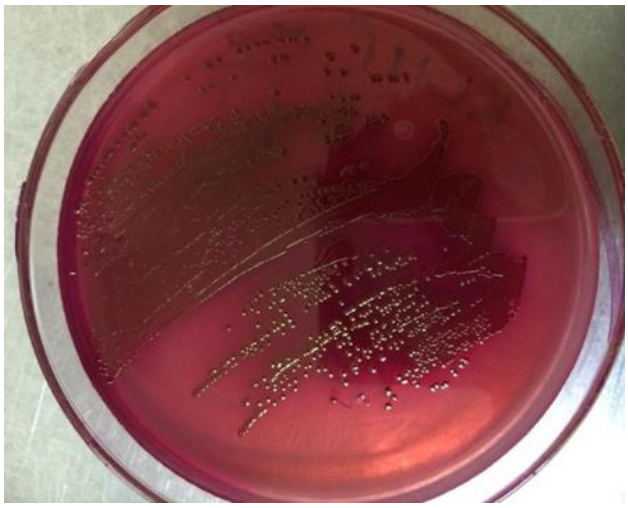


Figure 1 Probiotic *E. coli* strain's growing at the Endo agar: dark red colonies with metal shine.

The cultivation of the *E. coli* 64G strain on the media of MNA (pH 7.0-7.4) for bile and MPB (pH 7.0-7.4) for hydrochloric acid demonstrated the resistance of the tested microbe in them after cultivation for 18-48 h (Figure 2). The control mediums were inoculated by the tested strain, free of the supplement substances.

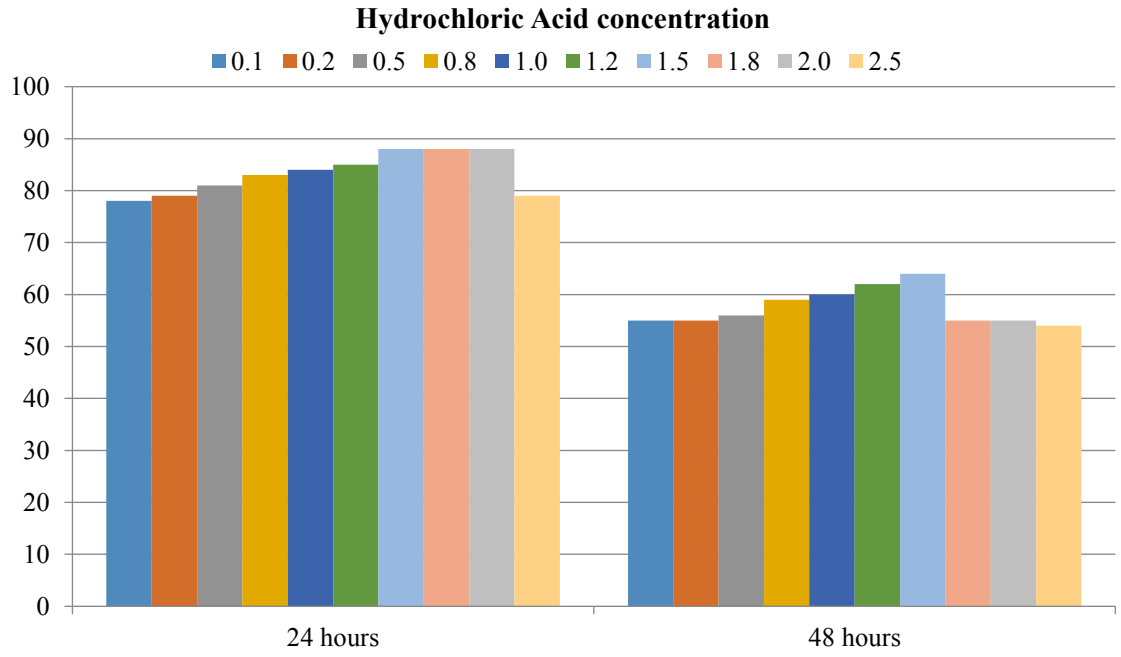


Figure 2 Results of studying the *E.coli* 64G strain's resistance to hydrochloric acid by the photocolorimetric method.

The results of the bile resistance study showed that the *E. coli* 64G strain is highly resistant to 1%, 5%, 10%, and 20% of bile. Escherichia strain has demonstrated high resistance to the various concentrations of hydrochloric acid (0.1%, 0.2%, 0.5%, 0.8%, 1.0%, 1.2%, and 1.5%) (Figure 3).

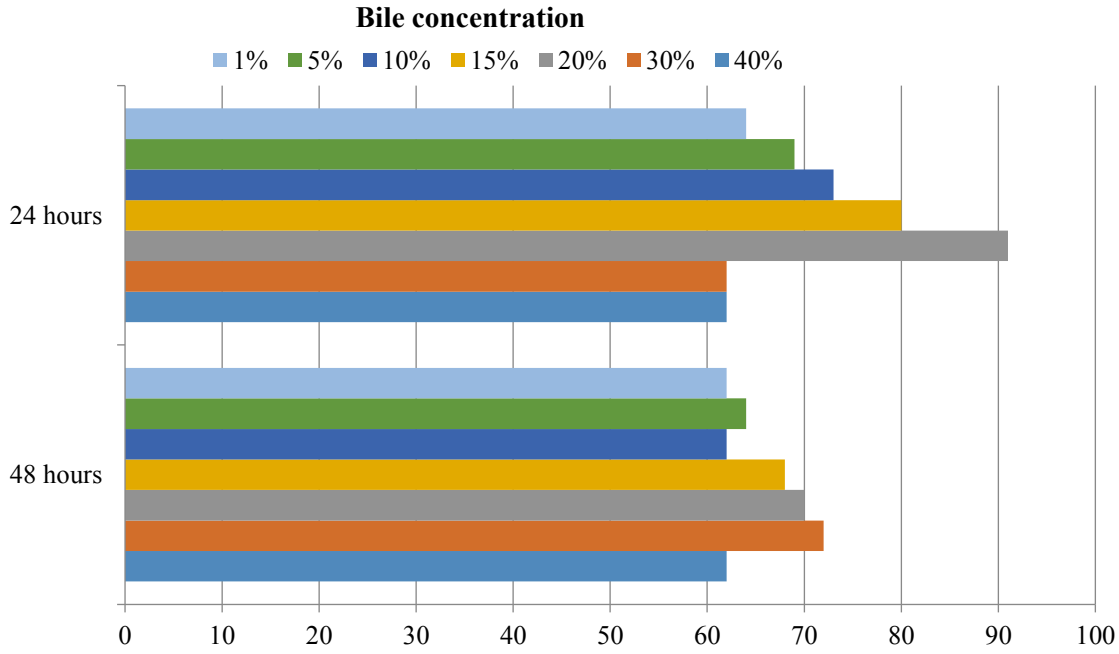


Figure 3 Determination of the sensitivity of the *E. coli* 64G strain to bile

So, tested strain can be passed through the aggressive conditions of the internal tract and can help to reduce age-related changes in immune status, bowel function, and suboptimal nutrition **[25]**. So, it can be used in real conditions of intestinal tract efficiency.

Study the antagonist action of *E. coli* 64G against different bacteria strains

In recent years, many probiotics have been proposed, and many papers characterizing the effectiveness of these drugs **[26]**. And this is important, considering statistical data that 75% of elderly people have different kinds of nutritional disorders **[18]**. In this case, different types of microorganisms and their combinations can be used in their creation, taking into account requirements for probiotic strains that allow them to compete with pathogenic and conditionally pathogenic microorganisms **[26]**. So, the antagonistic activity of the probiotic strain has a significant impact **[27]** and can replace antibiotic therapy in some cases **[28]**. In this way, the other task was - to test the efficiency of the *E. coli* 64G strain in its antagonistic action against pathogen bacteria. In testing the antagonistic activity of the probiotic strain *E. coli* 64G, MNA and the inoculation E. coli 64G strain on the microbe film was used. The results of checking the inhibition zones (mm) can be observed in Table 1. The best inhibition results were observed in *Escherichia coli v3 (6.2 mm), E. coli* v3 (5.8 mm), and *Salmonella abortus ovis* (4.8 mm). The smallest activity was noted against *Klebsiella pneumoniae* v1 (0.2 mm), *Klebsiella pneumoniae* v3 (0.4 mm), and *E. coli* v1 (0.6 mm)

Table 1 Result of the antagonistic activity *E. coli* 64G strain study relation to different bacteria strains.

Tested sample number	Wild strains from sick animals	Diameter of growth inhibition zones, mm
1	*Salmonella dublin*	1.6
2	*Salmonella abortus ovis*	4.8
3	*Salmonella typhymurium*	1.1
4	*Klebsiella pneumoniae* v1	0.2
5	*Klebsiella pneumoniae* v2	1.3
6	*Klebsiella pneumoniae* v3	0.4
7	*Streptococcus pneumoniae*	3.5
8	*Streptococcus pneumoniae* v2	4.3
9	*Streptococcus pneumoniae* v3	3.2
10	*Escerichia coli* v1	0.6
11	*Escerichia coli* v2	5.6
12	*Escerichia coli* v3	6.2

E. coli 64G strain had high antagonistic activity against virulent test cultures. The study found that before and after *E. coli* 64G strain application cultures of *Enterobacteriaceae* family (and *E. coli* as a part), *Enterococci spp.*, *Lactobacillus spp.,* and *Bifidiobacterium spp.* were isolated from the mice's intestines.

E. coli 64G strain had high antagonistic activity against virulent test cultures. The study found that before and after *E. coli* 64G strain application cultures of *Enterobacteriaceae* family (and *E. coli* as a part), *Enterococci spp.*, *Lactobacillus spp.,* and *Bifidiobacterium spp.* were isolated from the mice's intestines.

So, the tested strain can be used to normalize the gut microbiome because it is protective against harmful microbe action. There is a lot of data about using *Lactobacillus spp.* **[29]** as well as *Biphidobacillus spp.* **[30]** in normalized gut microbiome. Lee et al. **[29]** gave data that using *Lactobacillus acidophilus* NCFM decreased the visceral pain threshold in an experiment in rats by 44% via the opioid pathway and activated opioid receptors (OR) in humans. Stomach pain is truly actual in elderly people: Satayeva et al. **[18]**, in their last research, gave data that about 20% of elderly are overeaten, 60% of persons eat irrationally (males in the main) with dominant meat meal, flour products, and food with animal fats high content as well as sweets. At the same time, insufficient consumption of dairy products, fish, vegetables, and fruits fixes for these persons. Introducing *Bifidobacterium lactis* Bi-07 can be significantly less effective in increasing OP expression than *Lactobacillus acidophilus* NCFM. Two doses of *L. acidophilus* NCFM at 10^9 and 10^{10} CFU were recommended as clinically appropriate and applicable for use in functional products **[31]**. The effect of L. acidophilus NCFM on IBS symptoms has been determined. In 340 out of 391 volunteers in the groups with initially moderate to severe abdominal pain for 12 weeks of treatment, there was a significant decrease in points on the VAS scale (for probiotics versus placebo, $p = 0.046$). As well as Vinderola et al. **[32]** gave data that oral intake of specific strains of *Lactobacillus* induces the expression of L-opioid and cannabinoid receptors in intestinal epithelial cells and provides an analgesic effect similar to that of morphine. However, using Enterobacteriaceae bacteria is not common. However, there are data about the probiotic activity of *E. coli*. For example, Karim et al. **[33]** studied *E. coli* strains from 40 different samples. The probiotic and antagonist activity was determined for the *E. coli* O157:H7 strain.

But before using each probiotic strain, the safety and efficiency of the drug have been proved in vivo experiments.

Study of the Enterocol safety on the mice organism

Total bacteriological consisting was formed by *Salmonella spp*. (the main part), *Klebsiella spp.*, *E. coli*, and 1-2% of the undifferentiated bacteria of the *Enterobacteriaceae* family. *Enterococci spp.* in the intestinal chyme were mainly represented by *E. faecalis.*

Enterocol introduction has changed the intestinal chyme consisting. In 24 h the Escherichia's number increased by order of magnitude compared to the early study. Escherichia's counts increased 5 and 7 times on the second and third days, respectively ($p < 0.001$). Identifying Escherichia's cultures isolated from the mice's intestines showed that all cultures in their cultural - biochemical, antigenic, and adhesive properties correspond to the *E. coli* 64G strain.

The number of enterococci, lactobacilli, and bifidobacteria in the intestinal chyme of mice 24 h after taking drugs from *E. coli* slightly decreased compared to the control, then on the second and third days after excluding the test *E. coli* 64G strain from the diet increased 1 and 2 times than in the early study. The studies showed that a relatively short-term 2-day use of the bacteriocinogenic strain *E. coli* 64G had a noticeable regulatory effect on the intestinal microflora of mice.

In 48 and 72 h, the enterobacteria's content decreased by 3 and 4 times, respectively. The bacteriocinogenic strains had a significant inhibitory effect on the enterobacteria population. The number of enterococci, lactobacilli, and bifidobacteria in the intestinal chyme of mice on the second and third day increased up to two times.

Also, we have tested the harmlessness of the E. coli 64G strain to the mice's organism after oral income. All animals of the 4 experimental and control groups survived after three oral ingences of the tested probiotic in dosages of 0.5, 0.7, 1.0, 1.2, and 1.5 mL, as well as a control mice's group with the introduction of 1.0 mL NaCl. So, for the 20 days of observation, there were no changes in animal behavior. All white mice were active and mobile, ate well, and had no impaired physiological functions.

So, we can note that the created drug is safe for living organs.

Preventive efficiency of the Enterokol as an antagonistic drug

Prophylactic and therapeutic properties of Enterokol in mice (average age of an adult individual was 1.8 m) were determined by experimental infecting with virulent cultures (*E.coli* 25 strain; *Klebsiella pneumoniae* 30 strain; and *Salmonella typhimurium* 371 strain). In 10 days, mortality was observed for one mouse in the first and the second groups, and 100% of lethality was observed in the control group in all infected mice (Table 2).

Table 2 Preventive properties of the Enterokol tested in mice.

Animal's group	Results of infection with a virulent								
	E.coli 25			*Klebsiellapneumoniae*30			*Salmonella typhimurium* 371		
	Died	Recovered	Survived, %	Died	Recovered	Survived, %	Died	Recovered	Survived, %
First, animals	1	1	90	1	2	85	0	0	100
Second, animals	1	0	90	0	0	100	0	0	100
Third, animals	0	0	100	0	0	100	0	0	100
Fourth, animals	0	0	100	0	0	100	0	0	100
Control, animals	20	0	-	20	0	0	20	0	-

Table 2 data proved the possibility of using *E. coli* 64G strain as a probiotic component, and the best dose was determined to be 10^{10} (20 mL). We can note that that is a good result comparing the data with *Lactobacillus spp.* Frece, in 2005 **[34]**, noted that for *L. acidophilus* M92, *L. plantarum* L4, and *E. faecium* L3, the best-feeding probiotic daily dose was 2×10^{10} CFU. Moreover, we have to note that the antagonistic ability of the E. coli 64G strain, completed by its ability to become the allochthone microbe, makes it applicable to be used as a probiotic strain in functional products. This is actual and essential for elderly people taking care of the fact that in the absence of active microbiota, anticarcia immunity decreases **[35]**. Probiotic components, introduced in the human body by functional products, can significantly improve the patient's immune state **[12]**, **[35]**. These components are capable of activating phagocytes to eliminate early-stage cancer cells **[35]**, and give help in the treatment of necrotizing enterocolitis, colic in infants to constipation, IBS, and hepatic encephalopathy in adult patients **[36]**. Our data allow us to continue our research, taking care of the regulations formulated by Brinton in 1965 **[37]**. But we have to note that this is presented in the beginning research stage, and there is a need for future deep research of the proprieties E. coli 64G strain in manufacturing conditions.

CONCLUSION

The Enterocol drug can supplement the feeding fish diet with later semi-finished products manufactured from fish meat. The results of the studies indicate that the *E. coli* 64G strain, which is the base of the Enterocol, meets certain probiotic requirements: it is non-pathogenic and non-toxic. *E. coli* 64G strain has demonstrated a set of properties that made competing with pathogenic and conditionally pathogenic microorganisms possible. *E. coli* 64G-strain can be transported through the stomach because of its bile and hydrochloric acid resistance. All of these make it possible to use Enterocol like a probiotic. The Enterocol drug can supplement the feeding fish diet with later semi-finished products manufactured from fish meat. The results of the studies indicate that the *E. coli* 64G strain, which is the base of the Enterocol, meets certain probiotic requirements: it is non-pathogenic and non-toxic. E. coli 64G strain has demonstrated properties that made competing with pathogenic and conditionally pathogenic microorganisms possible. *E. coli* 64G strain can be transported through the stomach because of its bile and hydrochloric acid resistance. All of these make it possible to use Enterocol like a probiotic. *E. coli* 64G has demonstrated its activity in the gastrointestinal ecosystem in protecting from semi-pathogen microbes; it can adhere to the epithelium, take root in the digestive tract, and have no pathogen action on the internal organs (confirmed by histological studies). E. coli 64G-strain is stable and can remain viable under production conditions.

REFERENCES

1. Milner, J. A. (2002). Functional foods and health: a US perspective. In British Journal of Nutrition (Vol. 88, Issue S2, pp. S152–S158). Cambridge University Press (CUP). https://doi.org/10.1079/bjn2002680
2. Romero-Cortes, T., Lopez-Perez, P. A., Toledo, A. K. M., Perez-Espana, V. H., Aparicio-Burgos, J. E., & Cuervo-Parra, J. A. (2018). Nutrigenomics and Nutrigenetics in Functional Foods. In International Journal of Bio-resource and Stress Management (Vol. 9, Issue 6, pp. 661–672). Puspa Publishing House. https://doi.org/10.23910/ijbsm/2018.9.6.1922
3. Nechaev, A. P., Tarasova, V. V., Nikolaeva, Y. V., & Kuzhleva, A. A. (2018). Emulsified fatty products for functional purposes in modern nutrition. Food Processing Industry (Vol. 5, pp. 26–29). Food Industry.
4. Pushpangadan, P., George, V., Sreedevi, P., Bincy, A. J., Anzar, S., Aswany, T., ... & Ijinu, T. P. (2014). Functional foods and nutraceuticals with special focus on mother and child care. In Annals of Phytomedicine (Vol. 3, Issue 1, pp. 4–24). UKaaz.
5. Oleg, A. N. (2011). Analysis of country markets for functional foods. In University Bulletin (Issue 25, pp. 177–179). State University of Management.
6. Zaporozhskaya, S. P., Kasyanov, G. I., Kosenko, O. V., Jum, T. A., Ksenz, M. V., & Kucherova, S. I. (2021). Technology and commodity characteristics of extracts and oil cake for food enrichment. In IOP Conference Series: Earth and Environmental Science (Vol. 640, No. 4, p. 042017). IOP Publishing. https://doi.org/10.1088/1755-1315/640/4/042017
7. Hussein, M. F. (2021). Colibacillosis. In Infectious Diseases of Dromedary Camels (pp. 111–116). Springer International Publishing. https://doi.org/10.1007/978-3-030-79389-0_16
8. Heboyan, A., Avetisyan, A., & Vardanyan, A. (2019). Halitosis as an issue of social and psychological significance. In Journal of Research in Medical and Dental Science (Vol. 7, Issue 4, pp. 33–40). Amber Publication.
9. Peng, M., Tabashsum, Z., Anderson, M., Truong, A., Houser, A. K., Padilla, J., Akmel, A., Bhatti, J., Rahaman, S. O., & Biswas, D. (2020). Effectiveness of probiotics, prebiotics, and prebiotic-like components in common functional foods. In Comprehensive Reviews in Food Science and Food Safety (Vol. 19, Issue 4, pp. 1908–1933). Wiley. https://doi.org/10.1111/1541-4337.12565
10. Kilany, O. E., Youssef, F., Mabrouk, M., & Fares, I. M. (2018). Clinicopathological studies on the effect of some antibacterial medicinal plants in broilers. In Journal of Clinical Pathology Forecast (Vol. 1, Issue 1, pp. 1–6). Science Forecast.
11. Barrington, G. M., Gay, J. M., & Evermann, J. F. (2002). Biosecurity for neonatal gastrointestinal diseases. In Veterinary Clinics of North America: Food Animal Practice (Vol. 18, Issue 1, pp. 7–34). Elsevier BV. https://doi.org/10.1016/s0749-0720(02)00005-1
12. Saribayeva, D. A., Biyashev, K. B., Valdovska, A., Sansyzbai, A. R., & Biyashev, B. K. (2015). Study antagonistic activity, the level of resistance to hydrochloric acid and bile probiotic strain Escherichia coli. In Journal of Pure and Applied Microbiology (Vol. 9, Issue 1, pp. 573–578). CABI.
13. Eveno, M., Savard, P., Belguesmia, Y., Bazinet, L., Gancel, F., Drider, D., & Fliss, I. (2020). Compatibility, Cytotoxicity, and Gastrointestinal Tenacity of Bacteriocin-Producing Bacteria Selected for a Consortium Probiotic Formulation to Be Used in Livestock Feed. In Probiotics and Antimicrobial Proteins (Vol. 13, Issue 1, pp. 208–217). Springer Science and Business Media LLC. https://doi.org/10.1007/s12602-020-09687-y
14. Chung The, H., & Le, S.-N. H. (2022). Dynamic of the human gut microbiome under infectious diarrhea. In Current Opinion in Microbiology (Vol. 66, pp. 79–85). Elsevier BV. https://doi.org/10.1016/j.mib.2022.01.006
15. International Organization for Standardization. (2007). Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations (ISO Standard No. 7218:2007).
16. Bergey, D. H. (1994). Bergey's manual of determinative bacteriology. Ed. by Holt, J. G., Kreig, N. R., Sneath, P. H. A., Staley, J. T., Willams, S. T. 9th Edition, Lippincott Williams and Wilkins, Baltimore.
17. Snel, J., Harmsen, H. J. M., Van der Wielen, P. W. J. J., & Williams, B. A. (2002). Dietary strategies to influence the gastrointestinal microflora of young animals, and its potential to improve intestinal health. In Nutrition and health on the gastrointestinal tract (Vol. 37, pp. e69). CABI.
18. Satayeva, Z., Tayeva, A., Rskeldiyev, B., Zhaksylykova, G., & Akhmetova, N. (2022). Nutrition of older adults in the Republic of Kazakhstan. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 244–257). HACCP Consulting. https://doi.org/10.5219/1747
19. Utebekova, G., Akhmetova, N., & Gurinovich, G. (2023). The study of the nutritional and biological value of functional semi-finished fish products “fish balls.” In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 43–54). HACCP Consulting. https://doi.org/10.5219/1828

20. Lübbert, C., & Mutters, R. (2017). Gastrointestinale Infektionen. In Der Internist (Vol. 58, Issue 2, pp. 149–169). Springer Science and Business Media LLC. https://doi.org/10.1007/s00108-016-0183-y

21. Ringel-Kulka, T., Palsson, O. S., Maier, D., Carroll, I., Galanko, J. A., Leyer, G., & Ringel, Y. (2011). Probiotic Bacteria Lactobacillus acidophilus NCFM and Bifidobacterium lactis Bi-07 Versus Placebo for the Symptoms of Bloating in Patients With Functional Bowel Disorders. In Journal of Clinical Gastroenterology (Vol. 45, Issue 6, pp. 518–525). Ovid Technologies (Wolters Kluwer Health). https://doi.org/10.1097/mcg.0b013e31820ca4d6

22. Kryvda, M., & Rybachuk, Z. (2021).Antibiotic therapy in „One Health" conception. In Theoretical and Empirical Scientific research: Concept and Trends (Vol. 1, pp. 150–152). European Scientific Platform. https://doi.org/10.36074/logos-28.05.2021.v1.46

23. Sears, C. L. (2005). A dynamic partnership: Celebrating our gut flora. In Anaerobe (Vol. 11, Issue 5, pp. 247–251). Elsevier BV. https://doi.org/10.1016/j.anaerobe.2005.05.001

24. Kryvda, M., & Rybachuk, Z. (2022). Antibiotic susceptibility of Escherichia coli wild strains. In Bacterial Empire (Vol. 5, Issue 1, p. e353). SciCell. https://doi.org/10.36547/be.353

25. Kovaiou, R. D., Herndler-Brandstetter, D., & Grubeck-Loebenstein, B. (2007). Age-related changes in immunity: implications for vaccination in the elderly. In Expert Reviews in Molecular Medicine (Vol. 9, Issue 3, pp. 1–17). Cambridge University Press (CUP). https://doi.org/10.1017/s1462399407000221

26. Steinhoff, U. (2005). Who controls the crowd? New findings and old questions about the intestinal microflora. In Immunology Letters (Vol. 99, Issue 1, pp. 12–16). Elsevier BV. https://doi.org/10.1016/j.imlet.2004.12.013

27. Doan, H. V., Soltani, M., & Ringø, E. (2021). In vitro antagonistic effect and in vivo protective efficacy of Gram-positive probiotics versus Gram-negative bacterial pathogens in finfish and shellfish. In Aquaculture (Vol. 540, p. 736581). Elsevier BV. https://doi.org/10.1016/j.aquaculture.2021.736581

28. Nataraj, B. H., & Mallappa, R. H. (2021). Antibiotic Resistance Crisis: An Update on Antagonistic Interactions between Probiotics and Methicillin-Resistant Staphylococcus aureus (MRSA). In Current Microbiology (Vol. 78, Issue 6, pp. 2194–2211). Springer Science and Business Media LLC. https://doi.org/10.1007/s00284-021-02442-8

29. Yu, P., Ke, C., Guo, J., Zhang, X., & Li, B. (2020). Lactobacillus plantarum L15 Alleviates Colitis by Inhibiting LPS-Mediated NF-κB Activation and Ameliorates DSS-Induced Gut Microbiota Dysbiosis. In Frontiers in Immunology (Vol. 11). Frontiers Media SA. https://doi.org/10.3389/fimmu.2020.575173

30. Di Gioia, D., Aloisio, I., Mazzola, G., & Biavati, B. (2013). Bifidobacteria: their impact on gut microbiota composition and their applications as probiotics in infants. In Applied Microbiology and Biotechnology (Vol. 98, Issue 2, pp. 563–577). Springer Science and Business Media LLC. https://doi.org/10.1007/s00253-013-5405-9

31. Lee, J. G., Han, D. S., Jo, S. V., Lee, A. R., Park, C. H., Eun, C. S., & Lee, Y. (2019). Characteristics and pathogenic role of adherent-invasive Escherichia coli in inflammatory bowel disease: Potential impact on clinical outcomes. In E. Mizoguchi (Ed.), PLOS ONE (Vol. 14, Issue 4, p. e0216165). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0216165

32. Vinderola, G., Ouwehand, A., Salminen, S., & von Wright, A. (Eds.). (2019). Lactic acid bacteria: microbiological and functional aspects. Crc Press.

33. Karimi, S., Azizi, F., Nayeb-Aghaee, M., & Mahmoodnia, L. (2018). The antimicrobial activity of probiotic bacteria Escherichia coli isolated from different natural sources against hemorrhagic E. coli O157:H7. In Electronic Physician (Vol. 10, Issue 3, pp. 6548–6553). Knowledge Kingdom Publishing. https://doi.org/10.19082/6548

34. Frece, J., Kos, B., Beganović, J., Vuković, S., & Šušković, J. (2005). In vivo Testing of Functional Properties of Three Selected Probiotic Strains. In World Journal of Microbiology and Biotechnology (Vol. 21, Issues 8–9, pp. 1401–1408). Springer Science and Business Media LLC. https://doi.org/10.1007/s11274-005-5741-8

35. Górska, A., Przystupski, D., Niemczura, M. J., & Kulbacka, J. (2019). Probiotic Bacteria: A Promising Tool in Cancer Prevention and Therapy. In Current Microbiology (Vol. 76, Issue 8, pp. 939–949). Springer Science and Business Media LLC. https://doi.org/10.1007/s00284-019-01679-8

36. Liu, Y., Tran, D. Q., & Rhoads, J. M. (2018). Probiotics in Disease Prevention and Treatment. In The Journal of Clinical Pharmacology (Vol. 58, Issue S10). Wiley. https://doi.org/10.1002/jcph.1121

37. Brinton, C. C., Jr. (1965). The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria*. In Transactions of the New York Academy of Sciences (Vol. 27, Issue 8 Series II, pp. 1003–1054). Wiley. https://doi.org/10.1111/j.2164-0947.1965.tb02342.x

Funds:

The research was carried out in the range of the State grant under the PhD program of the Almaty Technological University (Order number UPO-01/38 dated August 25, 2017).

Acknowledgments:

We are grateful to the heads of Almaty Technological University for the possibility of conducting our research and to specialists LLP "AsylTasEngineering" for supporting our research activity and curating our actions during the research time.

Conflict of Interest:

No potential conflict of interest was reported by the authors.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Dinara Moldagaliyeva**, Asfendiyarov Kazakh National Medical University, Dinara Moldagaliyeva, 94, Tole Bi St., 050012, Almaty, Republic of Kazakhstan,
Tel.: 87775466446
E-mail: d_moldagalieva@mail.ru
ORCID: https://orcid.org/0000-0002-0915-2495

Nurzhan Sarsembaeva, Kazakh National Agrarian Research University, Veterinary and Sanitary Expertize Department, 8, Abay St., 050010, Almaty, Republic of Kazakhstan,
Tel.: 87775466446
E-mail: lady.nurzhan@inbox.ru
ORCID: https://orcid.org/0000-0002-3501-3720

Yasin Uzakov, Almaty Technological University, 100 Tole bi str.; 050010, Almaty, Republic of Kazakhstan, Tel. 87017441590
E-mail: uzakm@mail.ru
ORCID: https://doi.org/0000-0003-4626-2471

Bozhena Lozovicka, Plant Protection Institute, Food Safety Laboratory, 20, Władysława Węgorka St., 15195, Bialystok, Poland,
Tel.: 48856753419
E-mail: B.Lozowicka@iorpib.poznan.pl
ORCID: https://orcid.org/0000-0002-2760-3333

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 4.1.2024
Revised: 11.2.2024
Accepted: 16.2.2024
Published: 28.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 185-206
https://doi.org/10.5219/1949
ISSN: 1337-0960 online
www.potravinarstvo.com

Upcycling agricultural byproducts into eco-friendly food packaging

Natalya Oboturova, Sergey Povetkin, Natalya Nikulnikova, Natalya Lazareva, Anna Klopova, Nikita Lyubchanskiy, Ekaterina Sukhanova, Nadezhda Lebedeva

ABSTRACT

This investigation looks at the transformative potential of upcycling agricultural waste to make ecologically friendly food packaging. Agricultural wastes, which are frequently ignored, might be valuable resources in reversing the sustainable destiny of the packaging sector. We review recent research on plant-based byproducts, including proteins, polysaccharides, lipids, pigments, and minerals, that are isolated from agricultural waste. Creating edible and (bio)degradable packaging solutions that can include biobased active components, including flavorings, antioxidants, and antimicrobials, can begin with these compounds. Utilizing plant fibers from agricultural waste reduces environmental contamination while increasing packing efficiency. The review concentrates on packaging solutions that are good for the environment, like edible coatings and films with antioxidant and antibacterial qualities and active packaging made of phenolic chemicals. These innovations, derived from various foods and agricultural waste, satisfy customer demand for premium foods with longer shelf lives. A practical way to lessen the excessive use of non-biodegradable plastics is to create edible materials, especially in light of the global push for sustainability. These formulations can enhance food packaging performance since they are made from biowastes and biopolymers. Our comprehensive research synthesizes existing knowledge to shed light on the extraction, processing, and application of agricultural byproducts in packaging materials. The broad spectrum includes regulatory systems, processing techniques, biodegradability parameters, and the properties of various byproducts. By providing an all-encompassing viewpoint, this evaluation draws attention to current achievements and indicates avenues for more research and development. It provides a roadmap for the ecologically friendly upcycling of agricultural waste into sustainable food packaging, which helps to shift the packaging industry's paradigm continuously.

Keywords: upcycling, agricultural byproducts, eco-friendly, food packaging, sustainability

INTRODUCTION

Agricultural byproducts, frequently ignored, have a lot of potential and might drastically alter the food packaging sector while assisting the greater worldwide transition towards sustainability. There has been a lot of interest in turning these leftovers into environmentally acceptable food packaging materials due to the rising awareness of environmental issues and the demand for eco-friendly substitutes. Researchers have lately focused on developing novel food packaging materials extracted from agricultural waste using plant-based byproducts such as proteins, polysaccharides, lipids, pigments, micronutrients, and minerals. Researchers are considering

using these materials to create edible (bio)degradable packaging solutions that carry active biobased ingredients, including flavoring additives, antioxidants, and antimicrobials.

Researchers are considering using these materials to create edible (bio)degradable packaging solutions that carry active biobased ingredients, including flavoring additives, antioxidants, and antimicrobials. Furthermore, the use of plant fibers made from agricultural waste in environmentally friendly packaging has shown promising results, reducing pollution and increasing the effectiveness of food packaging **[1]**, **[2]**, **[3]**, **[4]**, **[5]**.

Much attention has been focused on using edible films/coatings with antibacterial and antioxidant qualities and active packaging based on phenolic compounds as innovative ways to meet customer expectations for high-quality, shelf-stable food products. These compounds, derived from various foods and agricultural waste, can enhance food products' oxidative state and antibacterial properties, indicating their efficacy in maintaining food quality and extending its shelf life. Developing innovative packaging materials, such as edible ones, is viewed as a global initiative to promote sustainability and lessen the use of polymers that deteriorate over time. Edible film and coating formulations based on biopolymers and active chemicals recovered from biowastes have potential due to the bioactivities of these compounds **[3]**, **[6]**, **[7]**, **[8]**, **[9]**.

This analysis aims to thoroughly examine how agricultural byproducts may be used to make food packaging less harmful to the environment. By examining the corpus of existing research, we want to clarify the current state of knowledge on the extraction, processing, and application of these byproducts in packaging materials. This initiative aims to draw attention to the innovative technologies and solutions pushing the packaging industry to adopt a more sustainable way of thinking. By integrating the most significant insights available, we hope to provide a comprehensive resource highlighting current successes and offering potential routes for future research and growth. Numerous subjects are covered in this examination, including a detailed assessment of the distinctive characteristics of various agricultural outputs deemed suitable for packing. The study also examines the many processing techniques employed to turn these wastes into functional and eco-friendly packaging materials. The environmental aspects of these materials' biodegradability and end-of-life concerns are thoroughly examined. The intricate topography of legislative frameworks and certification standards that regulate the development and use of ecologically friendly packaging solutions is also covered in the test. The primary objective is to thoroughly assess the current state of affairs, challenges faced, and prospects for the ground-breaking method of converting agricultural waste into ecologically favorable food packaging.

Agricultural Byproducts: Potential and Challenges

Sustainable methods may greatly benefit from the wide range of materials produced throughout the many farming and processing phases known as agricultural byproducts. These leftovers are a helpful resource that may support a more ecologically responsible approach, although being frequently overlooked of standard farm approaches. Agricultural byproducts have promise because of their quantity, adaptability, and capacity to reduce waste and advance the concepts of the circular economy when appropriately used. Unlocking this potential will be challenging since solving problems with integration into current supply chains and collection, processing, and integration call for careful thought and creative thinking.

It is necessary to define "agricultural byproducts" and categorize them based on their characteristics and area of origin before realizing their potential. While there are undoubtedly potential benefits to employing agricultural outputs, this section critically evaluates the environmental implications of current farming practices and the challenges associated with properly disposing of leftovers. Finding a balance between the positive impacts and any potential ecological downsides, such as soil erosion and water pollution, is essential. By addressing these problems, we want to pave the way for environmentally friendly practices that optimize agricultural waste's advantages while minimizing its disadvantages **[1]**, **[5]**, **[6]**, **[10]**, **[11]**. This section examines the many types of byproducts generated, such as organic waste and biomass, as well as processing leftovers and agricultural residues. By adequately categorizing these materials, we offer the basis for examining their many applications. The staggering number of agricultural byproducts generated globally is both an opportunity and a concern. This chapter uses statistical data and research findings to quantify the amount of these byproducts. It also looks at regional and seasonal variations in availability, shedding light on the practical concerns related to the extensive usage of these resources.

Environmental Impact of Packaging

Traditional Packaging Challenges

Conventional packing methods have long been connected to pollution and resource depletion, among other environmental issues. One of the primary reasons for concern is the excessive use of non-renewable resources, such as fossil fuels, in producing plastic packaging. In addition to exhausting limited resources, this causes the production process to generate greenhouse gases. Trash also builds up faster when traditional packaging, which

usually takes the form of single-use goods, is disposed of. They are endangering wildlife, ecosystems, and groundwater quality result of non-biodegradable packaging materials filling landfills. In particular, vast expanses of seas and streams are clogged with packaging waste due to the global spread of plastic pollution **[8]**, **[12]**, **[13]**, **[14]**.

Role of Packaging in Waste Management

Packaging significantly impacts waste streams' amount, composition, and recyclable nature, making it a crucial component of garbage management. The sequential "take-make-dispose" model of conventional packaging exacerbates waste management issues. Single-use packaging often ends up in landfills once it is abandoned, which makes the issue of overflowing waste disposal facilities worse. To shift this perspective, sustainable packaging promotes the concepts of the circular economy. The goal of sustainable packaging is to minimize the environmental impact of a product's end-of-life by emphasizing easily recyclable, biodegradable, or compostable materials. Using source reduction techniques and placing greater focus on reusability can help further lessen the burden on waste management systems **[12]**, **[15]**, **[16]**, **[17]**, **[18]**.

Carbon Footprint and Greenhouse Gas Emissions

The carbon footprint of packaging includes greenhouse gas emissions from production, transportation, use, and disposal. Traditional packing materials have an extensive lifetime carbon emission increase, especially those derived from fossil fuels. Large amounts of carbon dioxide and other greenhouse gases are emitted into the atmosphere when these resources are extracted, manufactured, and transported. Sustainable packaging employs energy-efficient production methods and environmentally beneficial materials to reduce carbon emissions. Modern technologies, renewable resources, and recycled materials all contribute to a total reduction in carbon footprint. Addressing greenhouse gas emissions in the packaging sector not only benefits the environment but also advances global efforts to combat climate change and transition to a low-carbon, more sustainable economy **[19]**, **[20]**, **[21]**.

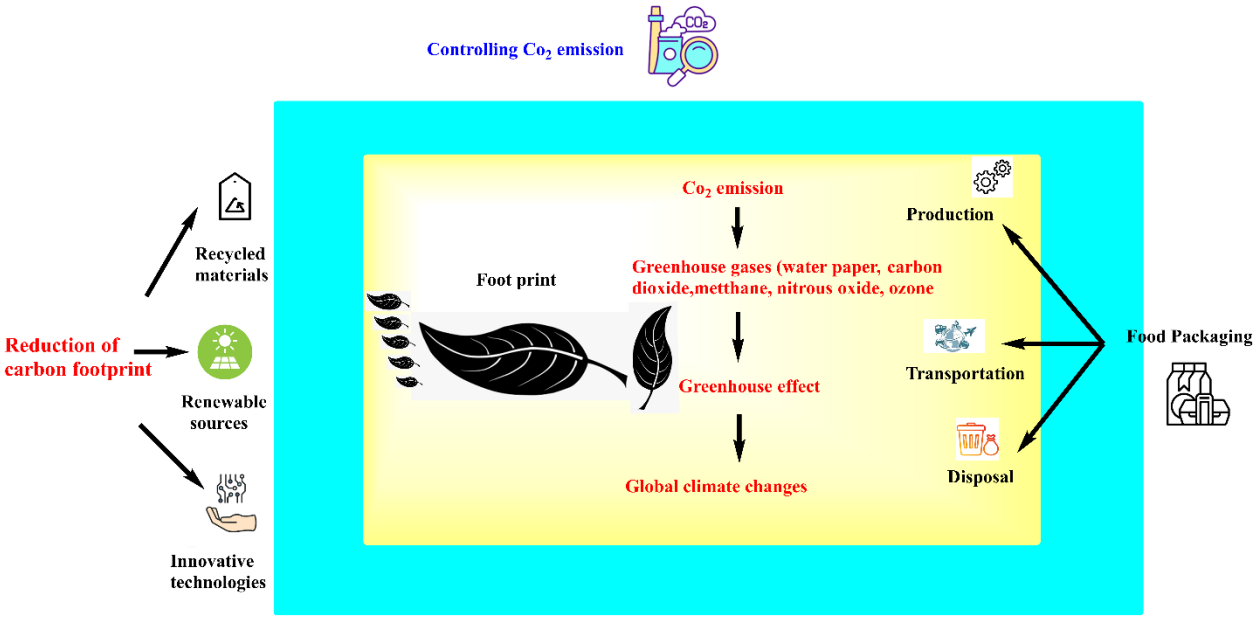


Figure 1 Potential methods for decreasing carbon footprint through material recycling to generate products with added value.

Biodegradable Materials

Compostable materials are vital to sustainable packaging because they offer a means of reducing the harmful environmental consequences of packaging waste. They are intrinsically able to undergo a biological breakdown process in a composting environment, transforming into very nutrient-dense organic matter under closely supervised conditions. Compostable materials are produced using renewable resources, such as plant-based polymers like PLA and agricultural waste, and they support a circular economy. Industrial composting facilities with controlled conditions that ensure efficient breakdown are the ideal fit for it. This is not the case with landfill disposal when the release of the potent greenhouse gas methane is caused by the absence of oxygen. Because compostable materials use less materials, they contribute to the concept of a circular economy.

Biodegradable packaging must include the need for an established composting infrastructure in order to yield environmental benefits. Noting the importance of consumer knowledge, some products could be suitable for composting at home. When statements on biodegradable packaging are backed up by accreditations such as the Biodegradable Products Institute (BPI) certification, they continue to have credibility. Public awareness is a major component in the success of biodegradable packaging. When consumers are given clear labeling and compostability information, they are better able to make informed decisions and dispose of packaging in an ethical manner. Packaging solutions that are compostable need to balance compostability with practicality, such longevity and shelf life. Packaging becomes more sustainable and regenerative and promotes responsible resource management when biodegradable materials are used **[22]**, **[23]**, **[24]**.

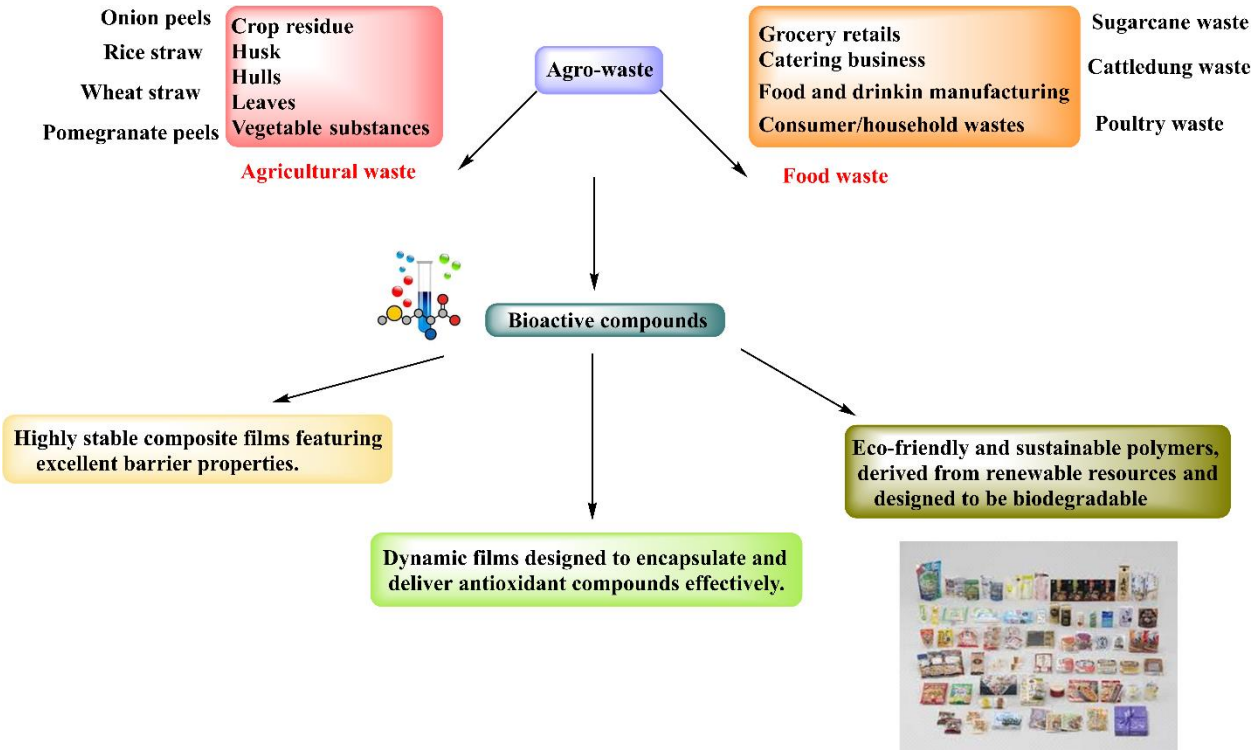


Figure 2. Various applications of agri-food waste materials in developing environmentally friendly and biodegradable packaging.

Minimalist Design and Lightweighting

Lightweighting and minimalist design are crucial components of sustainable packaging solutions since they aim to minimize waste and maximize resource efficiency to lessen their negative environmental effects. These approaches stand out for their characteristics that emphasize their commitment to material optimization, usability, and simplicity. To reduce the overall material footprint of packaging, minimalist design strongly stresses simplicity and resource economy. The objective is to encourage the prudent use of available resources by maximizing usefulness while limiting material consumption. This technique prioritizes the efficient use of resources throughout the package lifecycle, congruent with the circular economy principles. The simultaneous use of carefully selected materials to reduce weight and maintain packing integrity is known as lightweight. Often, to achieve the requisite strength and durability, innovative materials and design concepts must be used. This optimization contributes to the more general waste reduction goal by reducing the material required for packaging. Space efficiency is a critical component of minimalist design, which stresses maximizing available space to reduce unnecessary packing. This might lead to smaller packaging sizes, requiring less transportation and storage. Since optimizing spatial utilization minimizes the overall environmental effect, it aligns with sustainability goals. Lightweight design and minimalist packaging contribute to reducing waste by utilizing fewer materials. This highlights how important it is to manage resources responsibly throughout the packaging lifecycle, which aligns with the tenets of the circular economy.

Consumer appeal is another characteristic of minimalist design, characterized by its simplicity and clean lines. Packaging that adheres to minimalist design principles may draw in more environmentally conscious consumers and increase the allure of sustainable packaging solutions. Material selection, functionality, and safety must be addressed while implementing lightweight and minimalist design. Using lightweight, environmentally friendly

materials with an excellent strength-to-weight ratio is crucial. Maintaining packaging efficiency requires striking a compromise between minimalist design and practical requirements like product protection.

One significant benefit of lightweight packing is that it may reduce transportation-related fuel consumption and greenhouse gas emissions, increasing efficiency. This highlights the connection between effective supply chains and environmentally friendly packaging techniques and promotes broader environmental goals. End-of-life concerns and recyclable materials are two crucial elements of sustainable packaging. In pursuing minimalist design and lightweight, it is imperative to consider the recyclability or compostability of materials. This ensures that the environmental benefits will be realized throughout the package lifecycle. How lightweight and minimalist designs are perceived and communicated by consumers determines how well they work. Accurate labeling and marketing are essential to promote proper disposal methods and aid consumers in understanding the environmentally beneficial aspects of these strategies. It is vital to ensure that packaging techniques adhere to current norms and industry standards, with regulatory compliance being a critical concern. Meeting regulatory requirements increases the validity of sustainable packaging initiatives and fortifies a commitment to environmental responsibility **[19]**, **[20]**, **[25]**, **[26]**.

Extended Producer Responsibility (EPR)

Extended Producer Responsibility (EPR) is a critical component of sustainable packaging solutions that fundamentally alters the traditional approach to controlling a product's package lifespan. Since producers are fully accountable for the product's lifetime, they must intentionally minimize the packaging's adverse environmental effects. The characteristics and variables associated with EPR largely influence the development of a more circular and sustainable package management strategy. In essence, EPR is a representation of lifelong responsibility. The manufacturer is responsible for all aspects of a product's lifecycle, such as its design, production, use, and packaging treatment at the end of its useful life. By adopting this holistic perspective, producers are urged to consider the environmental consequences of their packaging decisions at every stage.

This includes establishing collection protocols, working with recycling facilities, and contributing funds to recycling programs. The goal is to establish a closed-loop system where producers actively participate in recovering and recycling their products and packaging. One of the critical objectives of EPR is recyclable design. Producers are urged to design packaging with clear labeling that instructs consumers on how to dispose of it correctly, is easy to recycle, and contains less mixed materials. This approach aligns with the overall goal of creating packaging that is environmentally sustainable over its entire lifecycle **[20]**, **[27]**, **[28]**.

Financial responsibility is a crucial element of EPR. The producer bears the expense of handling a product's end-of-life. This might be contributing to or paying for waste management facilities, recycling initiatives, or campaigns to reduce the environmental effects of packaging. The financial requirement encourages producers to use sustainable packaging practices. Consumer education is one of the critical components of EPR. The goal is to educate consumers about proper disposal techniques, recycling guidelines, and environmentally responsible waste management benefits. EPR aims to enhance the impact of sustainable packaging initiatives by cultivating a consumer base that is more informed and engaged. Effective EPR implementation requires regulatory frameworks and supporting legislation. Governments are crucial in ensuring manufacturers meet specific standards, targets, and timelines aligning with broader environmental goals. Collaboration between many entities, including recycling facilities, manufacturers, governments, and municipal governments, is essential.

Collaborations and partnerships provide EPR projects that are both effective and long-lasting **[27]**, **[28]**, **[29]**. EPR encourages innovation and research across the sector. Manufacturers are encouraged to devote funds to study to develop innovative, affordable, and environmentally friendly packaging solutions. This entails investigating cutting-edge packaging design strategies, innovative materials, and recycling technologies. EPR requires reporting and monitoring mechanisms. Producers participating in EPR programs must monitor and report on their progress toward predefined objectives. This transparency fosters responsibility, aids in assessing the effectiveness of EPR initiatives, and supports continuous improvement. Globally, there is a growing trend of interest in EPR concepts. Producers operating in different places must deal with different regulatory contexts, and their EPR strategies must be modified accordingly. The broad adoption of EPR demonstrates a commitment to more broadly addressing packaging waste and environmental problems. Extended producer responsibility is a game-changer in package management, allowing for a more sustainable and circular approach to product lifecycles. EPR makes producers accountable for the environmental impact of their packaging by promoting creativity, collaboration, and responsible resource management across the whole supply chain **[19]**, **[27]**, **[28]**.

Smart Packaging Technologies

Real-time data transfer is made possible by combining networking technologies like NFC and RFID with sensors in intelligent packaging. This feature enables the real-time monitoring of many characteristics, such as temperature, humidity, freshness, and integrity. Innovative packaging is quite helpful for things with particular needs regarding storage and transportation. It guarantees that goods like perishables are kept in the best possible storage. Authentication and traceability are the two main functions made possible by intelligent packaging technology. The supply chain may become more transparent by tracing a product's path from manufacturing to consumption using QR codes, RFID tags, and other identifying technologies. In addition to their usefulness, several interactive aspects of intelligent packaging solutions draw in customers.

Affordability and cost are crucial considerations, as incorporating these technologies may increase expenses. Achieving a balance between the benefits of more functionality and cost is vital for broad adoption. Energy consumption is another consideration because many innovative packaging systems rely on a power source to run their sensors or connectivity features. Reducing environmental impact requires finding a balance between energy efficiency and necessary functionality. Robust data security and privacy must be ensured since innovative packaging necessitates data collection and transfer. Encryption and secure data storage practices are required to safeguard consumer information. To guarantee seamless interaction with existing systems and foster adoption in international sectors with various technical infrastructures, compatibility, and standardization are also essential factors to consider.

Environmental issues arise from end-of-life concerns, especially for parts like batteries or electrical elements. These issues can be addressed using sustainable materials or designing with recyclability. Finally, customer education is crucial for smart packaging to be successfully used. Customer acceptability is influenced by conveying the advantages of the technology, making sure it's simple to use, and offering clear instructions on how to use intelligent features. In summary, innovative packaging technologies are a game-changer for the packaging sector, providing advantages including better supply chain visibility and customer experiences. For these technologies to be widely and sustainably implemented, cost, environmental effect, and user acceptance must all be carefully considered as they develop **[19]**, **[20]**, **[26]**.

Bioplastics and Bio-based Materials

Bioplastics and bio-based materials are gaining popularity in sustainable packaging as environmentally benign alternatives to traditional plastics derived from fossil fuels. These plant-based, cornstarch-and sugarcane-derived polymers provide a sustainable solution to the environmental issues that conventional plastics cause. The fact that bioplastics and bio-based materials come from sustainable resources is a notable characteristic that marks a departure from traditional plastics' reliance on non-renewable fossil fuels. This shift lowers greenhouse gas emissions during production, lowering the production's carbon footprint due to plants' growth-induced absorption of carbon dioxide. Compostability and biodegradability are other noteworthy attributes, particularly in regions with prevalent single-use plastics. Some bioplastics are designed to break down in specific conditions, offering a potential solution to the environmental persistence of plastic waste. This aligns with the overall goal of mitigating the adverse environmental impacts of packaging. Bioplastics and biobased materials may be easily adapted to many packaging types, including bottles, films, and containers. Combining this versatility with ongoing research and development yields fresh properties for the material. Bio-based materials may be modified to have unique properties such as increased strength, flexibility, or barrier capabilities, expanding their range of applications across several industries. However, using these materials requires carefully considering several factors. It is crucial to consider that manufacturing bio-based goods can compete with resources that might be used for food production. In sustainable sourcing, resource utilization needs to be balanced. End-of-life management is another important consideration, especially for bioplastics meant to be disposable or biodegradable. Proper disposal practices, including the need for industrial composting facilities, must be made clear to consumers through consumer education. Technical advancements, adherence to legal standards, compatibility with present recycling streams and separate processing facilities, and observance of current standards define the effective adoption of bioplastics and bio-based products **[22]**, **[24]**, **[30]**.

Characteristics of Packaging Reusability and Refill Systems

While bioplastics and bio-based materials offer promising prospects for eco-friendly packaging, their application necessitates a thorough assessment of several factors. One of the most important considerations is the potential for resource competition since producing bio-based products may involve using resources such as land, water, and other resources that could be used for food production instead. Achieving sustainable sourcing techniques means balancing resource usage to lessen competition with food crops. A further critical consideration for bioplastics is proper end-of-life management; even though some are intended to be compostable or

biodegradable, composting occasionally requires industrial infrastructure. Compatibility with existing recycling systems is a significant challenge since some bio-based products need independent processing facilities. Ensuring alignment with recycling infrastructure is crucial for promoting circularity in the packaging sector. Research and development are needed if bioplastics and other bio-based materials are to perform better and be more cost-effective. Technology advancement is crucial to reducing limitations and expanding the range of applications for these materials. Education and client impression are also important components. It is necessary to educate consumers about the benefits and limitations of bioplastics through appropriate labeling and communication to promote their acceptance and comprehension. Complying with regulatory standards and certifications, such as ASTM D6400 or EN 13432 for compostability, lends more credibility to bio-based products and ensures their environmental performance. When converting to sustainable packaging solutions, there are two things to consider: refill systems and reusable packaging. These innovative techniques promote container reuse and facilitate product refills to decrease packaging waste and promote a circular economy. Reusable containers, robust, long-lasting designs, and fewer single-use packaging are some of the features of these systems. Several factors are considered, including stakeholder participation, economic feasibility, safety and hygiene, supply chain logistics, package design and materials, and consumer behavior and uptake. By addressing these issues, a more circular and sustainable packaging ecosystem may be developed and successfully adopted **[22]**, **[24]**, **[30]**.

Characteristics of Agricultural Byproducts

Agricultural byproducts are unique in several ways, making them excellent choices for eco-friendly packaging solutions. Their primary attribute is that they are sustainable and renewable. These byproducts provide a reliable and sustainable supply since they are made from the organic matter generated during agricultural activities. Unlike finite resources like fossil fuels, agricultural byproducts can be produced seasonally, by sustainability principles. These biomass-derived byproducts from plants demonstrate how sustainable plants are since they can be cultivated in cycles, ensuring a consistent supply for various purposes. Utilizing packaging manufactured from agricultural waste lowers the carbon footprint compared to traditional materials. Since the carbon dioxide emitted during their decomposition is a component of the natural carbon cycle, they are a more environmentally friendly choice. Additionally, some farming practices and byproducts could be able to store carbon in the soil, which would double the mitigation of climate change. Various materials with customizable compositions may be used to satisfy different packaging requirements that fall under agricultural byproducts. Agricultural wastes like husks, leaves, stems, and shells help to create this type. These wastes provide a variety of unique raw materials. Because agricultural wastes include a wide range of intrinsic chemical properties, materials with specific properties may be created and tailored to meet the needs of different sectors. Combining different agricultural byproducts or mixing them with other materials enhances material attributes and improves usefulness and performance in packaging applications, which fosters innovation. Crop harvesting cycles and seasonal variations impact the availability of agricultural byproducts. It is crucial to comprehend these cycles to arrange and ensure a continuous supply of packaging materials. Due to its seasonal nature, agriculture requires careful planning for fluctuations in the availability of byproducts. viable harvesting practices are necessary to keep agricultural ecosystems viable. By utilizing methods that ensure crop regeneration, it is feasible to provide a long-term supply of byproducts while stopping environmental degradation. Crop rotation methods combined with the utilization of leftovers help promote sustainable packaging material supply and agricultural production. This method lessens the impact of agriculture on the environment while increasing soil fertility **[24]**, **[31]**.

Physical Properties

The physical attributes of agricultural products are essential in determining whether or not they are suitable for certain usage in packaging. Mechanical strength and durability are important considerations when protection and structural integrity are at stake. One agricultural waste that significantly improves the mechanical strength of packing materials is plant fiber. The packaging is more resilient to tearing, puncturing, and other mechanical forces that may occur during packaging and shipment because of the inherent strength of these fibers. Moreover, agricultural byproducts may be further treated to enhance their mechanical properties through fiber extraction and composite manufacture, expanding the industries in which they can be used for packaging. Flexible and malleable packaging materials are essential, especially when product shape conformity is needed. Some plant-based. Among the naturally flexible agricultural byproducts are several films generated from plants and natural fibers. This inherent quality allows packaging materials to adapt to the contours of objects.

Moreover, agricultural byproducts may be made more flexible and malleable by processing them using techniques like molding and extrusion, which opens up new opportunities for them in the packaging industry. Because of their malleability and flexibility, these byproducts may be used in form-fitting packaging, which is crucial when wrapping fragile or asymmetrically shaped items. The weight-to-strength ratio is crucial when

designing packaging since there are instances in which minimizing weight without compromising strength is required. Many agricultural byproducts have an excellent weight-to-strength ratio in packing because they are inherently light. This function helps reduce the overall amount of material used and the delivery cost. Agricultural leftovers can also be combined with other polymers or strengthening agents to create lightweight composites, maximizing the weight-to-strength ratio for specific packaging requirements. The low bulk of agricultural waste helps to improve packing efficiency and lessen the environmental effect of handling and transportation. Texture and surface features are significant aspects that impact the whole consumer experience, branding possibilities, and visual attractiveness of packaging materials. Packaging incorporating agricultural waste looks more natural and organic, appealing to eco-conscious consumers. Furthermore, because of their changeable surface textures, agricultural waste allows for customization based on desired tactile and visual characteristics. This includes smooth finishes, embossed patterns, and textured surfaces. Printability is influenced by the surface features and texture of agricultural outputs, which creates opportunities for effective product marketing through readable labeling and printing **[24]**, **[31]**, **[32]**.

Chemical Properties

The chemical properties of agricultural byproducts determine their suitability for various packaging purposes. Since many agricultural outputs naturally tend to break down over time due to microorganisms, biodegradability is an essential element to consider. This packaging waste's inherent biodegradability reduces its persistence in the environment, in line with environmental sustainability criteria. The concept of a circular economy is promoted as biodegradable agricultural wastes may be reintegrated into the ecosystem without causing long-term harm, especially when considering single-use packaging. Additionally, certain agricultural wastes can be processed to achieve controlled decomposition rates, allowing the biodegradability to be altered to suit specific application requirements. Chemical stability is a crucial chemical property of agricultural residues. These materials often demonstrate chemical resistance, ensuring their structural and functional properties are retained throughout time. This stability is necessary for packaging to remain dependable across various environmental conditions. Packaging materials made from agricultural wastes have the potential to properly protect enclosed objects while maintaining their shelf life and product quality due to their chemical stability. Because of their inherent chemical stability, agricultural wastes may package various goods with varying pH levels, moisture concentrations, or other chemical qualities. Packaging materials need to be waterproof and resistant to moisture intrusion to preserve the security and integrity of their contents. Some agricultural outputs' innate hydrophobic or hydrophilic properties determine how resistant they are to moisture. This quality is crucial for protecting products against external sources of moisture and humidity. Moreover, agricultural wastes can be coated or treated using natural or environmentally friendly processes to improve their water resistance, enhancing the overall sustainability of the packaging. Due to its moisture resilience, packaging made of agricultural wastes is great for various climates and transportation scenarios, especially for moisture-sensitive things. The reaction of packaging materials to ultraviolet (UV) light is also being studied. Certain agricultural goods, including some meals and pharmaceuticals, can benefit from the inherent UV light-filtering properties of various agricultural outputs. Agricultural byproducts can also be processed for UV stabilization or treated to boost their resistance to UV degradation. Packaging made of UV-resistant agricultural byproducts is suitable for outdoors or in places that receive a lot of sunlight since it ensures the material's continuous integrity and protects the contents. This is particularly crucial for products that, under some circumstances, require an extended shelf life **[6]**, **[32]**, **[33]**.

Thermal Properties

Due to their thermal properties, agricultural wastes may be employed in various packaging applications. The above-stated byproducts often exhibit thermal stability, ensuring the structural and functional integrity of packaging materials under various conditions. They can be used to pack goods for cold storage or to transport perishable goods because of their insulating properties and capacity to act as a barrier against heat transmission. Additionally, because some agricultural byproducts have higher melting points, increasing their thermal stability, they could be better suitable for applications that need endurance to high temperatures. Furthermore, many agricultural wastes are eligible for use in insulated packaging due to their poor thermal conductivity, which allows heat retention. These qualities make agricultural wastes more beneficial because they make it possible to preserve enclosed goods—like some foods or medications—sensitive to temperature fluctuations **[34]**, **[35]**.

Barrier Properties

Among the packaging materials derived from agricultural wastes are starch-based films, poly (lactic acid) (PLA) films, and poly (butylene adipate-co-terephthalate) (PBAT) films. These films display different barrier qualities essential for maintaining the quality and shelf life of enclosed products.

Gas Barrier Properties

Films made of starch and inexpensive, sustainably produced plasticizers have been created to enhance the gas barrier qualities of food packaging. Furthermore, PLA films have demonstrated improved gas barrier qualities when combined with lignin nanoparticles, successfully preventing the transmission of UV radiation **[4]**, **[36]**.

Moisture Barrier Properties

The characteristics of zeolite-incorporated PBAT films as moisture barriers have been investigated. It has been discovered that adding zeolite to composite films enhances their ability to withstand water vapor, which makes them appropriate for prolonging the ripening time of agricultural goods like bananas **[37]**.

Odor Barrier Properties

Improved odor barrier qualities have been demonstrated for starch-based films that contain defatted brownish water-soluble extract (BrE) derived from potato chip byproducts. These movies showed that they could keep packed goods smelling how they wanted, making them appropriate for cheese packing **[38]**.

Light Barrier Properties

PLA films modified with special stereocomplex (SC) networks and polyethylene glycol (PEG) have been developed to improve light barrier properties. These modified films have shown good light transmittance and potential for use in green packaging applications, including agricultural films **[39]**.

Processing Techniques for Agricultural Byproducts

Fiber Extraction

Research on the cellulose extraction process The viability of using chemical processes like acid hydrolysis, chlorination, alkaline extraction, and bleaching to extract cellulose from a variety of sources, including cotton, sisal, flax fibers, corn stover, and rice husk, has been demonstrated by nanowhiskers from natural fibers and agricultural byproducts. Research also recommended employing a low-cost household blender adapted to extract cellulose nanofiber from residual agricultural crop material to reduce production expenses. The extraction and purification process affects rice bran oil, high in tocopherols, tocotrienols, γ-oryzanol, and unsaturated fatty acids. The bioactive ingredients in rice bran have been shown to have antidiabetic, hypocholesterolemic, anti-inflammatory, anti-cancer, and anti-colitis properties **[35]**, **[40]**, **[41]**.

Polymer Extraction

Recovering polymers from agricultural waste is a critical step in producing sustainable packaging. Bio-based polymers from agricultural leftovers provide a sustainable and ecologically friendly alternative to traditional petroleum-based plastics. These polymers reduce the need for non-renewable fossil fuels, contributing to a closed-loop, sustainable solution. They are made using feedstocks generated from renewable plants, such as starch, cellulose, and sugar. They are consistent with broader sustainability objectives and promote environmental sustainability by reducing dependency on conventional polymers derived from fossil fuels. Additionally, they lessen the harm that the exploitation of non-renewable resources does to the ecosystem. One significant advantage that makes bio-based polymers environmentally acceptable substitutes for conventional polymers when their useful lifetimes are ending is their capacity to decompose or biodegrade. This is particularly beneficial for cutting down on pollution and plastic waste. Furthermore, because of their processing flexibility and ability to be formed using conventional plastic production techniques, bio-based polymers may be produced on a large scale and integrated into many packaging applications. Additionally, biodegradable and active films used to wrap food items are manufactured from bio-based polymers derived from residues from seafood preparation. These films can extend the shelf life of packaged foods due to their bioactivities, which proactively release antibacterial and antioxidant compounds into the food and offer sufficient barrier qualities. The development of environmentally friendly food packaging options might benefit from this tactic. Additionally, molded cellulosic pulp materials provide petroleum-based packaging methods with eco-friendly substitutes. They are used when cushioning is required, and the things in the pack must match in shape. Methods to increase output and lower energy usage are being researched to overcome water evaporation and dewatering challenges during molding. Furthermore, when

combined with chemical sensors and indicators, bio-based and biodegradable packaging has attracted a lot of attention since it may provide information about the actual freshness of food in addition to protection. It has proven possible to generate an effective, biodegradable polymer loaded with curcumin by combining poly (lactic acid) (PLA) and poly (propylene carbonate) (PPC). It is recyclable and may be used as a clever indicator of food degradation. Moreover, juice processing byproducts have been utilized to produce bioelastomers for food packaging that have antibacterial and antioxidant qualities, offering state-of-the-art technology for sustainable development **[42]**, **[43]**, **[44]**, **[45]**.

Chemical Processing

Chemical processing is crucial in converting agricultural waste into materials suitable for ecologically acceptable packaging. This involves using precisely selected chemical treatments to modify inherent properties and enhance the performance of packaging materials. Numerous chemical treatments, such as the addition of cross-linking agents, esterification, etherification, enzymatic hydrolysis, and alkaline treatment, are utilized to achieve specific changes in material properties. Remaining material can be made more flexible and cellulose more accessible by breaking down lignin and hemicellulose in alkaline solutions. Enzymatic hydrolysis is the process of enzymes breaking down complex carbohydrates to generate refined fibers with better mechanical and purity properties. Acid hydrolysis breaks down cellulose and hemicellulose to provide cellulose-rich fractions for various packaging applications. Esterification and etherification result in the addition of functional groups, which enhance water resistance and flexibility. Cross-linking agents increase dimensional stability and mechanical strength by strengthening the network inside the material structure. Chemical processing affects stability and biodegradability in addition to improving qualities. Certain methods increase biodegradability, which facilitates the organic decomposition processes that take place during end-of-life disposal. Concurrently, stability improvement ensures resistance to outside factors like moisture, temperature, or microbial degradation, enhancing the durability and functionality of packaging materials. Achieving a careful balance between enhanced durability and biodegradability is crucial, considering the potential toxicity of the chemicals used in the process to the environment. Reducing the environmental impact of these processes is necessary to maintain the packaging solution's overall sustainability **[42]**, **[43]**, **[45]**, **[46]**.

Mechanical Processing

Extrusion

A flexible processing method called extrusion is essential for turning agricultural waste into materials fit for environmentally friendly packaging. The ability of agricultural byproducts to be processed by an extrusion machine under pressure and heat, or extrudability, varies depending on the agricultural byproduct's composition, moisture level, and intrinsic structural properties. Extrudability is influenced by several parameters such as the ideal moisture level, uniform particle size distribution, and the chemical makeup of agricultural wastes. Throughout the extrusion process, issues including temperature sensitivity and the crucial function die design play must be considered carefully. Extrusion is a versatile process that uses agricultural leftovers as feedstocks to create sustainable packaging materials. Film production is a common application that yields thin, flexible films with specific barrier properties for packaging applications such as bags, pouches, and wraps. Sheet extrusion enables rigid and semi-rigid packaging solutions, including trays, containers, and other components. By forming cross-sectional profiles for packing structural components, profile extrusion enhances both design and usefulness. Because co-extrusion blends components, agricultural byproducts can be co-extruded with other polymers for better characteristics. Moreover, extrusion produces filaments for 3D printing, providing a sustainable alternative for creating intricate and customized package designs. In conclusion, agricultural byproducts may be modified into a range of packaging materials because to extrusion's versatility, which contributes to the creation of sustainable packaging solutions in several forms **[5]**, **[47]**, **[48]**.

Thermal Processing

Agricultural byproducts are thermoformed by first allowing the material to become malleable through heat. The exact temperature and heating duration are determined by the material's composition and the needed qualities of the final product. After that, the softer material is formed over a mold, imparting its contours to create precise and intricate packaging designs. The last step involves rapid cooling to maintain the molded shape; the stiffness and dimensional stability of the material are impacted by the cooling methods used. Using shaped agricultural waste, thermoforming is a versatile method that may be used for various sustainable packaging design applications. Trays and Containers: Thermoforming is a common method used to create trays and containers that may be used to package various things. Shaped agricultural byproducts provide rigid or semi-rigid structures that effectively exhibit and protect goods. Clamshells and Blister Packs: Thermoforming may be used to create blister

packs and clamshells, commonly used to package small consumer goods, pharmaceuticals, and electronics. Thermoforming may be tailored to match certain product shapes and sizes because to its flexibility. Disposable Packaging: Disposable bowls, plates, and silverware are made from thermoformed agricultural waste. This is an environmentally friendly option to single-use plastics. Customized Packaging Solutions: It is simpler to create packaging solutions using thermoforming that meet the requirements of a certain brand or product. Because of its adaptability, designers may create distinctive packaging that enhances a brand. Agricultural waste-derived thermoformed inserts offer a safe and reliable grip for objects enclosed in the packaging. These inserts reduce the need for additional packaging materials by stabilizing and cushioning items during shipment. Thermoforming is a crucial thermal processing technique that turns agricultural waste into sustainable packaging materials. Its applications span from conventional trays and containers to innovative, customized ones that encourage the development of practical and environmentally friendly packaging alternatives **[22]**, **[24]**, **[31]**.

Heat Treatment

Heat treatment, a thermal processing method, is essential to sustainable packaging when employing agricultural residues. Using this technique, materials are heated at certain temperatures for predefined durations of time to increase their general stability and change their properties in the right manner. When using heat treatment to alter the properties of agricultural outputs, precise temperature control is crucial. The temperature range and treatment length significantly impact the material's mechanical strength, chemical stability, and flexibility. Applying heat causes structural changes at the molecular and crystalline levels, enhancing tensile strength, dimensional stability, and resistance to external stimuli. Furthermore, by reducing the water content of agricultural wastes, heat treatment enhances their moisture resistance and increases material stability during storage and transportation. Moreover, agricultural outputs can alter their color and appearance by controlled heat treatment, allowing them to meet the aesthetic requirements of packaging applications. Thermal processing is used to strengthen the durability of agricultural leftovers. This technique also reduces their microbial activity, making them more suitable for packaging applications where biological factors might cause contamination or degradation. Agricultural products that have been heat-treated may eventually show improved resistance to degradation, increasing their shelf life and preserving their effectiveness for their intended use. Using heat treatment to reduce thermal expansion, packaging components may be kept dimensionally stable and free from warping or distortion. The impact of heat treatment on the material's biodegradability must be carefully considered. Heat treatment can alter a material's sensitivity to natural degradation processes, even though it enhances stability. This could affect the material's overall environmental compatibility. In conclusion, heat treatment is a versatile thermal processing method that may be applied to agricultural outputs to help them become more stable and shaped. By carefully controlling temperature settings, heat treatment aids in producing sustainable packaging materials with improved mechanical strength, moisture resistance, and shelf life. These benefits must be carefully weighed against issues like biodegradability and other environmental concerns to assess the material's sustainability Field fully **[24]**, **[32]**.

Surface Modification Techniques

Surface modification techniques are essential for customizing a material's qualities for a certain use. Surface modification techniques are utilized in sustainable packaging that uses agricultural waste to improve functionality and handle particular difficulties. In this area, barrier and functional coatings for packaging are the main discussion topics regarding coating as a surface modification approach.

Coating

Coating is a surface modification technique crucial to sustainable packaging since it involves applying a thin material layer to alter substrate properties and enhance performance. Coatings are applied to packaging materials composed of agricultural byproducts to impart certain functionalities that improve the overall effectiveness of the package. The aim of barrier coatings is to provide an impermeable barrier against external factors, including light, moisture, and gases. The freshness and quality of packaged items must be preserved, and there are several types of these coatings. Vapor barrier coatings are necessary in food packaging to stop water vapor from entering the product. Gas barrier coatings extend the shelf life of perishable items by preventing gases like oxygen and carbon dioxide from penetrating. On the other hand, UV barrier coatings prevent UV radiation from destroying light-sensitive products, preserving their flavor, color, and nutritional content. Agricultural byproducts including paper, cardboard, and bioplastics may be effectively protected from the elements by applying barrier coatings. Functional coatings are a different coating that provides additional advantages over simple protection. These coatings improve functioning by having antibacterial properties, improved adhesion, or other surface characteristics tailored to the demands of the packed product. Antimicrobial coatings are very useful for food packaging because

they stop bacteria from growing on the container's surface and preserve the contents' safety and freshness. Because anti-fog coatings prevent condensation from forming, temperature variations do not affect the contents of packaged items' visibility or aesthetic appeal. Adhesion capabilities are enhanced by adhesive coatings, which also improve the structural integrity of the package. When functional coatings are added to packaging materials manufactured from agricultural byproducts, certain demands or impediments are satisfied. For example, anti-fog coatings maintain visual attractiveness in various weather conditions, while antimicrobial coatings increase the safety of perishable goods **[19]**, **[49]**.

Lamination

Lamination is one of the most significant surface modification methods utilized to produce environmentally friendly packaging derived from agricultural waste. This method primarily aims to build a single, multi-layered structure by combining many material layers to improve strength, durability, and overall performance. Lamination is a technique to construct multi-layered structures in sustainable packaging created from agricultural waste. The material may be combined with other elements, such as those produced from bio-based polymers, recycled materials, or crops because each layer contributes special properties to the material overall.

This material mixing technique increases the final laminated material's versatility and utility. Furthermore, laminated structures can have layers with specific barrier properties to offer protection against light, gases, and moisture. This feature makes packaging from agricultural waste far more sustainable since it keeps packed goods in better condition and extends their shelf life. Lamination also facilitates the addition of layers for improved printability. This feature is especially useful for packaging applications where visual appeal and branding are important considerations. Selecting surface layers with print-friendly properties can help successfully exhibit vibrant graphics and product information. One of lamination's remarkable properties is its ability to significantly improve packing materials' mechanical properties and overall strength. Laminated materials combine layers with different structural properties to increase longevity while balancing flexibility, stiffness, and tear resistance. Along with resistance to external factors, including humidity, temperature fluctuations, and abrasions, this increased resilience guarantees the package maintains its integrity during storage and transportation **[24]**, **[32]**, **[33]**.

Adding layers with great puncture resistance is another notable feature of laminated packaging, which is especially useful for items with sharp edges or vulnerable to puncture damage. Additionally, lamination helps extend the shelf life of packed items by providing a robust barrier against the weather, which is crucial for perishables. Because laminated packaging prioritizes strength and durability, it is sustainable, but it also has to consider recycling and biodegradability. The laminating process should use environmentally friendly materials and adhesives to provide appropriate end-of-life outcomes **[19]**, **[33]**, **[50]**.

Printability and Labeling

Printability and labeling are essential components of packaging design to communicate information, branding, and aesthetic appeal to consumers. When it comes to sustainable packaging that uses agricultural waste, it is crucial to ensure that it works with different printing techniques. This section examines printability issues and how certain materials can work with flexography, offset printing, and digital printing.

Compatibility with Various Printing Methods

Flexible printing techniques, including flexography, offset printing, and digital printing, are suitable for materials created from agricultural waste used in packaging. Flexography is a widely used printing method that imprints ink on a substrate using flexible relief plates. Agricultural waste may be used to create a printed surface for various applications using the flexographic process. However, because some agricultural wastes are absorbent, specific ink formulae can be needed to achieve the optimum results. Offset Printing: A rubber blanket transfers ink from a plate to print on packaging materials.

Agricultural byproducts are a good fit for offset printing, especially if their surfaces are uniformly flat. Prints of the best quality require careful consideration of prepress setup and ink selection. This method makes direct printing from digital data possible, allowing for shorter print runs and greater customization possibilities. If appropriately prepared and managed, digital printing technology may be utilized with agricultural byproducts. This method works well for applications that include variable data and on-demand printing **[51]**, **[52]**.

Printability Considerations

For packaging materials manufactured from agricultural byproducts, certain surface treatments are often necessary to enhance printability. For a surface to be more consistent and smoother for printing, surface treatments like coatings or finishes are necessary. This enhances color reproduction and ink adhesion, which improves print

quality overall. It's crucial to consider ink compatibility since different ink formulations may react differently with agricultural waste.

Selecting inks that complement the characteristics of the material yields the best printing outcomes. These characteristics include absorption, drying qualities, and adherence. Efficient prepress preparation is necessary to produce prints of superior quality. This involves modifying pictures, changing color, and setting up plates. To get precise and colorful printing results, the prepress stage must consider the porosity and texture of agricultural byproducts. Durability and adhesion are additional crucial aspects to consider throughout the printing process. Sufficient ink adhesion to the substrate allows prints to withstand handling and use, extending their longevity. Using bio-based or environmentally friendly inks is one method to be environmentally mindful. This decreases the environmental impact of printing operations and adds to the overall eco-friendliness of the packaging—both of which align with sustainability goals. These materials are suitable for various printing processes since they may be made to provide the best printability possible when using agricultural waste. Their versatility in printing techniques enhances their value in developing environmentally friendly packaging **[19]**, **[53]**.

Label Adhesion

A key component of package design is label adhesion, which guarantees that labels are securely attached to the packing material. When it comes to labeling materials that are sustainable and use agricultural waste, the material's surface properties are critical. This section discusses particular labeling strategies designed for agricultural byproducts and the surface properties necessary for effective labeling. An essential condition for the best label adherence is smoothness. Agricultural byproducts with a consistent, smooth surface are better for labeling since they don't allow air pockets to develop and the label and package material stick together tightly. The surface's porosity also influences label adhesion, as a suitable porosity level promotes adhesive penetration and strengthens the binding. If the porosity is too high, more surface treatments could be needed to improve label adherence without sacrificing material integrity. Furthermore, the material's absorbency is a key element influencing the absorption and setting of adhesives. A balanced absorbency must be maintained to ensure correct label adherence without jeopardizing the adhesive's capacity to make a strong bond. Agricultural byproducts can benefit from surface treatments, such as coatings or finishes, to improve surface energy and smoothness and make them more compatible with label adhesives **[6]**, **[19]**, **[54]**.

Packaging commonly uses self-adhesive labels, also known as pressure-sensitive labels. These labels have pre-applied adhesive activated by pressure during application, so they function well with agricultural byproducts with the required surface properties. Wet glue labels, applied with a wet adhesive and let to dry to produce a solid bond, work best on agricultural goods with smooth surfaces and moderate absorbency. Before packaging material is molded, labels are integrated into the mold using in-mold labeling (IML), creating an integrative label-packing structure. For deployment to be effective, label materials need to work with agricultural waste materials. Since heat is used to transfer printed images from a surface, heat transfer labels need surfaces that can withstand heat and have strong adhesion. Direct printing on packaging material, in particular, eliminates the need for a separate labeling procedure by utilizing digital printing technology. A useful labeling alternative is offered by agricultural products that are directly printed. Efficient labeling of packaging materials made from agricultural byproducts requires appropriate labeling processes and careful consideration of surface aspects. The chosen strategy influences the branding and visual attractiveness of sustainable packaging, leading to a thorough and environmentally conscious package design **[6]**, **[19]**, **[54]**.

Environmental Considerations in Processing

An essential part of ecologically sustainable operations is the integration of sustainable energy sources, namely those derived from agricultural byproducts, into the packaging material production process. One component of this plan is using renewable energy sources, such as biomass, hydro, wind, and solar. Using solar energy using devices like photovoltaic systems and solar thermal collectors can help reduce dependency on conventional energy sources.

This is a viable choice. In a similar spirit, using wind energy solutions, which produce electricity via turbines, contributes to a cleaner, more sustainable energy balance. Produced from organic resources, such as agricultural wastes, biomass energy adheres to the principles of the circular economy. It provides sustainable energy solutions while minimizing waste. Furthermore, employing the hydropower generated by water movement is a dependable renewable energy source, especially for structures. The impact of this switch to sustainable energy sources is seen in the decrease in greenhouse gas emissions associated with the processing of packaging materials.

Emissions are nearly eliminated when sustainable energy technology is used, reducing the overall carbon footprint. This reduction aligns with the life cycle assessment (LCA) principles, which indicate that the application of sustainable energy practices positively affects the environmental performance of packaging materials from the

extraction of raw materials to end-of-life concerns. In addition, using sustainable energy shows a commitment to processing packaging materials in an environmentally responsible way and is compatible with carbon neutrality goals. Businesses striving for carbon neutrality actively offset or balance their carbon emissions, promoting a socially conscious approach. Following the rules and regulations that promote sustainable energy use and reduce carbon emissions is essential to this goal. Beyond merely adhering to regulations, this promise shows a commitment to environmental stewardship in managing packaging materials. Agricultural byproduct packaging may be easily implemented using sustainable energy technologies. By reducing their reliance on traditional energy sources, facilities may significantly minimize the carbon footprint associated with manufacturing and processing sustainable packaging solutions. By placing a high value on energy efficiency and renewable energy sources, the packaging industry is making a substantial contribution to environmental sustainability. This is consistent with broader initiatives to advance a circular economy and reach carbon neutrality **[19]**, **[20]**, **[55]**.

Application on Agricultural Byproducts

Sustainable practices align with the implementation of waste reduction techniques, such as closed-loop systems and recycling/reuse activities, for packaging materials made from agricultural wastes. Reducing the environmental effect of packaging materials is mostly achieved by closing the loop and actively engaging in recycling and reuse initiatives.

Life Cycle Assessment (LCA)

A thorough method for assessing a process or product's environmental impact throughout its life cycle is life cycle assessment or LCA. A life cycle assessment (LCA) must be performed to make sustainable and knowledgeable decisions about processing packaging materials, particularly those made from agricultural wastes. A life cycle assessment (LCA) is a systematic research method that extensively analyzes the environmental effect across the complete life of a process or product. This assessment begins with the extraction of raw materials and includes information on land and water usage. Production, delivery, and use phases come next; recycling or disposal happens at the end of the product's life. The Life Cycle Assessment (LCA) process consists of multiple steps, including determining the environmental impact of raw material extraction, assessing the sustainability and effectiveness of the production process, assessing the environmental impact of transportation and distribution, and comprehending the impact during the use phase. Life cycle assessment (LCA) considers landfill impact, recycling efficacy, and reuse prospects. It also looks at how a product should be recycled or disposed of at the end of its life cycle. Above all, LCA is based on quantitative data, which allows for an accurate and methodical evaluation of the environmental effect. This information makes it easier to compare various materials, procedures, or design options, highlighting areas for development and swaying choices in favor of more environmentally friendly solutions. LCA also considers other environmental parameters including resource depletion, carbon footprint, and water impact. These indicators offer a wide view of the effects on the environment, assisting in the development of well-informed decisions for a more environmentally conscious and sustainable strategy **[55]**, **[56]**, **[57]**.

Incorporating LCA in Processing Decisions

Data-driven decision-making is the cornerstone of sustainable practices, and life cycle assessment (LCA) is a particularly helpful tool for providing the data needed to make educated decisions at every stage of processing. Processors can use Life Cycle Assessment (LCA) data to lower environmental impact and enhance sustainability in operations, such as resource selection and manufacturing methods. Regarding packaging materials derived from agricultural leftovers, life cycle assessment (LCA) aids in the process by identifying the byproducts best suited for certain applications and have the least adverse impact on the environment. Furthermore, life cycle analysis (LCA) is an essential tool for process optimization since it may identify inefficiencies in the energy and manufacturing processes. Equipped with this information, processors may strategically optimize operations, reduce the overall environmental impact of their processes, and improve energy efficiency. Most importantly, LCA is not a one-time assessment; rather, it is a vehicle for continuous improvement. By routinely conducting Life Cycle Assessments (LCAs), processors may evaluate their progress toward sustainability objectives, implement improvements, and track changes in the environmental effect over time. Transparency and communication are critical components of sustainable practices, and LCA results may be a useful tool in fostering these attributes. Sharing LCA findings with customers and other stakeholders demonstrates a commitment to transparency and sustainability. Fostering sustainable branding and fostering trust are accomplished via transparently discussing the environmental impact of packaging materials derived from agricultural waste. A systematic approach to analyzing environmental impact in the specific context of agricultural waste is provided by incorporating life cycle assessment (LCA) into the packaging material manufacturing process. Processors can

utilize life cycle assessment (LCA) data to make educated decisions that reduce environmental impact, promote responsible practices, and align with sustainability objectives throughout the packaging materials' life cycle **[53]**, **[57]**, **[58]**.

Biodegradability and End-of-Life Considerations

Biodegradability is the ability of a substance to spontaneously break down into safe molecules as a consequence of bacteria. Biodegradable packaging departs from traditional, non-biodegradable materials that significantly worsen environmental contamination. The evolution of materials from conventional plastics to the development of biodegradable alternatives is charted in the history of biodegradability, providing insight into the causes of this paradigm shift. It examines the scientific theories underlying biodegradation, emphasizing bacteria, enzymes, and environmental factors' roles in the process. Studying biodegradability becomes more important as demand for sustainable methods increases. This section takes the reader through the major historical moments, cutting-edge scientific findings, and shifting consumer preferences that have shaped the discussion around biodegradable packaging materials. Knowing the historical context helps stakeholders understand the motivations behind the hunt for biodegradable solutions and the revolutionary consequences they want to achieve **[58]**, **[59]**.

Significance of End-of-Life Considerations in Packaging

Concerns about end-of-life packaging are significant because they highlight a more sustainable and circular strategy that goes beyond the straightforward paradigm of consumption and disposal. When packing materials reach the end of their useful life, their handling becomes an important consideration in evaluating the environmental impact of the materials. This section explains the significance of end-of-life concerns and covers a wide variety of subjects, from preventing ecological effects to reducing waste and conserving resources. Under the traditional linear paradigm, packaging materials often wind up in landfills, causing resource waste and environmental harm. The trend toward package design considering the end-of-life phase recognizes the need for appropriate waste management. To completely comprehend the importance, it is vital to examine the environmental consequences of several disposal options, including recycling, composting, and landfilling. The part also emphasizes how end-of-life practices are being more recognized by consumers, businesses, and regulatory bodies as essential to developing a sustainable and circular economy. The baseline knowledge on biodegradability and the significance of end-of-life considerations made it possible to conduct a thorough examination of sustainable packaging methods, with a focus on using agricultural wastes to find biodegradable alternatives **[58]**, **[60]**.

Importance of Biodegradable Packaging

Biodegradable packaging is a pioneer in ecological techniques, offering advantages over traditional packaging materials. It is significant because it will address plastic pollution, decrease the negative environmental effects, and adjust to shifting consumer preferences and industry changes. Environmental Impact Reduction: Biodegradable packaging reduces the need for virgin materials, uses agricultural waste, and complies with sustainable standards, all of which help save natural resources. Biodegradable packaging may need less energy to make than packaging made of traditional polymers, which lessens both the package's environmental effect and total energy usage. Additionally, because biodegradable packaging created from agricultural waste decomposes naturally and emits less greenhouse gases than conventional plastics, it can reduce carbon emissions. Conventional plastics are persistent and harm the environment over time. Alternatives that are biodegradable breakdown more quickly, offering a solution to the issue of microplastics that can damage ecosystems and jeopardize marine life. Furthermore, these materials offer a more environmentally friendly alternative to non-biodegradable ones by keeping waste out of landfills. Consumer Preferences and Industry Developments: People are actively searching for products with a lower environmental effect as their knowledge of the environment rises. Biodegradable packaging satisfies these demands and is committed to sustainability, appealing to growing environmentally conscious consumers. Companies that use biodegradable packaging stand to gain from consumers' increasing desire for environmentally friendly and sustainable goods in a market where sustainability trends are driving change. Furthermore, the relevance of biodegradable packaging is highlighted by the need to comply with evolving regulations and industry standards as governments and organizations globally encourage sustainable habits. At a time when environmental sustainability is crucial, companies that prioritize biodegradable packaging enhance their brand image by exhibiting corporate responsibility and cultivating customer loyalty **[58]**, **[59]**.

End-of-Life Considerations

The final decisions taken regarding the disposal of packing materials have a big influence on how environmentally friendly they are overall. This section examines several end-of-life situations for packaging

materials, focusing on materials derived from agricultural waste. It offers a comprehensive summary of potential results, looks at the ramifications of dumping trash in a landfill, looks at composting as a workable end-of-life solution, and evaluates the potential for recycling agricultural waste. By comparing the traditional linear model—which is defined as a linear process from production to use and disposal, with the circular model, which integrates end-of-life concerns into a comprehensive framework, this section elucidates the implications for environmental sustainability. The circular economy strongly focuses on recycling, composting, and reuse. This section emphasizes the need to maintain nutrient cycles, lower the long-term environmental impact through natural decomposition, and dispose of agricultural wastes responsibly. Landfill disposal of packaging waste presents several challenges, particularly about non-biodegradable materials. Prolonged decomposition rates cause trash to accumulate, which in turn causes issues including methane emissions, soil contamination, and aesthetic degradation. The detrimental impacts of landfill dumping on ecosystems and the ensuing environmental ramifications are discussed in this section. Toxic substances are discharged into the soil and water beyond what is immediately noticeable, negatively impacting ecosystems. Remaining materials in landfills obstruct natural processes, which is bad for biodiversity and ecosystem health overall **[6]**, **[53]**, **[59]**, **[61]**.

Composting as an End-of-Life Option

One essential component of sustainable waste management techniques is the examination of the compatibility of biodegradable packaging materials, especially those derived from agricultural leftovers, with composting processes. Because these materials are naturally biodegradable, they easily fit into composting systems and break down to produce nutrient-rich compost. This end-of-life solution meets the waste reduction need and significantly contributes to soil enrichment, promoting increased agricultural output. Biodegradable packaging and composting complement each other for goals beyond waste minimization. Composting is an end-of-life alternative that is very beneficial to the environment. The most notable of these advantages is the enhanced soil structure that results in increased water-retention capabilities. This composting component is consistent with farming practices that use less water. Additionally, composting lessens reliance on synthetic fertilizers, consistent with ecologically friendly farming methods. In essence, properly selecting composting as a biodegradable packaging material end-of-life strategy is a comprehensive and sustainable method with positive ecological consequences **[6]**, **[53]**, **[59]**, **[61]**.

Recycling Potential of Agricultural Byproducts

The thorough assessment of the potential for recycling that comes with agricultural leftovers in the context of packaging materials requires careful consideration of opportunities and obstacles. This talk explores the unique characteristics of these materials in detail and considers how recycling procedures may be carefully designed to meet the goals of waste reduction and resource recovery. Agricultural byproducts must be seamlessly incorporated into closed-loop systems to maximize recycling possibilities. This strategic strategy requires packaging materials to be intentionally designed with recycling at the forefront, which promotes cooperative collaboration across the supply chain.

Active involvement in recycling initiatives is necessary to guarantee the efficient reintroduction of materials into the production cycle and to show that one is firmly committed to the circular economy's tenets. The topic of cutting-edge recycling systems created particularly for agricultural trash is explored in the next section of the talk. Only two examples of how ongoing research and development initiatives greatly increase the sustainability factor of recycling operations are innovations in material recovery facilities and processing methods. These innovations show a commitment to the packaging industry's responsible and efficient treatment of agricultural byproducts, even beyond their ability to boost productivity. By deliberately concentrating on challenges, this scientific study elucidates recycling agricultural wastes as a critical step on the road towards more sustainable, scientifically informed, and ecologically sensitive packaging paradigms **[5]**, **[6]**, **[48]**, **[53]**, **[62]**, **[63]**.

Future Prospects and Innovations

Future technological developments, shifting consumer preferences, and governmental initiatives might create a completely new dynamic environment for sustainable packaging. Using agricultural leftovers in packaging materials will significantly shape this future by fostering environmental sustainability and resilience. innovative Materials: More study and development should lead to the creation of innovative materials derived from agricultural waste. They might be more effective, durable, and adaptable for various packaging applications than current materials. Innovative materials include improved water resistance, barrier properties, and flexibility. Integration of Packaging Materials into Circular Economy Models: This integration is projected to gain momentum. Improved recycling technologies, closed-loop systems, and circular supply networks are necessary for a sustainable future. Since agricultural byproducts are one of the main forces behind circularity, they will be crucial to cutting waste and maximizing resource efficiency. Consumer Involvement and Instruction: This will be

given greater consideration from a forward-looking perspective. Businesses and brands will invest financial resources in transparent communication to educate consumers about the environmental impact of packaging choices. Rising environmental consciousness is anticipated to drive demand for eco-friendly products and change consumer behavior. International Collaboration for Sustainability: International cooperation is necessary because environmental challenges are interrelated. An integrated strategy to lowering the ecological footprint of packaging materials is predicted to be shaped by international cooperation, agreements, and shared sustainability goals. Cooperation in research and sharing of knowledge will help create solutions that apply to everybody **[6]**, **[15]**, **[16]**, **[17]**, **[18]**.

Conclusion

In conclusion, utilizing agricultural waste as a starting point for packaging is a fantastic move toward sustainable methods. Analyzing these materials' environmental benefits, biodegradability, and end-of-life concerns paints a complete picture of how they will impact packaging as we advance. The importance of biodegradable packaging in reducing plastic pollution and environmental effect underscores the necessity for its widespread use. Recycling and composting at end-of-life scenarios emphasize the significance of appropriate waste management practices. Future changes are expected to be exciting and driven by regulations that encourage ethical conduct, technological advancements, and consumer demand for sustainability. The use of agricultural waste in packaging materials is a step toward a more resilient and sustainable future, especially when businesses, sectors of the economy, and consumers adopt the concepts of a circular economy. This shift to sustainable packaging will need the collaboration of all supply chain participants, continued research and development, and a shared commitment to environmental stewardship. By embracing these concepts, we pave the way for a time when packaging is both a protective barrier for commodities and an advocate for the environment's health for coming generations.

REFERENCES

1. Uthpala, T. G. G., Navaratne, S. B., Thibbotuwawa, A., Jayasinghe, M., & Wanigasinghe, R. S. (2021). Agricultural By-Product Proteins: Emerging Source for Packaging Applications. In International Journal of Food Science and Agriculture (Vol. 5, Issue 3, pp. 355–362). Hill Publishing Group Inc. https://doi.org/10.26855/ijfsa.2021.09.003
2. Das Purkayastha, M., Manhar, A. K., Das, V. K., Borah, A., Mandal, M., Thakur, A. J., & Mahanta, C. L. (2014). Antioxidative, Hemocompatible, Fluorescent Carbon Nanodots from an “End-of-Pipe” Agricultural Waste: Exploring Its New Horizon in the Food-Packaging Domain. In Journal of Agricultural and Food Chemistry (Vol. 62, Issue 20, pp. 4509–4520). American Chemical Society (ACS). https://doi.org/10.1021/jf500138f
3. Singh, A. K., Kim, J. Y., & Lee, Y. S. (2022). Phenolic Compounds in Active Packaging and Edible Films/Coatings: Natural Bioactive Molecules and Novel Packaging Ingredients. In Molecules (Vol. 27, Issue 21, p. 7513). MDPI AG. https://doi.org/10.3390/molecules27217513
4. Lauer, M. K., & Smith, R. C. (2020). Recent advances in starch-based films toward food packaging applications: Physicochemical, mechanical, and functional properties. In Comprehensive Reviews in Food Science and Food Safety (Vol. 19, Issue 6, pp. 3031–3083). Wiley. https://doi.org/10.1111/1541-4337.12627
5. Soleimanian, Y., Sanou, I., Turgeon, S. L., Canizares, D., & Khalloufi, S. (2021). Natural plant fibers obtained from agricultural residue used as an ingredient in food matrixes or packaging materials: A review. In Comprehensive Reviews in Food Science and Food Safety (Vol. 21, Issue 1, pp. 371–415). Wiley. https://doi.org/10.1111/1541-4337.12875
6. Hamed, I., Jakobsen, A. N., & Lerfall, J. (2021). Sustainable edible packaging systems based on active compounds from food processing byproducts: A review. In Comprehensive Reviews in Food Science and Food Safety (Vol. 21, Issue 1, pp. 198–226). Wiley. https://doi.org/10.1111/1541-4337.12870
7. Kozlu, A., & Elmacı, Y. (2021). Aktif Bileşenler ile Zenginleştirilmiş Yenilebilir Film ve Kaplamaların Taze ve İşlem Görmüş Et ve Balık Ürünlerine Uygulanması. In Turkish Journal of Agriculture - Food Science and Technology (Vol. 9, Issue 5, pp. 868–877). Turkish Science and Technology Publishing (TURSTEP). https://doi.org/10.24925/turjaf.v9i5.868-877.4125
8. Perera, K. Y., Sharma, S., Pradhan, D., Jaiswal, A. K., & Jaiswal, S. (2021). Seaweed Polysaccharide in Food Contact Materials (Active Packaging, Intelligent Packaging, Edible Films, and Coatings). In Foods (Vol. 10, Issue 9, p. 2088). MDPI AG. https://doi.org/10.3390/foods10092088
9. Yong, H., & Liu, J. (2021). Active packaging films and edible coatings based on polyphenol-rich propolis extract: A review. In Comprehensive Reviews in Food Science and Food Safety (Vol. 20, Issue 2, pp. 2106–2145). Wiley. https://doi.org/10.1111/1541-4337.12697

10. Ababor, S., Tamiru, M., Alkhtib, A., Wamatu, J., Kuyu, C. G., Teka, T. A., Terefe, L. A., & Burton, E. (2023). The Use of Biologically Converted Agricultural Byproducts in Chicken Nutrition. In Sustainability (Vol. 15, Issue 19, p. 14562). MDPI AG. https://doi.org/10.3390/su151914562
11. Brandt-Williams, S., Pillet, G. (2003). Fertilizer co-products as agricultural emternalities Quantifying Environmental Services Used in Production of Food. In Biennial Emergy Analysis Conference (pp. 1-16). University of Florida.
12. Joachimiak-Lechman, K., Selech, J., & Kasprzak, J. (2018). Eco-efficiency analysis of an innovative packaging production: case study. In Clean Technologies and Environmental Policy (Vol. 21, Issue 2, pp. 339–350). Springer Science and Business Media LLC. https://doi.org/10.1007/s10098-018-1639-7
13. Rodríguez, M. del P., Vázquez-Vélez, E., Martinez, H., & Torres-Islas, A. (2023). Life Cycle Analysis of a Novel Process from the Automotive Industry in Mexico for Recycling Nylon 6,6 into Polymeric Coatings. In Sustainability (Vol. 15, Issue 12, p. 9810). MDPI AG. https://doi.org/10.3390/su15129810
14. Mandal, P.K.; Choi, K.; Min, S.; Lee, C.-H. Application of Nanotechnology in Food Packaging: An Overview. 2009.
15. Meherishi, L., Narayana, S. A., & Ranjani, K. S. (2019). Sustainable packaging for supply chain management in the circular economy: A review. In Journal of Cleaner Production (Vol. 237, p. 117582). Elsevier BV. https://doi.org/10.1016/j.jclepro.2019.07.057
16. Singh, A. K., Itkor, P., Lee, M., Shin, J., & Lee, Y. S. (2022). Promoting sustainable packaging applications in the circular economy by exploring and advancing molded pulp materials for food products: a review. In Critical Reviews in Food Science and Nutrition (Vol. 63, Issue 32, pp. 11010–11025). Informa UK Limited. https://doi.org/10.1080/10408398.2022.2088686
17. Iacovidou, E., & Gerassimidou, S. (2018). Sustainable Packaging and the Circular Economy: An EU Perspective. In Reference Module in Food Science. Elsevier. https://doi.org/10.1016/b978-0-08-100596-5.22488-8
18. Circular economy for sustainable packaging. Food Science and Technology 2021. A division of Northern Technologies International Corporation.
19. A Comprehensive Study of the Market Determinants Impacting Sustainable Packaging. International Journal of Sustainable Economies Management 2021.
20. Lekesiztürk, D., & Oflaç, B. S. (2022). Investigating sustainable packaging practices: a framework approach. In Present Environment and Sustainable Development (pp. 171–186). Editura Universitatii Alexandru Ioan Cuza din Iasi. https://doi.org/10.47743/pesd2022161013
21. Poovarodom, N., Ponnak, C., & Manatphrom, N. (2011). Comparative Carbon Footprint of Packaging Systems for Tuna Products. In Packaging Technology and Science (Vol. 25, Issue 5, pp. 249–257). Wiley. https://doi.org/10.1002/pts.975
22. Varžinskas, V., & Markevičiūtė, Z. (2020). Sustainable Food Packaging: Materials and Waste Management Solutions. In Environmental Research, Engineering and Management (Vol. 76, Issue 3, pp. 154–164). Kaunas University of Technology (KTU). https://doi.org/10.5755/j01.erem.76.3.27511
23. Santi, R., Elegir, G., & Del Curto, B. (2020). Designing for sustainable behaviour practices in consumers: a case study on compostable materials for packaging. In Proceedings of the Design Society: DESIGN Conference (Vol. 1, pp. 1647–1656). Cambridge University Press (CUP). https://doi.org/10.1017/dsd.2020.150
24. Farias, N. C., Devine, D. M., Fournet, M. B., Pezzoli, R. (2020). Development of sustainable flexible packaging solutions using novel biodegradable and compostable polymer blends. In Bio Innovation a Circular Economy for Plastics (1 p.). AIMPLAS – Instituto.
25. Bianchi, E., Guidotti, G., Soccio, M., Siracusa, V., Gazzano, M., Salatelli, E., & Lotti, N. (2023). Biobased and Compostable Multiblock Copolymer of Polylactic acid) Containing 2,5-Furandicarboxylic Acid for Sustainable Food Packaging: The Role of Parent Homopolymers in the Composting Kinetics and Mechanism. In Biomacromolecules (Vol. 24, Issue 5, pp. 2356–2368). American Chemical Society (ACS). https://doi.org/10.1021/acs.biomac.3c00216
26. Roy, R. (2022). Sustainable Packaging Practices Across Various Sectors: Some Innovative Initiatives Under the Spotlight. In Operations and Supply Chain Management: An International Journal (pp. 461–473). OSCM Forum. https://doi.org/10.31387/oscm0510359
27. Jalalipour, H., Ahmadi, M., Jaafarzadeh, N., Morscheck, G., Narra, S., & Nelles, M. (2021). Provision of extended producer responsibility system for products packaging: A case study of Iran. In Waste Management & Research: The Journal for a Sustainable Circular Economy (Vol. 39, Issue 10, pp. 1291–1301). SAGE Publications. https://doi.org/10.1177/0734242x211040327

28. Pruess, J. T. (2023). Unraveling the complexity of extended producer responsibility policy mix design, implementation, and transfer dynamics in the European Union. In Journal of Industrial Ecology (Vol. 27, Issue 6, pp. 1500–1520). Wiley. https://doi.org/10.1111/jiec.13429

29. Lorang, S., Yang, Z., Zhang, H., Lü, F., & He, P. (2022). Achievements and policy trends of extended producer responsibility for plastic packaging waste in Europe. In Waste Disposal & Sustainable Energy (Vol. 4, Issue 2, pp. 91–103). Springer Science and Business Media LLC. https://doi.org/10.1007/s42768-022-00098-z

30. Müller, G., Hanecker, E., Blasius, K., Seidemann, C., Tempel, L., Sadocco, P., Pozo, B. F., Boulougouris, G., Lozo, B., Jamnicki, S., & Bobu, E. (2012). End-of-life Solutions for Fibre and Bio-based Packaging Materials in Europe. In Packaging Technology and Science (Vol. 27, Issue 1, pp. 1–15). Wiley. https://doi.org/10.1002/pts.2006

31. Sastre, R. M., Zeni, C. F., De Paula, I. C., Hauser, G., & Da Conceição, S. (2023). The use of organic residues to develop packaging: tests in molded pulp. In Proceedings of the Design Society (Vol. 3, pp. 3543–3550). Cambridge University Press (CUP). https://doi.org/10.1017/pds.2023.355

32. Matheus, J. R. V., Dalsasso, R. R., Rebelatto, E. A., Andrade, K. S., Andrade, L. M. de, Andrade, C. J. de, Monteiro, A. R., & Fai, A. E. C. (2023). Biopolymers as green-based food packaging materials: A focus on modified and unmodified starch-based films. In Comprehensive Reviews in Food Science and Food Safety (Vol. 22, Issue 2, pp. 1148–1183). Wiley. https://doi.org/10.1111/1541-4337.13107

33. Khan, A., & Tandon, P. (2018). Realizing the End-of-life Considerations in the Design of Food Packaging. In Journal of Packaging Technology and Research (Vol. 2, Issue 3, pp. 251–263). Springer Science and Business Media LLC. https://doi.org/10.1007/s41783-018-0041-6

34. Holt, G. A., Chow, P., Wanjura, J. D., Pelletier, M. G., & Wedegaertner, T. C. (2014). Evaluation of thermal treatments to improve physical and mechanical properties of bio-composites made from cotton byproducts and other agricultural fibers. In Industrial Crops and Products (Vol. 52, pp. 627–632). Elsevier BV. https://doi.org/10.1016/j.indcrop.2013.11.003

35. Ludueña, L. N., Vecchio, A., Stefani, P. M., & Alvarez, V. A. (2013). Extraction of cellulose nanowhiskers from natural fibers and agricultural byproducts. In Fibers and Polymers (Vol. 14, Issue 7, pp. 1118–1127). Springer Science and Business Media LLC. https://doi.org/10.1007/s12221-013-1118-z

36. Boarino, A., Schreier, A., Leterrier, Y., & Klok, H.-A. (2022). Uniformly Dispersed Poly(lactic acid)-Grafted Lignin Nanoparticles Enhance Antioxidant Activity and UV-Barrier Properties of Poly(lactic acid) Packaging Films. In ACS Applied Polymer Materials (Vol. 4, Issue 7, pp. 4808–4817). American Chemical Society (ACS). https://doi.org/10.1021/acsapm.2c00420

37. Threepopnatkul, P., Wongsuton, K., Jaiaue, C., Rakkietwinai, N., Stittatrakul, A., & Kulsetthanchalee, C. (2020). Effect of Zeolite on Mechanical and Barrier Properties of PBAT Films for Life Extension of Agricultural Products. In Key Engineering Materials (Vol. 861, pp. 176–181). Trans Tech Publications, Ltd. https://doi.org/10.4028/www.scientific.net/kem.861.176

38. Peixoto, A. M., Petronilho, S., Domingues, M. R., Nunes, F. M., Lopes, J., Pettersen, M. K., Grøvlen, M. S., Wetterhus, E. M., Gonçalves, I., & Coimbra, M. A. (2023). Potato Chips Byproducts as Feedstocks for Developing Active Starch-Based Films with Potential for Cheese Packaging. In Foods (Vol. 12, Issue 6, p. 1167). MDPI AG. https://doi.org/10.3390/foods12061167

39. Jiang, Y., Yan, C., Wang, K., Shi, D., Liu, Z., & Yang, M. (2019). Super-Toughed PLA Blown Film with Enhanced Gas Barrier Property Available for Packaging and Agricultural Applications. In Materials (Vol. 12, Issue 10, p. 1663). MDPI AG. https://doi.org/10.3390/ma12101663

40. Nakagaito, A. N., & Takagi, H. (2018). Easy cellulose nanofiber extraction from residue of agricultural crops. In International Journal of Modern Physics B (Vol. 32, Issue 19, p. 1840080). World Scientific Pub Co Pte Lt. https://doi.org/10.1142/s0217979218400805

41. Sadovoy, V. V., Selimov, M. A., Shchedrina, T. V.,& Nagdalian, A. A. (2017). Nutritional supplement for control of diabetes. In Journal of Excipients and Food Chemicals (Vol. 8, Issue 2, pp. 31–38). IPEC-Americas.

42. Gulzar, S., Tagrida, M., Prodpran, T., Li, L., & Benjakul, S. (2023). Packaging films based on biopolymers from seafood processing wastes: Preparation, properties, and their applications for shelf-life extension of seafoods—A comprehensive review. In Comprehensive Reviews in Food Science and Food Safety (Vol. 22, Issue 6, pp. 4451–4483). Wiley. https://doi.org/10.1111/1541-4337.13230

43. Debnath, M., Sarder, R., Pal, L., & Hubbe, M. A. (2022). Molded pulp products for sustainable packaging: Production rate challenges and product opportunities. In BioResources (Vol. 17, Issue 2, pp. 3810–3870). BioResources. https://doi.org/10.15376/biores.17.2.debnath

44. Cvek, M., Paul, U. C., Zia, J., Mancini, G., Sedlarik, V., & Athanassiou, A. (2022). Biodegradable Films of PLA/PPC and Curcumin as Packaging Materials and Smart Indicators of Food Spoilage. In ACS Applied Materials & Interfaces (Vol. 14, Issue 12, pp. 14654–14667). American Chemical Society (ACS). https://doi.org/10.1021/acsami.2c02181

45. Lee, K. H., Chun, Y., Jang, Y. W., Lee, S. K., Kim, H. R., Lee, J. H., Kim, S. W., Park, C., & Yoo, H. Y. (2020). Fabrication of Functional Bioelastomer for Food Packaging from Aronia (Aronia melanocarpa) Juice Processing By-Products. In Foods (Vol. 9, Issue 11, p. 1565). MDPI AG. https://doi.org/10.3390/foods9111565

46. Simonini, E. (2021). Bio-plastics and technologies for eco-sustainable packaging. Amsdottorato. https://doi.org/10.48676/UNIBO/AMSDOTTORATO/9698

47. Nida, S., Moses, J. A., & Anandharamakrishnan, C. (2022). 3D Extrusion Printability of Sugarcane Bagasse Blended with Banana Peel for Prospective Food Packaging Applications. In Sugar Tech (Vol. 24, Issue 3, pp. 764–778). Springer Science and Business Media LLC. https://doi.org/10.1007/s12355-021-01095-y

48. Bacoup, F., Mahieu, A., Vitaud, A., Drone, P., Gattin, R., & Leblanc, N. (2019). Various methods of binding light agricultural byproducts. Academic Journal of Civil Engineering, Vol 37 No 2 (2019): Special Issue-ICBBM 2019. https://doi.org/10.26168/ICBBM2019.2

49. Choudhary, U., Kumar Bhinchhar, B., Kumar Paswan, V., Kharkwal, S., Prakash Yadav, S., & Singh, P. (2022). Utilization of Agro-Industrial Wastes as Edible Coating and Films for Food Packaging Materials. In A Glance at Food Processing Applications. IntechOpen. https://doi.org/10.5772/intechopen.99786

50. Robertson, G. L. (1992). Food Packaging: Principles and Practice. 676 p. Marcel Dekker Inc.

51. Mostafa, N. M. M. (2016). Digital Printing as an Alternative Printing System for Short Run Label Production. In مجلة التصميم الدولية (Vol. 6, Issue 1, pp. 151–157). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.12816/0036436

52. Bates, I., Plazonić, I., Dzimbeg-Malcic, V., Banić, D. (2017). Influence of straw pulp in printing substrate on stability of digital prints. In Proceedings of International Conference on Innovative Technologies IN-TECH 2017 (pp. 113–116). Tehnički fakultet Sveučilišta u Rijeci.

53. Nechai, A., Krvavych, A., & Konechna, R. (2021). Prospects for the use of plant materials in creating biodegradable eco-friendly packaging. In IOP Conference Series: Earth and Environmental Science (Vol. 915, Issue 1, p. 012003). IOP Publishing. https://doi.org/10.1088/1755-1315/915/1/012003

54. Chmyrev, V. P., Polutina, T. N., Moiseev, A. V., Oboturova, N. P., Kolesnikov, R. O., Baklanov, I. S., & Baklanova, O. A. (2022). Analysis of the state of food safety in the Russian federation. In Journal of Hygienic Engineering and Design (Vol. 38, pp. 111–118). Consulting and Training Center KEY.

55. Šuput, D., Popović, S., Ugarković, J., & Hromiš, N. (2022). Application of life cycle assessment in the packaging sector for the environmental assessment of polymer and biopolymer based materials: A review. In Journal on Processing and Energy in Agriculture (Vol. 26, Issue 2, pp. 75–78). Centre for Evaluation in Education and Science (CEON/CEES). https://doi.org/10.5937/jpea26-39342

56. Ndizeye, Y., Dongjie, N., Umuhoza, M., Head, S., Mulwa, F., & Collins, M. (2021). Integrated LCA and OPEN LCA-CML baseline analysis on environmental impact associated with the plastic packaging waste management system of Rubavu city Rwanda. In International Journal Of Scientific Advances (Vol. 2, Issue 2). International Journal of Scientific Advances. https://doi.org/10.51542/ijscia.v2i2.13

57. Desole, M. P., Aversa, C., Barletta, M., Gisario, A., & Vosooghnia, A. (2021). Life cycle assessment (LCA) of PET and PLA bottles for the packaging of fresh pasteurised milk: The role of the manufacturing process and the disposal scenario. In Packaging Technology and Science (Vol. 35, Issue 2, pp. 135–152). Wiley. https://doi.org/10.1002/pts.2615

58. Kakadellis, S., Lee, P.-H., & Harris, Z. M. (2022). Two Birds with One Stone: Bioplastics and Food Waste Anaerobic Co-Digestion. In Environments (Vol. 9, Issue 1, p. 9). MDPI AG. https://doi.org/10.3390/environments9010009

59. Chandrajith, A. V. (2022). Verification Study of Food Packaging Materials Recoverable Through Composting and Biodegradation. In International Journal of Conformity Assessment (Vol. 1, Issue 1). International Accreditation Service. https://doi.org/10.55459/ijca/v1i1/av

60. Gvozdenko, A. A., Siddiqui, S. A., Blinov, A. V., Golik, A. B., Nagdalian, A. A., Maglakelidze, D. G., Statsenko, E. N., Pirogov, M. A., Blinova, A. A., Sizonenko, M. N., Simonov, A. N., Zhukov, R. B., Kolesnikov, R. O., & Ibrahim, S. A. (2022). Synthesis of CuO nanoparticles stabilized with gelatin for potential use in food packaging applications. In Scientific Reports (Vol. 12, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-022-16878-w

61. Itävaara, M., Vikman, M., & Venelampi, O. (1997). Windrow Composting Of Biodegradable Packaging Materials. In Compost Science & Utilization (Vol. 5, Issue 2, pp. 84–92). Informa UK Limited. https://doi.org/10.1080/1065657x.1997.10701877
62. Loffredo, E. (2022). Recent Advances on Innovative Materials from Biowaste Recycling for the Removal of Environmental Estrogens from Water and Soil. In Materials (Vol. 15, Issue 5, p. 1894). MDPI AG. https://doi.org/10.3390/ma15051894
63. Siddiqui, S. A., Zannou, O., Bahmid, N. A., Fidan, H., Alamou, A.-F., Nagdalian, A. A., Hassoun, A., Fernando, I., Ibrahim, S. A., & Arsyad, M. (2022). Consumer behavior towards nanopackaging - A new trend in the food industry. In Future Foods (Vol. 6, p. 100191). Elsevier BV. https://doi.org/10.1016/j.fufo.2022.100191

Funds:
This research received no external funding.
Acknowledgments:
-
Conflict of Interest:
The authors declare no conflict of interest.
Ethical Statement:
This article does not contain any studies that would require an ethical statement.
Contact Address:

Natalya Oboturova, Department of Food Technology and Engineering, Faculty of Food Engineering and Biotechnology, North Caucasus Federal University, Pushkin Str., 1, 355017, Stavropol, Russia,
Tel.: +79614700778
E-mail: noboturova@ncfu.ru
ORCID: https://orcid.org/0000-0001-6204-607X

***Sergey Povetkin**, Laboratory of Food and Industrial Biotechnology, Faculty of Food Engineering and Biotechnology, North Caucasus Federal University, Pushkina Street 1, 355017, Stavropol, Russia,
Tel.: +7-918-350-08-89
E-mail: serenkiy7@yandex.ru
ORCID: https://orcid.org/0000-0002-8293-3984

Natalya Nikulnikova, Department of Food Technology and Engineering, Faculty of Food Engineering and Biotechnology, North Caucasus Federal University, Pushkin Str., 1, 355017, Stavropol, Russia,
Tel.: +79187607409
E-mail: nnikulnilova@ncfu.ru
ORCID: https://orcid.org/0000-0002-2700-1690

Natalia Lazareva, North Caucasus Federal University, Institute of Economics and Management, Department of Economic Security and Auditing, Pushkina Street, 1, 355017, Stavropol, Russia,
Tel.: +79624592272
E-mail: nlazareva@ncfu.ru
ORCID: https://orcid.org/0000-0002-5490-9936

Anna Klopova, Don State Agrarian University, Krivoshlykova Street 24, 346493, Persyanovsky village, Russia,
Tel.: +7-988-118-31-44
E-mail: anna-gorbatovskaya@mail.ru
ORCID: https://orcid.org/0000-0001-5201-7128

Nikita Lyubchanskiy, North Caucasus Federal University, Faculty of Food Engineering and Biotechnology, Department of Food Technology and Engineering, Kulakova Street, 2, 355017, Stavropol, Russia,
Tel.: +79187615190
E-mail: m.a.d.999@inbox.ru
ORCID: https://orcid.org/0000-0002-0989-4560

Ekaterina Sukhanova, North Caucasus Federal University, Faculty of Food Engineering and Biotechnology, Department of Food Technology and Engineering, Kulakova Street, 2, 355017, Stavropol, Russia,
Tel.: +79880989489
E-mail: lady.suha@yandex.ru
ORCID: https://orcid.org/0000-0003-2788-3897

Nadezhda Lebedeva, North Caucasus Federal Agrarian Centre, Department of Breeding and Primary Seed Production of Forage and Medicinal Herbs, Nikonova Street, 49, 356241, Mikhaylovsk, Russia,
Tel.: +79614426343
E-mail: nadezhda8358@gmail.com
ORCID: https://orcid.org/0000-0002-3113-3274

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 10.10.2023
Revised: 23.1.2024
Accepted: 2.2.2024
Published: 28.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 207-222
https://doi.org/10.5219/1930
ISSN: 1337-0960 online
www.potravinarstvo.com

Biometric analysis of food products of hybrid hypoophthalmichthys (*Hypophthalmichthys* spp.) to determine their nutritional value and use in the food industry

Alina Makarenko, Nataliia Rudyk-Leuska, Ruslan Kononenko, Melaniia Khyzhniak, Iryna Kononenko, Ganna Kotovska, Petro Shevchenko, Mykhailo Leuskyi

ABSTRACT

This scientific work describes research, the purpose of which was to study the spectrum of nutrition and the composition of the food lump, as studies aimed at assessing specific weight (%) of essential nutrients (glycogen, proteins, and lipids) in particular organs and tissues of different size and mass groups of the hybrid of Silver carp and Bighead carp in ponds and reservoirs in different periods of the year. In 2018, and 2019, the juveniles of the hybrid of Silver carp and Bighead carp in ponds and reservoirs mainly consumed phytoplankton organisms (from 30 to 90% by mass), among which green, diatom, and euglena algae predominated by mass in the food group. Zooplankton occupied an insignificant place in fish nutrition (up to 5%), even though its quantity and biomass were sufficient in reservoirs. The hybrid of Silver carp and Bighead carp does not hurt zooplankton communities, so it can be included in the stocking volume of Silver carp. The feeding spectrum and rations of different groups of Silver carp and Bighead carp in ponds and reservoirs had a well-defined seasonal character related to the composition of feed objects. In all size and mass groups of the hybrid of silver and bighead carp from ponds and reservoirs in 2018, and 2019, mostly satisfactory values of general metabolism indicators were found – glycogen, proteins, and lipids in the liver, gills, and muscles of fish. The difference found in the availability of essential nutrients in the body of the studied fish indicates a change in the intensity and direction of their metabolic processes.

Keywords: nutrition value, carp, phytoplankton, zooplankton, liver, white skeletal muscle

INTRODUCTION

Fish and fish products are important for ensuring the normal development and vital activity of the human body, as they are a source of necessary vitamins, macro-, and microelements, and complete proteins of animal origin **[1]**, **[2]**, **[3]**.

One of the promising objects of cultivation in reservoirs of various types is a hybrid of Silver carp and Bighead carp because it has a fast rate of mass gain until the end of the cultivation period. High plasticity in the choice of food objects (phytoplankton, zooplankton, detritus, and in ponds, in addition, the remains of the dusty fraction of carp compound feed) allows hybrids to have significantly less tension in competition, compared to other species **[4]**, **[5]**, **[6]**.

The main factor affecting the growth of fish is providing it with nutritious food in sufficient quantity with effective use. The presence of natural food in the diet of fish is the main condition for normal growth and development. It should be noted that a lot of natural protein is necessary for fish growth. After all, the protein component is the building block for a living organism. The need for fish in a natural feed base, which includes animal protein, is 30% higher than the permissible need for this component of farm animals. There is also a great

need for fish in vitamin and mineral nutrition, formed by their natural habitat. The fodder base of reservoirs should contain the necessary percentage of natural biologically active substances (amino acids, enzymes). Only in this case, can you count on the active growth of fish and an increase in the quality indicators of individuals. When the content of phytoplankton, zooplankton, and zoobenthos in the reservoir is insufficient, the following is often observed: slow growth of individuals; increase in fish departure; violation of metabolic processes in the organisms of individuals; fish diseases and a decrease in the quality of produced fish products; non-compliance of the grown product with modern environmental requirements. According to many authors, the minimum provision of natural food for fish should be at least 25-30%, and for young fish – up to 50% **[7]**.

One of the main components in the body of fish is protein, and its content depends on the rate of linear growth. In addition, in fish, proteins can be used as alternative energy sources, which, if necessary (with the participation of aminotransferases) play a role in the processes of adaptation to the negative factors of the environment **[8]**. Studies of internal organs, particularly the liver, provide objective information that can be used to determine the general physiological state of the body **[9]**.

The level of lipid accumulation is directly dependent on the fatness of fish, and the direction of lipid metabolism varies depending on the stage of ontogenesis, sex, and phase of the reproductive cycle **[10]**. Lipids are the basis for all intracellular membranes and play a significant role in cell metabolism **[11]**.

The glycogen content in the fish's liver tissues may decrease under the influence of pollution or deficiency of water-soluble oxygen caused by significant energy expenditure to overcome stress **[12]**. Under adverse conditions, detoxification and antioxidant protection systems change in fish tissues. Stimulation of detoxification mechanisms requires additional energy expenditure **[13]**, usually accompanied by suppression of energy metabolism. However, in the end, the effective work of regulatory and coordinating mechanisms ensures the organism's adaptation to changing conditions **[14]**.

Carps are a valuable industrial fish with high taste qualities. The meat is tender and tasty and belongs to the medium-fat group **[15]**, **[16]**, **[17]**.

Due to the characteristics of carp nutrition, the meat contains amino acids and polyunsaturated fatty acids of the omega-3 and omega-6 groups. Regular consumption, they help prevent the development of malignant tumors, and nervous disorders, improve the functioning of the heart, and strengthen the walls of blood vessels, to lower cholesterol and blood pressure in patients with hypertension **[18]**, **[19]**.

Scientific Hypothesis

The obtained data on fish nutrition can serve as an assessment of the supply of the body with food components that are the main, i.e., their favorite food, a characteristic of fish diets and can provide a deeper insight into the use of feed resources by fish in reservoirs and the influence of fish on their diversity and population density, and in the final result in general on the functional state of the ecosystem.

The difference in the availability of essential nutrients (glycogen, proteins, and lipids) in the body of the silver and bighead carp hybrid may indicate a change in the intensity and direction of their metabolic processes. At the same time, among relevant research, this direction remains insufficiently studied. However, earlier works were widely carried out, which included the collection of ichthyological material with the subsequent study of fish nutrition and the chemical composition of their organs and tissues.

MATERIAL AND METHODOLOGY

The research was conducted in the spring, summer, and autumn periods from 2018 to 2019 in ponds based on the training-research-production laboratory of fish farming of the National University of Life and Environmental Sciences (TRPLF NULES of Ukraine) of Ukraine, village Nemishayevo, Kyiv region (zone Polissya); State Enterprise "Experimental Farm" Nyvka " of the Institute of Fisheries of the National Academy of Agrarian Sciences (SEEF "Nyvka" IF NAAS) of Ukraine, Kyiv is located on the border of the zones (it is on the river Nivka that the Forest-Steppe is divided -to the south and Polissya -to the north); Bila Tserkva Experimental Hydrobiological Station of the Institute of Hydrobiology of the National Academy of Sciences (BEHS IHB NAS) of Ukraine, Bila Tserkva (Forest-steppe zone), Kosiv, Kyiv region (Forest-steppe zone) and Velykoburlutsky, Kharkiv region (Forest-steppe zone) reservoirs.

Samples

The collection of ichthyological material was carried out during the stocking and catching of fish in ponds and reservoirs. The material for the study was: young-of-the-year, biennials, and triennials of the hybrid of Silver carp and Bighead carp (Figure 1, 2, 3).

Figure 1 A young-of-the-year hybrid of Silver carp and Bighead carp, which was caught in the spring from the wintering pond No. 2 of the NNVLR of the NULES of Ukraine.

Figure 2 Collection of ichthyological materials (biennials of the hybrid of Silver carp and Bighead carp) in the spring period during fishing of wintering pond No. 119 of the "Nyvka" DPDG of the IRG of the National Academy of Sciences of Ukraine.

Figure 3 Catching biennials of the hybrid of Silver carp and Bighead carp in the winter pond No. 119 DPDG "Nyvka" IRG of the National Academy of Sciences of Ukraine.

Chemicals

Formaldehyde (CH_2O, producer «Inter-Synthesis» Limited Liability Company, Ukraine, chemically pure for analysis).

Formalin (water solution formaldehyde, producer«Inter-Synthesis» Limited Liability Company, Ukraine).

Potassium hydroxide (KOH), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Anthrone ($C_{14}H_{10}O$), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Concentrated sulfuric acid (H_2SO_4), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Sodium hydroxide (NaOH), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Sodium carbonate (Na_2CO_3), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Potassium sodium tartaric acid ($KNaC_4H_4O_6x_4H_2O$), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Folin–Ciocalteu reagent (FC), (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).
Vanillin reagent (produced by "Inter-Synthesis" Limited Liability Company, Ukraine).

Animals, Plants and Biological Materials

75 specimens of the hybrid of Silver carp and Bighead carp of different sizes and mass groups (young-of-the-year, biennials, and triennials) caught from ponds and reservoirs were processed.

Instruments

Set of grids with a mesh step from 0.03 to 0.1 m («CrayFish» Limited Liability Company, Finland).
Electronic laboratory scales (TBE-0.15-0.001-a-2, «Inter-Synthesis» Limited Liability Company, Ukraine).
Technical electronic scales (BTHE-6-H1K-1, "Inter-Synthesis" Limited Liability Company, Ukraine).
Measuring tape (1.5 m, "Inter-Synthesis" Limited Liability Company, Ukraine).
Laboratory filter paper (0.6 x 0.52 m, "Laboratory equipment" Limited Liability Company, Ukraine).
Counting chamber of Najotta ("Laboratory equipment" Limited Liability Company, Ukraine).
Binocular microscope (XSP-139B LED Ulab, "Laboratory equipment" Limited Liability Company, Ukraine).
Bogorov counting chamber ("ADS-Lab" Limited Liability Company, Ukraine).
Stereoscopic microscope (MBS-9, producer "Laboratory equipment" Limited Liability Company, Ukraine).
Spectrophotometer Unico 280 UV/VIS(ALTALAB Limited Liability Company, Ukraine).

Laboratory Methods

Processing of ichthyological materials was performed according to standard methods generally accepted in ichthyology **[20]**.

Determination of the taxonomic composition of algae was carried out according to the determinants **[21]**.

Phytoplankton biomass was determined by the calculation-volume method **[21]**. In-house processing of samples was carried out by the conventional hydrobiology counting-weight method in the Bogorov counting chamber under a stereoscopic microscope MBS-9. Phytoplankton samples were examined in a special Najott counting chamber 1.10^{-6} m under a light microscope, all detected algae species were determined and counted at 0.1 m^3.

Zooplankton organisms were identified to the species using determinants **[21]**.

The content of total proteins in tissue samples was determined by the method of Lowry et al. **[22]**, and the content of total lipids was determined using a phosphorovaniline reagent **[23]**. The anthrone method determined glycogen content in fish tissues **[24]**.

Description of the Experiment

Sample preparation: During the study of carp, 5 fish of different weights and ages were taken from ponds and reservoirs at certain time intervals.

Number of samples analyzed: We analyzed 45 gastrointestinal tracts of fish caught from ponds, and 30 from reservoirs, to determine the content of food lumps.

135 samples of tissues and organs (liver, white muscles, and gill petals) were taken from the fish caught in the ponds, and 90 samples were taken from the reservoirs to determine the number of proteins, fats, and carbohydrates.

Number of repeated analyses: Experiments were repeated once in experimental ponds and reservoirs.

Number of experiment replication: The number of repetitions of each experiment to determine one value was 5 times.

Design of the experiment: The gastrointestinal tract was cut from the oesophagus to the anus, and the degree of filling of the gastrointestinal tract was determined in points. Weighed on an electronic scale, measured the length with a measuring tape, and removed the contents by fixing with 4% formalin (1 part of 40% formalin to 9 parts of water). The fixed material was dried on filter paper until the trace of moisture disappeared and weighed on a scale. The content was weighed, mixed in water, and processed as a normal plankton sample. The species composition, number, and biomass of intact phytoplankton cells and zooplankton organisms were determined in the intestines of the studied fish **[25]**. Food similarity indices were calculated separately for groups of food organisms **[25]**.

The content of total proteins in tissue samples was determined by the method of Lowry et al. **[22]**. Briefly, 0.1 g of tissue and organ was hydrolyzed for 1 hour in 10 mL of 10% NaOH at a temperature of 60 °C. To 0.1 mL of the hydrolysate was added to 10 mL of solution No. 3, and staining was carried out for 15 minutes. Then, the sample added 1.0 mL of Folin's reagent diluted 1:1 with distilled water. The staining was carried out for 30 minutes. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 720 nm against control. The amount of protein was set according to the calibration schedule. Solution No. 3 was prepared from solutions No. 1 and No. 2 in a ratio of 9:1. Solution No. 1 was prepared based on 0.1 n NaOH with the addition of 0.02 g Na_2CO_3 and 0.005 kg of potassium, and sodium tartaric acid. Solution No. 2 contained 1 g CuSO4 per 1 liter of distilled water. The content of total lipids was determined using a phosphorovaniline reagent. Briefly, 100 mg of tissue was hydrolyzed in 1.5 mL of concentrated sulfuric acid for 15 minutes. About, 0.1 mL of the hydrolysate was added with 3 mL of vanillin reagent (10 mmol L^{-1} of vanillin and 11.5 mmol L^{-1} of phosphoric acid). The solution was stained for 40 min. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 530 nm against control. The amount of lipid was set according to the calibration schedule. The content of glycogen was determined by the anthrone method. Briefly, 0.1 g of tissue was hydrolyzed for 1 hour in 3 mL of 30% KOH at a temperature of 100 °C, then 0.9 mL of distilled water and 3 mL of 0.2% anthrone were added to 0.1 mL of the hydrolysate. Then the sample was boiled at 100 °C for 10 minutes. The extinction of the solution was determined on a spectrophotometer Unico 280 UV/VIS at 620 nm against control. The amount of glycogen was established according to the calibration graph.

Statistical Analysis

The results were evaluated by standard methods using statistical software Statgraphics Centurion XVII (StatPoint, USA) – multifactor analysis of variance (MANOVA), LSD test. Statistical processing was performed in Microsoft Excel 2016 in combination with XLSTAT. The statistical reliability of the results of the research was provided by analyzing samples with the number of fish 5 specimens.

RESULTS AND DISCUSSION

Scientists have proven that to fully support the body, a person should consume fish and fish products in the amount of 20 kg **[26]**, but, in recent years, it has been established thanks to an independent survey of the population that people consume no more than 10 kg of fish per year **[27]**, which is half below the required consumption rate **[28]**.

In modern conditions of fish farming, when growing fish, they mainly use a polyculture of carp and herbivorous fish, which ensures a balanced consumption of the entire complex of feed organisms and the formation of maximum fish productivity **[29]**.

The use of herbivorous fish cannot be limited to growing them only in ponds, these species are no less promising for reservoirs and other reservoirs of complex purposes.

All life processes occurring in the body of fish **[30]** are closely related to the external environment and are under its direct influence **[31]**, **[32]**.

An important place in fish farming belongs to the biotic conditions of the growing environment **[33]**.

Among the main biotic factors of the environment, that determine the efficiency of fish farming, is the natural fodder base of reservoirs **[34]**, which in terms of nutrient content and amino acid composition significantly exceeds the nutritional value of artificial fodder **[35]**.

The availability of feed resources is one of the main factors in the formation of optimal (from the ecological and fishery point of view) qualitative and quantitative characteristics of ichthyofauna **[36]** and maintaining its high industrial stock **[37]**.

Like any other reservoirs, the functioning and productivity of reservoirs are determined by the variety of trophic levels **[38]**, the main role of which belongs to the autotrophic, which is formed due to phytoplankton **[39]**.

By assimilating solar radiation and turning it into organic matter in photosynthesis, it creates primary products, due to which other hydrophones (organisms living in water) exist through food chains **[40]**.

When examining the contents of the intestines of young-of-the-year fish caught in the spring of 2018 from wintering pond No.101 and biennial fish caught in the spring of 2019 from wintering pond No.119 of the "Nyvka" DPDG of the National Academy of Sciences of Ukraine, it was a partially mucous mass. The share of algae in the biomass was estimated to be 40-50%.

17 species of algae were registered in the intestines of young-of-the-year fish, of which diatoms (4 species) and green, mainly flagellated (from the genera *Chlamydomonas*, *Pandorina*) – only 9 species, which abundantly vegetated in the reservoir. At the same time, euglenoids were practically not observed in the intestines (except for individual houses of the genus *Trachelomonas*), possibly due to rapid destruction. The shares in the biomass of

the algal component of the contents of fish intestines were: diatoms – 40%, green – 55%, others – 5%, while the content in ponds was: diatoms – 58%, and only green – 19% (Figure 4).

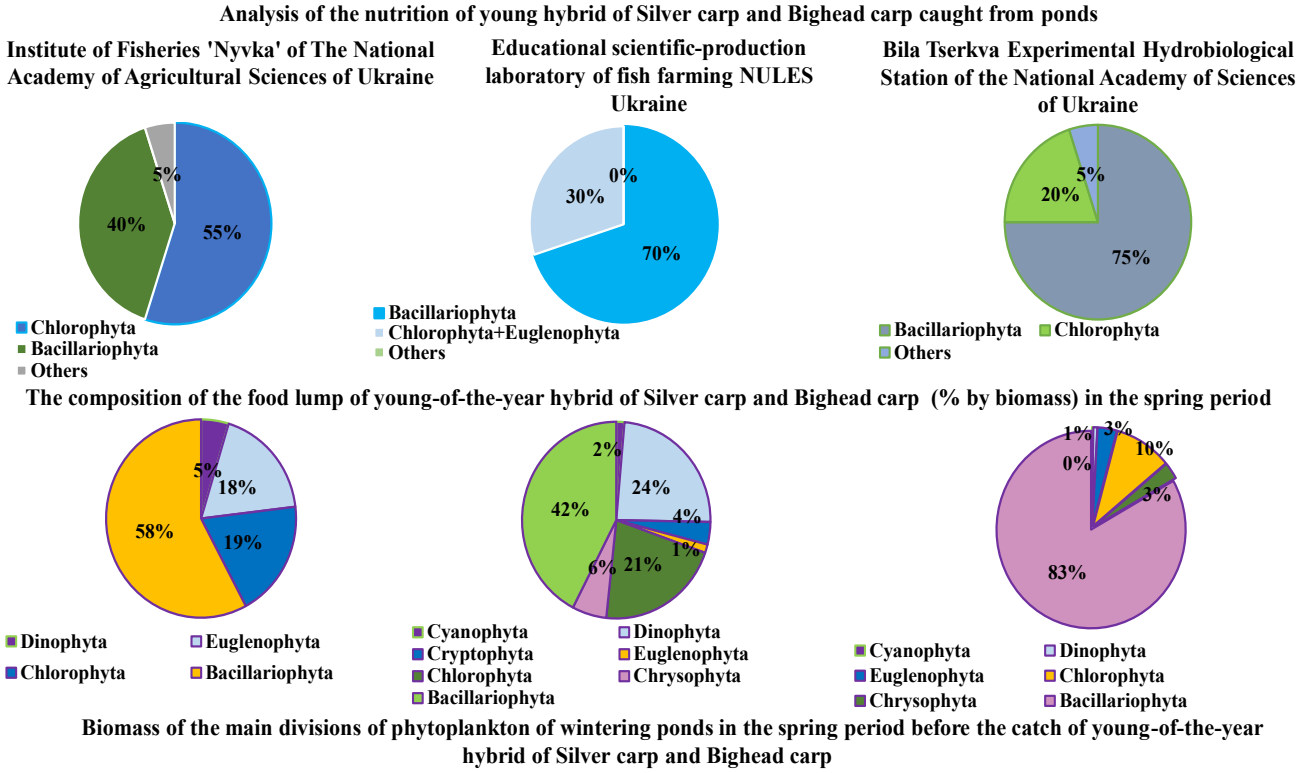


Figure 4 The composition of the food lump (% of the total mass) and phytoplankton biomass (%) of a young-of-the-year hybrid of Silver carp and Bighead carp in the wintering ponds of the studied farms in the spring of 2018.

In addition to 18 types of microscopic algae, the contents of the intestines of the biennial fish also included small fragments of filamentous green algae. Since no such fragments were found in the phytoplankton samples, it is most likely that they were not captured with the main mass of phytoplankton, but consumed "consciously". In the biomass of the algal component of the contents of the intestines of fish, the share of diatoms was 30% (in the pond only 7%), green microscopic – 30%, and green filamentous – 40% (in the pond 60%) (Figure 5).

The index of similarity between the species composition of phytoplankton and the contents of intestines for individuals of both age groups was 0.56.

The intestines of young-of-the-year fish caught in the spring of 2018 from the wintering pond No. 2 of the NNVLR of the NULES of Ukraine included about 30% of unicellular algae, mainly diatoms. The insignificant amount of green and euglenoids suitable for determination is most likely caused by rapid destruction when entering the digestive tract of fish. At the same time, not a single cell of dinophytic algae was noted in the contents of the intestines, although they have large sizes, and a rather strong shell, and their number in the reservoir was noticeable. The share of diatoms in the biomass of the algal component of the contents of fish intestines was 70% (in the pond – 42%), green + euglena – 3% (in the pond – 21+6%), others – <1% (Figure 4).

The contents of the intestines of small fish caught in the spring of 2019 from the wintering pond No. 1 of the NNVLR of the NULES of Ukraine had a yellowish tint and a significant proportion of mucus. The proportion of single-celled algae in the biomass did not exceed 20%, exclusively diatoms (8 species). The share of diatoms in the biomass of the algal component of the contents of the intestines of fish was 100% (in the pond – 58%) (Figure 5).

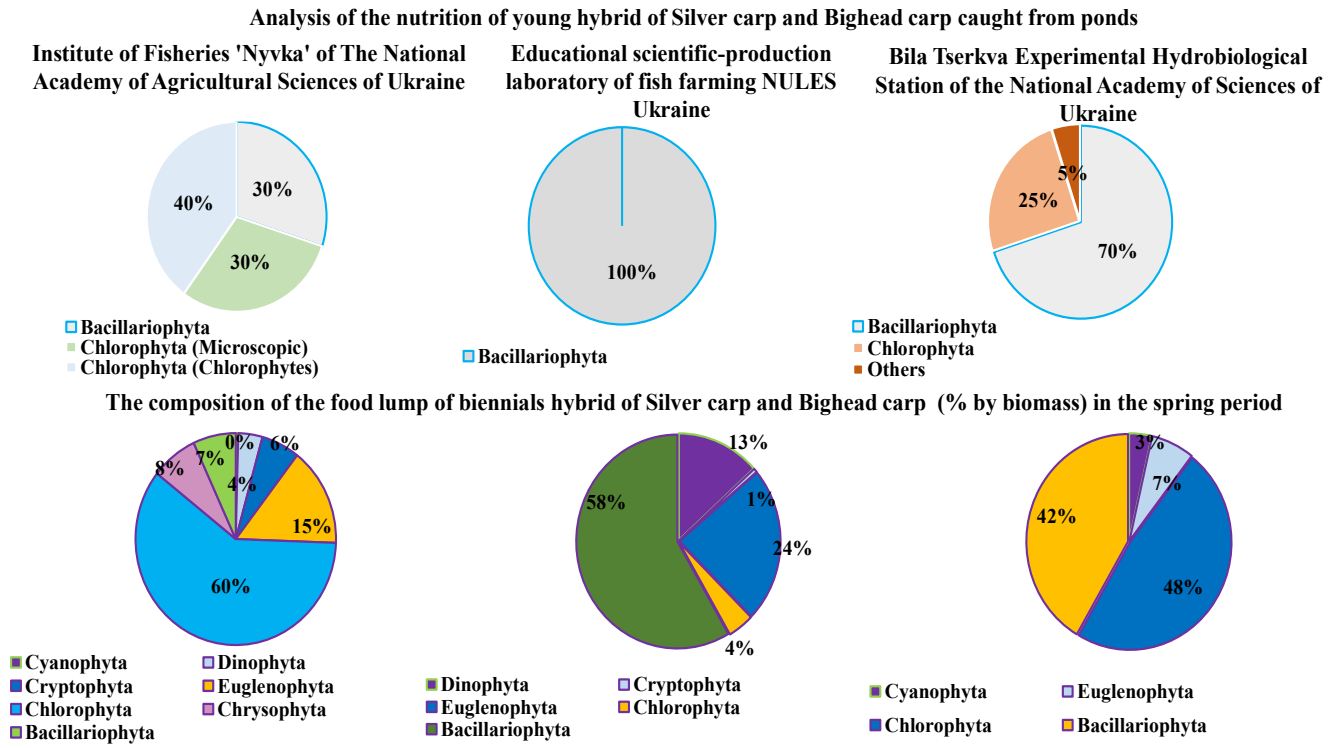


Figure 5 The composition of the food lump (% of the total mass) and the biomass of phytoplankton (%) in the yards of the Silver carp and Bighead carp hybrid in the studied farms' wintering ponds in the spring of 2019.

The index of similarity between the species composition of phytoplankton and the contents of intestines for young-of-the-year fish was 0.46, and for biennials fish – 0.26.

In the spring of 2018, the contents of the intestines of young-of-the-year fish caught from the wintering pond No. 5 of the National Agricultural Research Service of the National Academy of Sciences of Ukraine were green mass, 90% of which consisted of unicellular algae. At the same time, the number of diatom species even exceeded their number in the phytoplankton sample, possibly due to the consumption of benthic algae that did not enter the water column and, therefore into the phytoplankton samples (in particular, *Synedra ulna*, *Gyrosigma acuminatum*, *Caloneis silicula* and other large-celled forms were noted in the contents of the intestines). The shares in the biomass of the algal component of the contents of fish intestines were: diatoms – 75% (in the pond – 83%), green – 20% (in the pond – 10%), others – 5% (in the pond – 7%) (Figure 4).

The contents of the intestines of small fish caught in the spring of 2019 from wintering pond No. 11 of the BEGS IGB of the National Academy of Sciences of Ukraine were similar. At the same time, it should be noted that the estimated weight of the intestine sample of individuals of the two age groups was very close, which, given the larger body weight of small fish, indicates a less intensive consumption of phytoplankton, or a smaller share of it in the diet. The share of diatoms in the biomass of the algal component of fish intestines was 70% (in the pond – 42%), green – 25% (in the pond – 48%), others – 5% (in the pond – 10%) (Figure 5).

The index of similarity between the species composition of phytoplankton and the contents of intestines for individuals of both age groups was 0.76.

Hypophthalmichthys molitrix (Silver Carp) are abundant in the Mississippi River system, where they consume phytoplankton. There is concern that Silver Carp may influence phytoplankton community structure with cascading effects on other trophic levels. Information is needed regarding Silver Carp phytoplankton consumption rates and prey selection to assess their potential impact on the food web in the river. Investigated Silver Carp diets in a Lower Mississippi River backwater lake to quantify phytoplankton prey selectivity. Made measurements on 4 dates over a 2-y period, which spanned a range of hydrologic connectivity between the lake and the river and various fish sizes. Quantified selection by comparing phytoplankton community composition in the lake to prey in foreguts of captured Silver Carp using Vanderploeg and Scavia's relativized selection index. With a possible exception of diatoms on 1 date, there was no relationship between sample date or fish size on prey selection. However, there was a consistent pattern in prey selection: euglenoid algae were positively selected, the selection of colonial algae and diatoms was variable, and flagellates and filamentous cyanobacteria were negatively

selected. Results are discussed in a conceptual model for Silver carp phytoplanktivory that incorporates the roles of habitat selection, prey availability, prey capture and processing, and digestive physiology **[41]**.

The average values of the share of glycogen in most organs and tissues of young-of-the-year of the hybrid of silver and bighead carp in 2018 were at the level of 0.5% and below. Exceptions were the indicators of the specific gravity of glycogen in the liver of bighead carp: in the winter pond No. 101 – above 2%; in winter ponds No. 2 and No. 5 – less than 2%. Thus, a higher level of glycogen in the liver of young-of-the-year fish is evident compared to its presence in the muscles and gills of the youth of the year of silver and bighead carp hybrids. The average values of the specific weight of protein in most organs and tissues of young-of-the-year hybrid of silver and bighead carp in 2018 were almost evenly distributed in them, at the level of 10-14%. Exceptions were the indicators of the proportion of protein content in winter pond No. 5 – they were lower (about 10%). The average values of the specific weight of lipids in most organs and tissues of a young-of-the-year hybrid of silver and bighead carp in 2018 were, like glycogen, at 0.5% and below. Exceptions were the indicator of the proportion of lipids in the liver of bighead carp: in winter pond No. 101 – above 3.5%; in winter ponds No. 2 and No. 5 – about 3%. Thus, a higher level of lipids (as well as glycogen) is present in the liver of young-of-the-year fish compared to its content in the muscles and gills of the youth of the year of the hybrids of silver and bighead carp. Thus, according to the results of studies conducted in 2018, it was found that the concentration of glycogen, total protein, and lipids in fish organs and tissues of fish from SEEF "Nyvka" IF NAAS and TRPLF NULES of Ukraine was satisfactory. The results indicate that the young-of-the-year hybrid of silver and bighead carp in the winter significantly reduces or stops the trophic activity and switches partially or completely to endogenous nutrition **[42]**.

The average glycogen values in most organs and tissues of biennials of the hybrid of silver and bighead carp caught from winter ponds in 2019 were about and above 0.5%. Exceptions were glycogen content in the liver of bighead carp wintering ponds: No. 119 about 3%; No. 1 more than 2.5%, and No. 11 more than 2%. As a result, a higher level of glycogen was found in the liver of biennial than its presence in the muscles and gills of the youth of the year hybrids of silver and bighead carp. The average values of the proportion of protein in most organs and tissues of biennials of the hybrid of silver and bighead carp caught from winter ponds in 2019, as well as biennials, fluctuated markedly, and in particular organs and tissues: in muscle levels of 14-14.5%; in the liver at the level of 12.5-14% (the highest pond fish No. 2); in gills at least at the level of less than 12.5-13%. The average lipids values in most organs and tissues of biennials of the hybrid of silver and bighead carp caught from winter ponds in 2019, biennials, were at 1% and below. The results of the biennials of the hybrid of silver and bighead carp in 2019 indicate that their physiological state at the time of the study was within the physiological norm **[42]**.

In the spring of 2019, during the stocking of the Kosiv Reservoir, it was noted that the proportion of single-celled algae in the intestines of young-of-the-year was about 30%, mainly diatoms and blue-green. Also, a small share was the remains of green and euglena algae, unsuitable for determination. About 30% of the mass of intestinal contents was formed by fragments of green filamentous algae and higher aquatic plants. In the contents of the intestines of fish, the share of the plant component was: diatoms – 40% (in the reservoir – only 7%), green filamentous + higher plants – 55% (in the reservoir – 60%), green + euglena – 5% (in the reservoir – 15 %) (Figure 6).

At the same time, when catching triennial fish (stocking with yearlings in 2018), the contents of the intestines showed a dark mass with a significant proportion of mucus and fat droplets. The share of unicellular algae in the contents of the intestines did not exceed 15%, and fragments of green filamentous algae and higher plants – 10%. In the contents of fish intestines, the share of diatoms in the plant component was 15% (in the reservoir – 21%), green microscopic ones – 78%, and fragments of green filamentous algae and higher plants – 7% (in the reservoir – 54%) (Figure 6).

In the fall of 2019, when triennial fish were caught (stocking in 2019), the mass of plant components in the intestinal contents was 70%, of which about 25% were single-celled algae and about 45% were fragments of green filamentous algae and higher aquatic plants. In the contents of the intestines of fish, the share of diatoms in the plant component was 25% (in the reservoir – 21%), green microscopic – 15%, and green filamentous + higher – 60% (in the reservoir – 54%) (Figure 6).

The index of similarity between the species composition of phytoplankton and the contents of intestines for young-of-the-year was 0.55, triennial fish (stocking with biennial in 2019) – 0.40, triennial fish (stocking with young-of-the-year in 2018) – 0.30.

Hypophthalmichthys molitrix (Silver carp) is an invasive fish that threatens ecosystem function by consuming basal food web resources. This study quantifies the gut contents of 83 Silver carp in the mainstem reservoir ecosystem of Kentucky Lake, Kentucky, Tennessee River Valley, United States. Silver carp guts contained phytoplankton (63.5%), zooplankton (33.8%), and heterotrophic flagellates (2.7%) based on volume. Study indicates that Silver Carp are planktivorous and consume organisms within multiple lower trophic levels across

various habitats. However, shows that Silver carp diets differ at finer taxonomic scales and suggests these differences may be driven by forage availability **[43]**.

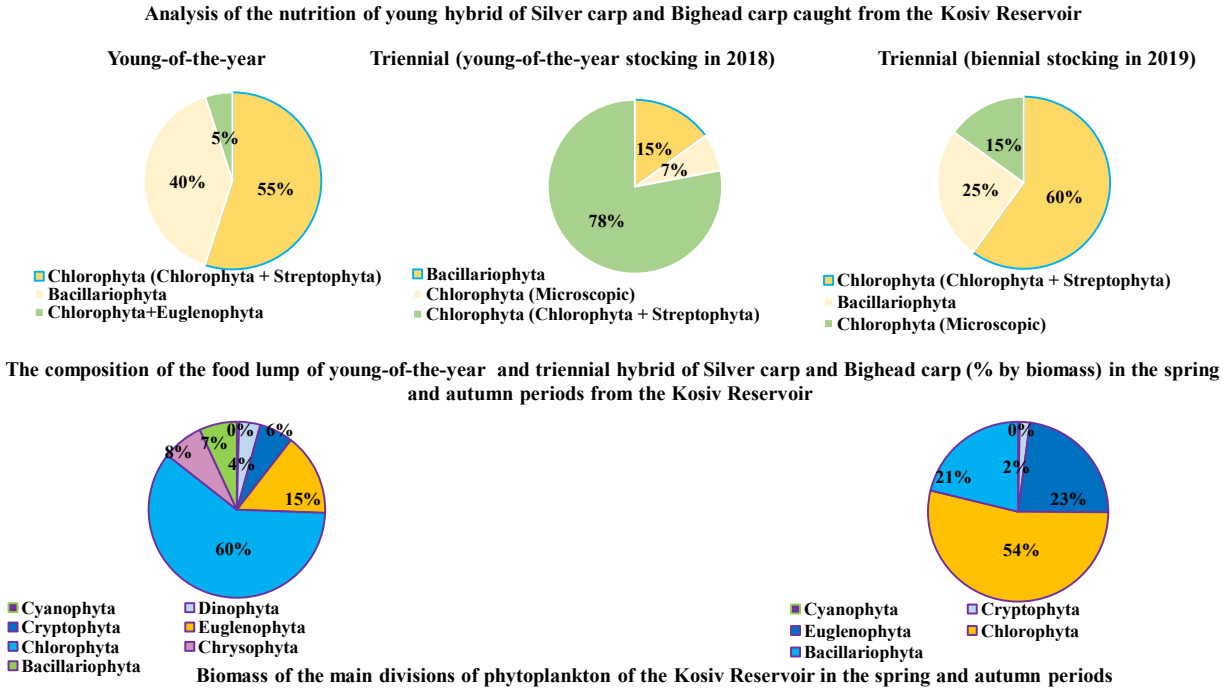


Figure 6 The composition of the food lump (% of the total mass) and phytoplankton biomass (%) of young-of-the-year and triennial hybrid of Silver carp and Bighead carp of the Kosiv Reservoir of the studied farms in the spring and autumn of 2018, 2019.

The average glycogen values in most organs and tissues of young-of-the-year and triennials of the hybrid of silver and bighead carp of the Kosiv Reservoir in 2019 were up to 0.4% and 1%, respectively. Exceptions were glycogen content in the liver of bighead carp of the Kosiv Reservoir, which was slightly more than 2% in one-year-olds and 5-5.5% in triennials in both years of research. Thus, a higher level of glycogen in the liver of annual fish is evident compared to its presence in the muscles and gills of the youth of the year of the hybrid of silver and bighead carp **[42]**.

The average values of protein content in most organs and tissues of young-of-the-year and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir in 2019 were lower in some organs and tissues in annuals, 12.5%, and higher in triennials, 15-22%. Moreover, the highest content was in the muscles of all age groups of fish, and the lowest protein content was in the gills. The average lipid content in the muscles and gills of young-of-the-year and triennials of the hybrid of silver and bighead carp from the Kosiv Reservoir in 2019 was at the level of annuals less than 0.5% and triennials about 1% or more. The lipid content in the liver was much higher: in young-of-the-year fish, they were about 3%, and in triennials from stocking in 2018, about 6%, and from stocking in 2019, more than 7% **[42]**.

The results of the young-of-the-year hybrid of silver and bighead carp in 2019 indicate that their physiological state at the time of the study was within the physiological norm. The studied triennial hybrids of silver and bighead carp from the Kosiv Reservoir in 2019 were marked by significant fluctuations in total protein content in muscles (20.04-22.16%), liver (18.00-18.56%), and gills (13.44-15.50%). The amount of glycogen in the liver (1.79-1.84 times) and lipids in the gills (1.19-1.36 times) increased slightly. A certain heterogeneity of the general physiological state of fish can explain the obtained data. This can be caused by hereditary factors determining a certain diversity of fish composition in reservoirs and the conditions of fish keeping **[42]**.

In the autumn of 2018, during the fishing of the Velikoburlutsky Reservoir, the contents of the intestines of biennial fish included about 45% of single-celled algae, mainly diatoms (12 species), Chlorococcum, and euglenoids, the latter of which were unsuitable for identification. In addition, a significant proportion of filamentous algae and fragments of higher aquatic plants were found in the intestines. From the weight of the contents of fish intestines, the share of diatoms was 50% (in the reservoir – 41%), green or *Chlorococcum* – 15% (in the reservoir – 6%), euglena – 10% (in the reservoir – 12%), filamentous algae and fragments of higher – 25%

(in the reservoir was not studied) (Picture 7). In addition to the mentioned algae, there was a significant share of blue-green biomass in the reservoir – 40%.

Considering the feeding characteristics of herbivorous fish, their use for melioration is the most effective for reducing areas of overgrowth and "blooming" of water **[44]**.

It should be noted that blue-green algae – *Microcystis*, and *Anabaena* emit toxins that negatively affect the natural self-cleaning of water bodies **[45]**.

At high air temperatures, intensive "blooming" of water occurs, condensation of water bodies occurs, and further increase has negative consequences for sanitary and biological water quality **[35]**, resulting in a deficit of oxygen in the lower water horizon and at night. The well-known organic and inorganic minerals are formed, including calcareous and toxic ones. **[46]**.

Blue-green algae species are the most resistant to temperature fluctuations: *Microcystis aeruginosa Kutz. emend. Elenkin i Anabaena flos-aquae* sp. **[47]**, **[48]**.

It was experimentally established that the toxins of blue-green algae inhibit the vital activity of nitrifying bacteria **[49]**, in connection with which the mineralization process ends in the first phase, which is accompanied by a decrease in the concentration of mineralized forms of nitrogen in the water **[50]**, as well as mass suffocation of fish in reservoirs. Some species of cyanobacteria produce toxins that affect people's health **[51]** when they consume contaminated water, fish, molluscs, or swim in a reservoir.

Freshwater cyanobacteria (blue-green algae) – *Microcystis aeruginosa* and *Anabaena flos-aquae* can produce hepatotoxic peptides that cause signs of poisoning in mice (LD50, 50 μg/kg) **[51]**, **[52]**.

In the summer of 2019, when triennial fish were caught, the intestinal contents also contained about 25% of diatoms, *Chlorococcum*, and euglena algae, while the share of filamentous algae and higher aquatic plants was much higher (up to 40%). In the contents of the intestines of fish, the share of diatoms by weight was 35% (in the reservoir – 54%), green or Chlorococcum – 15% (in the reservoir – 8%), euglena – 10% (in the reservoir – 6%), filamentous algae and fragments higher – 40% (in the reservoir was not studied) (Figure 7). There was also a significant share of blue-green biomass in the reservoir in summer – 31%.

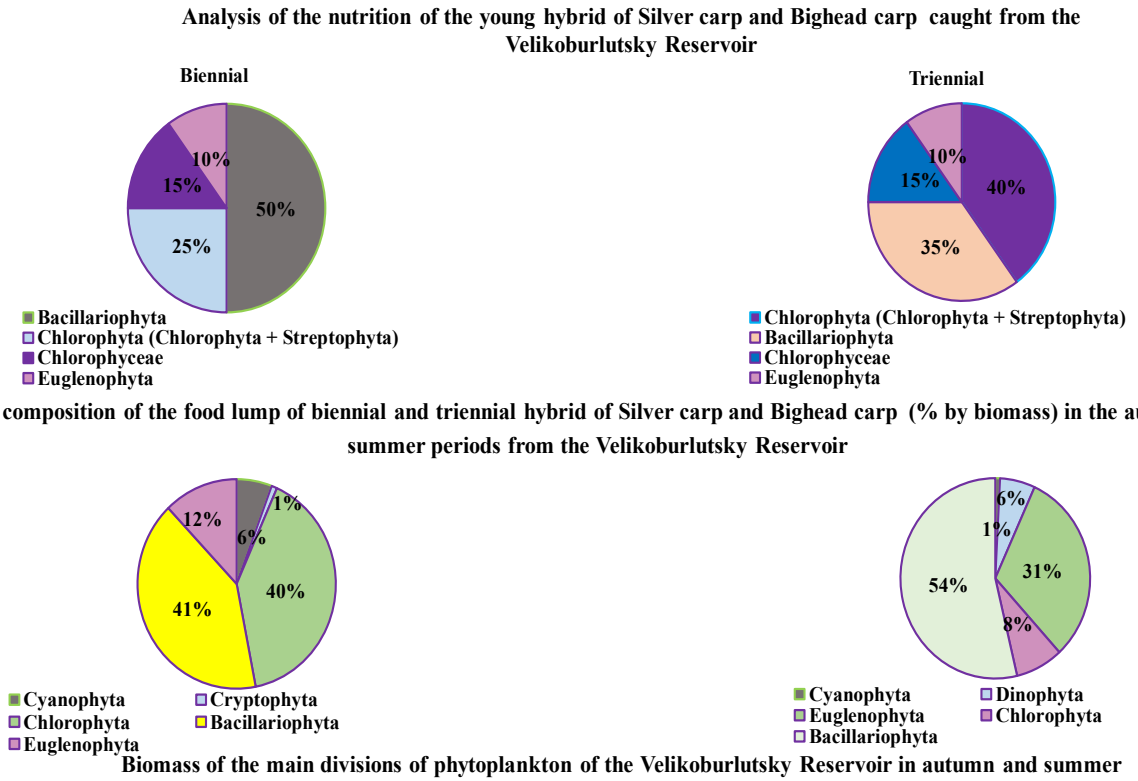


Figure 7 The composition of the food lump (% of the total mass) and phytoplankton biomass (%) of biennials and triennials of the hybrid of Silver carp and Bighead carp of the Velikoburlutsky Reservoir of the investigated farms in the fall of 2018 and summer of 2019.

The index of similarity between the species composition of phytoplankton and the contents of intestines for biennial fish was 0.48, triennial fish was 0.33.

Thus, the analysis of the food lumps of Silver carp and Bighead carp hybrid showed that phytoplankton plays a leading role (from 30 to 90% by mass) in its nutrition in all studied water bodies. Accordingly, when calculating the stocking volumes of the hybrid of Silver carp and Bighead carp, it can be attributed to phytophages, and their stocking at the expense of Silver carp will not hurt the zooplankton community, as an important food object for young fish and an agent of self-purification of water.

Common for individuals of both age groups was the presence of blue-green algae in the intestines, accumulated in the form of microscopic lumps covered with mucus. This was not observed in other reservoirs, even where the proportion of blue-greens in the biomass was higher.

The average values of glycogen in most organs and tissues of the youth of the year of the hybrid of silver and bighead carp of the Velykoburlutsky Reservoir in 2018, and 2019 increased from young-of-the-year to triennials and were, respectively, at the level: in muscle up to 0.5% or more; in gills 0.8% and more; the highest in the liver – 2.0-3.5% and more. The average values of protein content in most organs and tissues in biennials and triennials of hybrid of silver and bighead carp from VelykoburlutskyReservoir in 2018, and 2019 also increased with age and fluctuated in some organs and tissues of fish: in the highest muscles more than 12-17%; in the gills 12-14%; in the liver 12-16%. The average lipid content in the liver, muscles, and gills of the biennials and triennials hybrid of silver and bighead carp from the Velykoburlutsky Reservoir in 2018, and 2019 gradually increased from the youth of the year to triennials. It was at a lower level in muscles 0.2-1.2% and gills 0.2-1.5%. Lipid content in the liver was much higher: in biennials, 5.3%, and in triennials, more than 6%. Satisfactory levels of essential nutrients were characteristic of the experimental groups from the Velykoburlutsky Reservoir, not considering the slight excess of glycogen content in the gills of triennials **[42]**.

The feeding spectrum and rations of different groups of hybrid Silver carp and Bighead carp in ponds and reservoirs have a well-defined seasonal character related to the composition of feed objects. The composition of food largely depended on the qualitative composition and quantitative development of plankton only in ponds, in the conditions of reservoirs the indicator of "avoidance preference" for phytoplankton was expressed to a greater extent.

Thus, for the study, different size and weight groups of the hybrid of silver and bighead carp, caught from ponds and reservoirs are mainly characterized by satisfactory values of overall metabolic rates.

CONCLUSION

The analysis of the food lumps of the hybrid of Silver carp and Bighead carp showed that phytoplankton played a leading role (from 30 to 90% by mass) in its nutrition in all studied water bodies. Accordingly, when calculating the stocking volumes of the hybrid Silver carp and Bighead carp, it can be attributed to phytophages, and their stocking at the expense of white bullhead will not hurt the zooplankton community, as an important food object for young fish and an agent of self-purification of water. The feeding spectrum and rations of different groups of Silver carp and Bighead carp in ponds and reservoirs had a well-defined seasonal character related to the composition of feed objects. Food composition largely depended on the qualitative composition and quantitative development of plankton only in ponds. In the conditions of reservoirs, the "avoidance-advantage" indicator for phytoplankton was expressed to a greater extent. In all size and mass groups of the hybrid of silver and bighead carp from ponds and reservoirs in 2018 and 2019, mostly satisfactory values of general metabolism indicators were found - glycogen, proteins, and lipids in the liver, gills, and muscles of fish. In annual fish of winter ponds, total protein and glycogen content in all organs and tissues was slightly reduced. The organisms of biennial fish from feeding ponds were characterized by fluctuations in the content of glycogen in the liver (it was the highest in fish, 3.28-3.33%). Significant fluctuations in the total protein content of muscle, liver, and gills and a slight excess of glycogen in the liver and lipids in the gills of three-year-olds were observed in the reservoirs. The difference found in the availability of essential nutrients in the body of the studied fish indicates a change in the intensity and direction of their metabolic processes. The obtained results can be used for further research: studying the chemical composition of food products; research of functional characteristics of food products; optimization of the processes of production and storage of food products to ensure their maximum quality and preservation; studying the potential for creating new, functional food products and developing standards and quality control systems to ensure the safety and standardization of food products.

REFERENCES

1. Horobets, A. O. (2019). Vitamins and microelements as specific regulators of physiological and metabolic processes in the body of children and adolescents. In Ukrainian Journal of Perinatology and Pediatrics (Issue 4(80), pp. 75–92). Group of Companies Med Expert, LLC. https://doi.org/10.15574/pp.2019.80.75
2. Pukalo, P. Ja., Bozhyk, L. Ja., Dumych, O. Ja., & Tonkonozhenko, S. M. (2020). Conditions for carp growing in feeding ponds of the Yaniv fishery. In Scientific Messenger of LNU of Veterinary Medicine and

Biotechnologies (Vol. 22, Issue 93, pp. 35–39). Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. https://doi.org/10.32718/nvlvet-a9306

3. Dontsova, I. V., & Lebedynets, V. T. (2020). Food raw materials of animal origin: classification, properties and use. In Herald of Lviv University of Trade and Economics. Technical sciences (Issue 23, pp. 121–127). Publishing House Helvetica (Publications). https://doi.org/10.36477/2522-1221-2020-23-16
4. Molnár, T., Lehoczky, I., Edviné Meleg, E., Boros, G., Specziár, A., Mozsár, A., Vitál, Z., Józsa, V., Allele, W., Urbányi, B., Fatle, F. A. A., & Kovács, B. (2021). Comparison of the Genetic Structure of Invasive Bigheaded Carp (Hypophthalmichthys spp.) Populations in Central-European Lacustrine and Riverine Habitats. In Animals (Vol. 11, Issue 7, p. 2018). MDPI AG. https://doi.org/10.3390/ani11072018
5. Zheplinska, M., Mushtruk, M., Vasyliv, V., Slobodyanyuk, N., & Boyko, Y. (2021). The Main Parameters of the Physalis Convection Drying Process. In Lecture Notes in Mechanical Engineering (pp. 306–315). Springer International Publishing. https://doi.org/10.1007/978-3-030-77823-1_31
6. Makarenko, A., Mushtruk, M., Rudyk-Leuska, N., Kononenko, I., Shevchenko, P., Khyzhniak, M., Martseniuk, N., Glebova, J., Bazaeva, A., & Khalturin, M. (2021). The study of the variability of morphobiological indicators of different size and weight groups of hybrid silver carp (Hypophthalmichthys spp.) is a promising direction of development of the fish processing industry. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 181–191). HACCP Consulting. https://doi.org/10.5219/1537
7. Mytiai, I. S., Degtyarenko, O. V., Khomych, V. V., & Semenyuk, S. K. (2020). Hydroecological characteristics of malooleksandrivskogo reservoir of r. inhulets in relating to the restoration of the mini-hes operation. In Animal Science and Food Technology (Vol. 11, Issue 1, pp. 56–66). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2020.01.056
8. Zheplinska, M., Mushtruk, M., Vasyliv, V., Kuts, A., Slobodyanyuk, N., Bal-Prylypko, L., Nikolaenko, M., Kokhan, O., Reznichenko, Y., & Salavor, O. (2021). The micronutrient profile of medicinal plant extracts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 528–535). HACCP Consulting. https://doi.org/10.5219/1553
9. Brito, M. F. G., Daga, V. S., & Vitule, J. R. S. (2020). Fisheries and biotic homogenization of freshwater fish in the Brazilian semiarid region. In Hydrobiologia (Vol. 847, Issue 18, pp. 3877–3895). Springer Science and Business Media LLC. https://doi.org/10.1007/s10750-020-04236-8
10. Khomenchuk, V. O., Lyavrin, B. Z., Rabchenyuk, O. O., & Kurant, V. Z. (2020). Lipid metabolism in the body of fish under the action of the environmental aquatic factors. In Scientific Issue Ternopil Volodymyr Hnatiuk National Pedagogical University. Series: Biology (Vol. 80, Issues 3–4, pp. 126–138). Ternopil Volodymyr Hnatiuk National Pedagogical University. https://doi.org/10.25128/2078-2357.20.3-4.16
11. Kofonov, A., Potrokhov, O., & Hrynevych, N. (2020). Changes in the biochemical status of common carp juveniles (Cyprinus carpio L.) exposed to ammonium chloride and potassium phosphate. In Ukrainian Journal of Ecology (Vol. 10, Issue 4, pp. 137–147). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/2020_181
12. Kruzhylina, S. V., Buzevych, I. Y., Rudyk-Leuska, N. Y., Khyzhniak, M. I., & Didenko, A. V. (2021). Changes in the structure and dominance of the zooplankton community of the Kremenchuk Reservoir under the effect of climate changes. In Biosystems Diversity (Vol. 29, Issue 3, pp. 217–224). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/012127
13. Kuts, U. S., Kurinenko, H. A., & Tuchapsky, Y. V. (2021). Analysis of piscicultural-biological indicators and physiological condition of yearlings of common carp x Amur carp hybrids of different genesis. In Water bioresources and aquaculture (Issue 2, pp. 36–50). Kherson State Agricultural University. https://doi.org/10.32851/wba.2021.2.4
14. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/1711
15. Bober, A., Liashenko, M., Protsenko, L., Slobodyanyuk, N., Matseiko, L., Yashchuk, N., Gunko, S., & Mushtruk, M. (2020). Biochemical composition of the hops and quality of the finished beer. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 307–317). HACCP Consulting. https://doi.org/10.5219/1311
16. Holembovska, N., Slobodianiuk, N., & Israelian, V. (2021). Improving technology of fish semi-finished products with the addition of non-traditional raw materials. In Animal Science and Food Technology (Vol. 12, Issue 2). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.02.002

17. Yin, C., Wang, J., Qian, J., Xiong, K., & Zhang, M. (2022). Quality changes of rainbow trout stored under different packaging conditions and mathematical modeling for predicting the shelf life. In Food Packaging and Shelf Life (Vol. 32, p. 100824). Elsevier BV. https://doi.org/10.1016/j.fpsl.2022.100824
18. Jawdhari, A., Mihăilescu, D. F., Fendrihan, S., Jujea, V., Stoilov-Linu, V., & Negrea, B.-M. (2022). Silver Carp (Hypophthalmichthys molitrix) (Asian Silver Carp) Presence in Danube Delta and Romania – A Review with Data on Natural Reproduction. In Life (Vol. 12, Issue 10, p. 1582). MDPI AG. https://doi.org/10.3390/life12101582
19. Klymkovetskyi, A., & Khalturyn, M. (2022). Ichthyofauna of the Bilotserkivskyi lower reservoir on the Ros river. In Animal Science and Food Technology (Vol. 13, Issue 3). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(3).2022.20-27
20. Sabaj, M. H. (2020). Codes for Natural History Collections in Ichthyology and Herpetology. In Copeia (Vol. 108, Issue 3). American Society of Ichthyologists and Herpetologists (ASIH). https://doi.org/10.1643/asihcodons2020
21. Honcharova, O., Bekh, V., & Glamuzina, B. (2023). Physiological and biochemical aspects of the carp organism in conditions of increasing their viability when stocking water bodies. In Animal Science and Food Technology (Vol. 14, Issue 2). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.2.2023.28
22. Pico, J., Pismag, R. Y., Laudouze, M., & Martinez, M. M. (2020). Systematic evaluation of the Folin–Ciocalteu and Fast Blue BB reactions during the analysis of total phenolics in legumes, nuts and plant seeds. In Food & Function (Vol. 11, Issue 11, pp. 9868–9880). Royal Society of Chemistry (RSC). https://doi.org/10.1039/d0fo01857k
23. Encyclopedia of Clinical Laboratory Tests. (2000). Red. TitsaN. M.: Labinform, 452 p.
24. Polishchuk, N. V., Kovalenko, B. Yu., Vovk, N. I., Kononenko, I. S., & Kovalenko, V. O. (2021). Study of the effect of feed additive of sodium humate for sterlet by the cage method of growing. In Animal Science and Food Technology (Vol. 12, Issue 4). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.04.006
25. Parfeniuk, I., Grokhovska, Y., & Martseniuk, V. (2022). The state of use of fish resources in small reservoirs of the Rivne Oblast. In Animal Science and Food Technology (Vol. 13, Issue 3). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(3).2022.35-42
26. Shanina, O., Minchenko, S., Gavrysh, T., Sukhenko, Y., Sukhenko, V., Vasyliv, V., Miedviedieva, N., Mushtruk, M., Stechyshyn, M., & Rozbytska, T. (2020). Substantiation of basic stages of gluten-free steamed bread production and its influence on quality of finished product. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 189–201). HACCP Consulting. https://doi.org/10.5219/1200
27. Nazarenko, S., Bublyk, A., & Nazarova, E. (2019). Sanitary evaluation of fishes fished from the ponds of the Sumy region. In Bulletin of Sumy National Agrarian University. In The series: Veterinary Medicine (Vol. 3, Issue 46, pp. 54–60). Publishing House Helvetica (Publications). https://doi.org/10.32845/bsnau.vet.2019.3.8
28. Koval, O., Holubenko, O., Rud, V., & Tarasenko, L. (2021). Veterinary and sanitary assessment of fish quality and safety in the southern region of Ukraine (review article). In Agrarian Bulletin of the Black Sea Littoral (Issue 99). Odessa State Agrarian University. https://doi.org/10.37000/abbsl.2021.99.06
29. Orel, S. (2020). Fauna protection practices for Ukrainian military training area given NATO guidance documents. In Journal of Defense Resources Management (Vol. 11, Issue 2, pp. 222–232). Regional Department of Defense Resources Management Studies.
30. Zargar, A., Taheri Mirghaed, A., Mirzargar, S. S., Ghelichpour, M., Yousefi, M., & Hoseini, S. M. (2020). Dietary ginger administration attenuates oxidative stress and immunosuppression caused by oxytetracycline in rainbow trout (Oncorhynchus mykiss). In Aquaculture Research (Vol. 51, Issue 10, pp. 4215–4224). Hindawi Limited. https://doi.org/10.1111/are.14763
31. Rudenko, O. P., Paranyak, R. P., Kovalchuk, N. A., Kit, L. P., Hranovich, N. I., Gutyj, B. V., Kalyn, B. M., Sukhorska, P. P., Butsiak, A. A., Kropyvka, S. I., Petruniv, V. V., & Kovalska, L. M. (2019). Influence of seasonal factors on carp fish immune reactivity. In Ukrainian Journal of Ecology (Vol. 9, Issue 3, pp. 168–173). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/2019_75
32. Grynevych, N. E., Vodianitskyi, O. M., Khomiak, O. A., Svitelskyi, M. M., & Zharchynska, V. S. (2021). Monitoring of glycogen content of predatory fish species at the juvenile stage of development due to changes in the temperature and oxygen regime of the reservoir. In Water bioresources and aquaculture (Issue 1, pp. 49–61). Publishing House Helvetica (Publications). https://doi.org/10.32851/wba.2021.1.5

33. Hryhorenko, T., Mushyt, S., & Bazaieva, A. (2020). Productivity of nursery ponds under the complex exposure to their ecosystem. In Ribogospodars′ka nauka Ukraïni (Vol. 3, Issue 53, pp. 19–32). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka). https://doi.org/10.15407/fsu2020.03.019
34. Hryhorenko, T. V., Shumyhai, I. V., Dobrianska, O. P., & Bazaieva, A. M. (2019). Ecological status of fishery ponds for growing populations of Antoninsko-Zozulinetsky scaly carp. In Agroecological Journal (Vol. 0, Issue 4, pp. 65–73). Institute of Agroecology and Environmental Management NAAN. https://doi.org/10.33730/2077-4893.4.2019.189460
35. Ivaniuta, A. O., Slobodianiuk, N. M., & Cumachenko, I. P. (2019). Consumer properties of structure forming substances based on secondary raw fish with cystoseira. In Animal Science and Food Technology (Vol. 10, Issue 3, pp. 12–19). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2019.03.012
36. Hubanova, N. L. (2019). Production of zoobenthos in various areas of the Dnipro (Zaporizhzhia) reservoir. In Agrology (Vol. 2, Issue 3, pp. 156–160). Dnipro State Agrarian and Economic University. https://doi.org/10.32819/019023
37. Biliavtseva, V. (2021). Methods of wastewater treatment with the help of aquatic organisms. In Colloquium-Journal (Vol. 17, Issue 104, pp. 54–63). Vinnytsia National Agrarian University. https://doi.org/10.24412/2520-6990-2021-17104-54-63
38. Zheplinska, M., Mushtruk, M., Kos, T., Vasyliv, V., Kryzhova, Y., Mukoid, R., Bilko, M., Kuts, A., Kambulova, Y., & Gunko, S. (2020). The influence of cavitation effects on the purification processes of beet sugar production juices. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 451–457). HACCP Consulting. https://doi.org/10.5219/1284
39. Rahman, M. M., & Hamidah, H. (2020). Water quality influences the phytoplankton and bacteria abundance: a comparison between shallow freshwater and saltwater ponds. In Desalination and Water Treatment (Vol. 188, pp. 436–443). Desalination Publications. https://doi.org/10.5004/dwt.2020.25306
40. Zheplinska, M., Mushtruk, M., Vasyliv, V., & Deviatko, O. (2019). Investigation of the process of production of crafted beer with spicy and aromatic raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 13, Issue 1, pp. 806–814). HACCP Consulting. https://doi.org/10.5219/1183
41. Ochs, C. A., Pongruktham, O., Killgore, K. J., & Hoover, J. J. (2019). Phytoplankton Prey Selection by Hypophthalmichthys molitrix Val. (Silver Carp) in a Lower Mississippi River Backwater Lake. In Southeastern Naturalist (Vol. 18, Issue 1, p. 113). Humboldt Field Research Institute. https://doi.org/10.1656/058.018.0108
42. Makarenko, A., Mushtruk, M., Rudyk-Leuska, N., Kononenko, R., Shevchenko, P., Khyzhniak, M., Martseniuk, V., Kotovska, G., Klymkovetskyi, A., & Glebova, J. (2022). Investigation of internal organs and additive tissue of hybrid hypophthalmichthys (Hypophthalmichthys spp.) as a promising raw material for the production of dietary nutritional products. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 411–430). HACCP Consulting. https://doi.org/10.5219/1760
43. Benjamin B. Tumolo and Michael B. Flinn. Diet of Invasive Silver Carp (Hypophthalmichthys molitrix) in a Mainstem Reservoir Ecosystem. (2019). In Journal of the Kentucky Academy of Science (Vol. 79, Issue 1, p. 3). Kentucky Academy of Science. https://doi.org/10.3101/1098-7096-79.1.3
44. Mishra, P., Garg, V., & Dutt, K. (2019). Seasonal dynamics of phytoplankton population and water quality in Bidoli reservoir. In Environmental Monitoring and Assessment (Vol. 191, Issue 3). Springer Science and Business Media LLC. https://doi.org/10.1007/s10661-019-7185-x
45. Yan, M., Chen, S., Huang, T., Li, B., Li, N., Liu, K., Zong, R., Miao, Y., & Huang, X. (2020). Community Compositions of Phytoplankton and Eukaryotes during the Mixing Periods of a Drinking Water Reservoir: Dynamics and Interactions. In International Journal of Environmental Research and Public Health (Vol. 17, Issue 4, p. 1128). MDPI AG. https://doi.org/10.3390/ijerph17041128
46. Francé, J., Varkitzi, I., Stanca, E., Cozzoli, F., Skejić, S., Ungaro, N., Vascotto, I., Mozetič, P., Ninčević Gladan, Ž., Assimakopoulou, G., Pavlidou, A., Zervoudaki, S., Pagou, K., & Basset, A. (2021). Large-scale testing of phytoplankton diversity indices for environmental assessment in Mediterranean sub-regions (Adriatic, Ionian and Aegean Seas). In Ecological Indicators (Vol. 126, p. 107630). Elsevier BV. https://doi.org/10.1016/j.ecolind.2021.107630
47. Kondratiuk, V. M. (2020). Productivity of rainbow trout depending on protein levels in productive feed. In Animal Science and Food Technology (Vol. 11, Issue 4, pp. 45–53). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2020.04.045
48. Rudyk-Leuska, N., Leuskyi, M., Makarenko, A., & Yevtushenko, N. (2022). Current position of phytoplankton species diversity and water quality assessment of Kremenchuk reservoir according to probity

index. In Bulletin of Sumy National Agrarian University. The series: Agronomy and Biology (Vol. 48, Issue 2, pp. 139–147). Publishing House Helvetica (Publications). https://doi.org/10.32845/agrobio.2022.2.19

49. Shevchuk, S., Vyshnevskyi, V., Shevchenko, I., & Kozytskyi, O. (2019). Research of water objects in Ukraine using the data of remote sensing of the earth. In Land Reclamation and Water Management (Vol. 2, pp. 146–156). Institite of Water Problems and Land Reclamation OF NAAS of Ukraine.

50. Nikolenko, Y., & Fedonenko, O. (2021). Seasonal dynamics of phytoplankton indicators of the Zaporizhzhia (Dni-pro) reservoir phytoplankton of the Zaporizhzhia reservoir. In Ukrainian Journal of Ecology (Vol. 0, Issue 0, pp. 121–128). Bogdan Chmelnitskiy Melitopol State Pedagogical University.

51. Rudyk-Leuska, N., Leuskyi, M., Yevtushenko, M., Leuskyi, M., Khyzhnyak, M., & Makarenko, A. (2022). Phytoplankton and water quality of the Kakhovka reservoir in the summer period. In Ecological Sciences (Vol. 44, Issue 5, pp. 83–93). State Ecology Academy of Postgraduate Education and Management. https://doi.org/10.32846/2306-9716/2022.eco.5-44.13

52. Khyzhniak, M. I., Rudyk-Leuska, N. Y., Yevtushenko, N. Y., Leuskyi, M. V., Dudnyk, S. V., Danchuk, O. V., Dumych, O. Y. (2020). Development and structure of phytoplankton in the middle part of Kremenchuk reservoir. In Ukrainian Journal of Ecology (Vol. 10, Issue 4, pp. 132–136). Bogdan Chmelnitskiy Melitopol State Pedagogical University.

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank Dr. Alina Makarenko.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

According to Protocol No. 10 of 18.04.2020 at the meeting of the Ethics Commission of the Faculty of Livestock Raising and Water Bioresources, National University of Life and Environmental Sciences of Ukraine, Act No. 3 and 4 were signed during the experimental research, i.e. in the process of the slaughter of cattle " all the rules of the current legislation of Ukraine were observed, following DSTU 4673: 2006.

Contact Address:

*__Alina Makarenko__, National University of Life and Environmental Sciences of Ukraine, Faculty of Animal Husbandry and Aquatic Bioresources, Department of Hydrobiology and Ichthyology, Horihuvatskyi path Str., 19, Kyiv, 03041, Ukraine,
E-mail: almakarenko@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-2166-8566

Nataliia Rudyk-Leuska, National University of Life and Environmental Sciences of Ukraine, Faculty of Animal Husbandry and Aquatic Bioresources, Department of Hydrobiology and Ichthyology, Horihuvatskyi path Str., 19, Kyiv, 03041, Ukraine,
E-mail: rudykleuska@nubip.edu.ua
ORCID: https://orcid.org/0000-0003-4355-7071

Ruslan Kononenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Animal Husbandry and Aquatic Bioresources, Department of Hydrobiology and Ichthyology, Horihuvatskyi path Str., 19, Kyiv, 03041, Ukraine,
E-mail: ruslan_kononenko@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-7818-2583

Melaniia Khyzhniak, National University of Life and Environmental Sciences of Ukraine, Faculty of Animal Husbandry and Aquatic Bioresources, Department of Hydrobiology and Ichthyology, Horihuvatskyi path Str., 19, Kyiv, 03041, Ukraine,
E-mail: m_khyzhnjak@nubip.edu.ua
ORCID: https://orcid.org/0000-0003-2350-1919

Iryna Kononenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Animal Husbandry and Aquatic Bioresources, Department of Aquaculture, Horihuvatskyi path Str., 19, Kyiv, 03041, Ukraine,
E-mail: iryna_kononenko@nubip.edu.ua
ORCID: https://orcid.org/0000-0003-3906-3650

Ganna Kotovska, National University of Life and Environmental Sciences of Ukraine, Faculty of Animal Husbandry and Aquatic Bioresources, Department of Hydrobiology and Ichthyology, Horihuvatskyi path Str., 19, Kyiv, 03041, Ukraine,
E-mail: gannkot@nubip.edu.ua
ORCID: https://orcid.org/0000-0001-7155-1086

Petro Shevchenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Animal Husbandry and Aquatic Bioresources, Department of Hydrobiology and Ichthyology, Horihuvatskyi path Str., 19, Kyiv, 03041, Ukraine,
E-mail: p.shevchenko@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-5996-4328

Mykhailo Leuskyi, National University of Life and Environmental Sciences of Ukraine, Faculty of Animal Husbandry and Aquatic Bioresources, Department of Aquaculture, Horihuvatskyi path Str., 19, Kyiv, 03041, Ukraine,
E-mail: leuskyi@nubip.edu.ua
ORCID: https://orcid.org/0000-0001-5646-8524

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 11.11.2023
Revised: 4.2.2024
Accepted: 9.2.2024
Published: 28.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 223-238
https://doi.org/10.5219/1937
ISSN: 1337-0960 online
www.potravinarstvo.com

Challenges and ways forward for the Malaysian SMEs in the Halal food industry: a systematic review

Sorna Umme Saima, Radin Badaruddin Radin Firdaus, Sarjiyanto

ABSTRACT

With its rich Islamic cultural heritage, Malaysia has been at the forefront of the Halal food industry, presenting opportunities and challenges for its small and medium enterprises (SMEs). This study offers a critical review of the multifaceted challenges faced by these SMEs in the competitive landscape of the Halal food sector. Through a review of 79 scholarly articles from Scopus and Google Scholar databases covering the years 2013 to 2023, we unearthed a spectrum of challenges. Key among them are inconsistent Halal standards and a labyrinthine certification process, compounded by barriers in international trade, financing conundrums, evolving marketing paradigms, innovation deficits, and branding complexities. In response, this research outlines strategies tailored for industry resilience and growth. Central to our recommendations is the imperative to bolster Malaysia's stature as a global Halal food nexus, emphasising cutting-edge branding techniques, leveraging the potential of e-commerce, and strategically positioning for increased Halal food exports. Drawing these insights, the study furnishes SMEs, researchers, and policymakers with a roadmap for industry evolution and underscores the importance of research in this domain.

Keywords: Halal food industry, SMEs, challenges, strategies, Malaysia

INTRODUCTION

Situated amidst a rich blend of cultural and ethnic diversity, Malaysia boasts a majority population adhering to Islamic principles. The country is firmly dedicated to protecting Muslim rights by building a competitive Halal industry and becoming a potential leader globally. The Halal food sector's impact on Malaysia's economic landscape is evidenced by its substantial contribution to its gross domestic product (GDP), accounting for approximately 7.4 per cent in 2022 **[1]**.

Central to Islamic principles is the emphasis on Halal food sources, which are pivotal in shaping Muslim well-being and behavior **[2]**. The burgeoning demand for Halal products is anchored in Muslim consumers' increased awareness of their religious obligations, steering clear of haram foods as dictated by the Qur'an, Sunnah, and the consensus of Muslim jurists (Ijma). This awareness and socio-economic factors have transformed the Halal food industry into a standard requirement in domestic and international arenas **[3]**. The growing number of educated and affluent Muslims is linked to the increasing demand for Halal food **[4]**, valued at USD 346.7 billion annually **[5]**, and the global Halal market reached 3 trillion USD in 2023, of which Malaysia has captured 20 billion USD **[6]**.

The global preference increasingly favours Halal-certified products, transforming perceptions from mere religious compliance to a symbol of quality. The Halal lifestyle is recognized as an economic force and a powerful marketing strategy for big corporations or SMEs. In Malaysia, SMEs are well-positioned to seize opportunities in the growing Halal sector, supported by the government's vision to position Malaysia as a global Halal hub **[7]**. The rise of Halal certifications, particularly in food commodities, has accelerated this trajectory in Malaysia **[8]**.

Despite industry growth, SMEs in Malaysia's Halal food sector face challenges, including adhering to Shariah principles, processing standards, branding, logistics, and export barriers **[9]**, **[10]**. With a rising global Muslim population and increased Halal food demand, the sector offers growth opportunities **[11]**. However, SMEs must overcome key structural barriers. This review explores challenges in Malaysia's Halal food industry, focusing on SMEs, and recommends potential strategies.

HALAL FOOD INDUSTRY

Halal, a term deeply entrenched in Islamic doctrine, signifies what is permissible. Within the domain of consumables, Halal food is defined as those that align with the stipulations set forth by Islamic law **[12]**. Beyond the religious tenets, the Halal certification process underscores meticulous standards that encapsulate hygiene, quality control, and strict adherence to the teachings of Islam **[13]**. This extends to raw materials, semi-finished goods, and even the equipment used in the food production chain, ensuring an uncompromised commitment to Shariah law **[14]**. To further solidify its sanctity, the production process must be supervised by a Muslim inspector endowed with the requisite knowledge and competence **[15]**.

Diverse cultural landscapes and interpretations of religious beliefs have led to a multifaceted understanding of what constitutes 'Halal.' Consequently, this has birthed various Halal standards, wherein a certification from one Islamic authority might not necessarily gain acceptance in another jurisdiction **[16]**. This pluralism in certification is evident in Malaysia. As of 17th March 2023, the Department of Islamic Development Malaysia (JAKIM) recognizes 84 certification bodies spanning 46 nations **[17]**. The quintessence of Islam revolves around obedience to Allah's divine will and laws. This transcends rituals and forms the bedrock of a Muslim's daily life, encapsulating every action, including food consumption, as a devout act of worship. In its unique stance, Islam extends this sanctity to the commercial world, emphasizing the legitimacy of certifying, labeling, and branding goods and services, aligning them with the faith's teachings **[18]**. Continuing into the Halal food industry, Wilson **[19]** offers a comprehensive classification, dividing it into 11 categories based on commodities and consumption. These range from utilities and livestock to professional services and communication channels. While showcasing the industry's potential, such a broad spectrum is challenging. A significant concern stems from modifying the term 'Halal' in branding, which is often driven by profit motives. Unfortunately, this has led to food fraud in Malaysia, especially cross-contamination in meat-based supply chains, which has considerably eroded consumer trust in the Halal label **[20]**.

As shown in Figure 1, in 2015, a total of 6,138 establishments in Malaysia obtained JAKIM Halal certification, exhibited an average annual growth rate of 13.0 per cent over the specified period with the gross output value reached RM 208 billion, indicating a yearly growth of 9.0 per cent **[21]**. In the same year, the value-added amounted to RM 42.2 billion, representing a significant increase from RM 23.9 billion in 2010 and highlighting an average annual growth rate of 12.0 per cent. Furthermore, the total exports of Halal products in 2015 amounted to RM 39.4 billion. Given the burgeoning potential of the Halal sector, it is unsurprising that Malaysia has been working continuously to expand and solidify its position in the global Halal market. With a commendable growth trajectory, Malaysia's aspirations in the Halal domain are further buoyed by the robust performance of its SMEs. Recognizing their potential, several national planning initiatives have been tailored specifically to cater to Halal SMEs. To further bolster this growth, a slew of agencies, including the Small and Medium Industries Development Corporation (SMIDEC) and Malaysian Outer Exchange Improvement Company (MATRADE), among others, have been instituted, working in tandem to support and execute government policies.

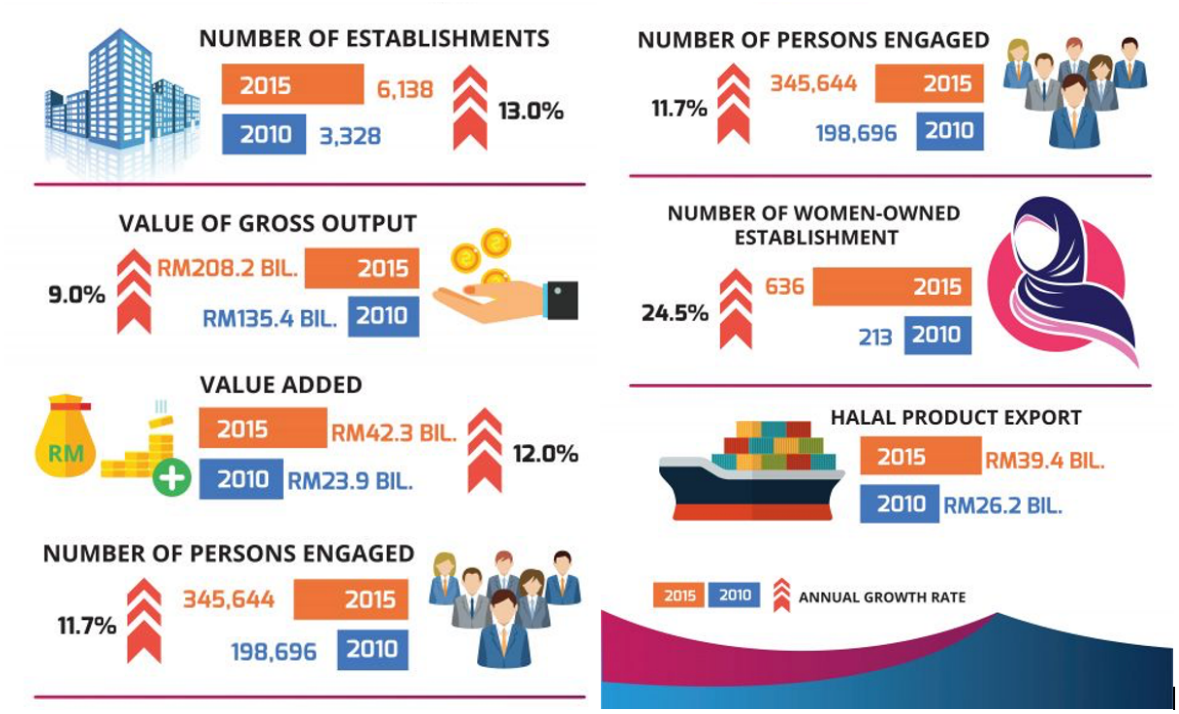


Figure 1 Malaysia Halal statistics, 2010 and 2015. Note: Source: Department of Statistics Malaysia **[21]**.

SMALL AND MEDIUM ENTERPRISES (SMES)

Tracing the origin of most global corporate behemoths reveals humble beginnings, often as SMEs **[22]**. Yet, the definition of SMEs is not universally constant. It is shaped by myriad factors, including annual turnover, equity, or workforce size, and is often tailored to each nation's economic and industrial nuances **[23]**. In Malaysia, the definition of SMEs is predicated on sales turnover and workforce size (Table 1).

SMEs form the foundation of Malaysia's economy, comprising 97.2 per cent of all business establishments, contributing 38.2 per cent to the GDP, and offering employment to 7.3 million individuals **[24]**. Their increasing importance in the Halal industry cannot be overstated, given their sizable contribution to the Malaysian economy and the potential to be forerunners of growth in the sector. Despite the optimism, challenges abound. A significant portion of SMEs in Malaysia, constituting 80 per cent, are Halal-certified. Yet, an alarming 75 per cent falter in their initial certification attempts, primarily attributed to gaps in understanding and preparedness **[25]**. This highlights the need for a more streamlined and comprehensible certification process. Furthermore, the increasing global recognition of Halal products, not just for their religious alignment but also for their safety and quality assurance, offers a lucrative market that Malaysian SMEs can tap into. Yet, doing so requires a dual focus: catering to the domestic market while casting an eye on international horizons **[26]**, **[27]**.

In short, the confluence of Malaysia's Halal industry and SMEs offers a distinctive blend of opportunities and challenges. As Malaysia strives to strengthen its standing in the global Halal market, the pivotal role of SMEs cannot be overstated. Successfully addressing the challenges, they encounter and leveraging their potential will shape Malaysia's trajectory for success in this domain.

Table 1 National Standard of SMEs in Malaysia.

Size	Manufacturing	Services and Other Sectors
Small	From RM300,000 to RM15 million From 5 to < 75 employees	From RM300,000 to < RM3 million From 5 to < 30 employees
Medium	From RM15 million to ≤ RM50 million From 75 to ≤ 200 employees	From RM3 million to ≤ RM20 million From 30 to ≤ 75 employees

Note: Source: SME Corporation Malaysia **[28]**.

MATERIAL AND METHODOLOGY

To comprehensively understand the dynamics of SMEs in the Halal food industry, we embarked on a systematic exploration. This was strategically designed to unearth and analyse studies targeting SMEs operating within the Halal food sectors. The initial step involved deploying key search terms to streamline the research process. These terms encompassed "SMEs," in conjunction with "Halal food industry," "Halal industry," "Halal food SMEs," and "issues and strategies." This precision targeting was deemed necessary to ensure that the research yield was relevant and significant.

Two primary databases were chosen for this investigation: Scopus and Google Scholar. These platforms are renowned for their expansive collection of scholarly articles, ensuring a diverse and comprehensive pool of research material. Our primary focus was to identify papers centered on the operations, challenges, and prospects of SMEs in the Halal food industry, with a special emphasis on the Malaysian context. To ensure the research was current and pertinent, we confined our search to papers published within a specific timeframe, from 2013 to August 2023. This timeframe was carefully chosen to encompass the most recent trends, challenges, and developments in the studied industry. Our initial search, leveraging the keywords, yielded 186 articles.

For systematic organization and management of these scholarly articles, they were collated and stored using Endnote X7. Given the emphasis on comprehensiveness, our consideration set was strictly limited to published papers, either online or in print. We consciously excluded conference papers, books, book chapters, and published theses from our analysis to maintain the focus and rigor of the study. Post this, a meticulous screening process was initiated. Articles published outside the stipulated timeframe or not aligned with our core research interest were promptly removed. This rigorous vetting process considerably narrowed our research pool, leaving us with 79 articles. At this juncture, a collaborative approach was adopted. Each team member thoroughly reviewed these articles, evaluating them against our predefined inclusion criteria. Through this collective endeavor, irrelevant articles were manually culled, leaving us with a final set of 54 published articles (Figure 2).

To structure our findings and discussions, the selected papers were bifurcated into two dominant themes: challenges faced by SMEs in the Halal food industry (elaborated in Section 4.0) and potential strategies and pathways for their growth and sustainability (detailed in Section 5.0). This structured methodology ensured a comprehensive, relevant, organized exploration of SMEs in the Halal food industry.

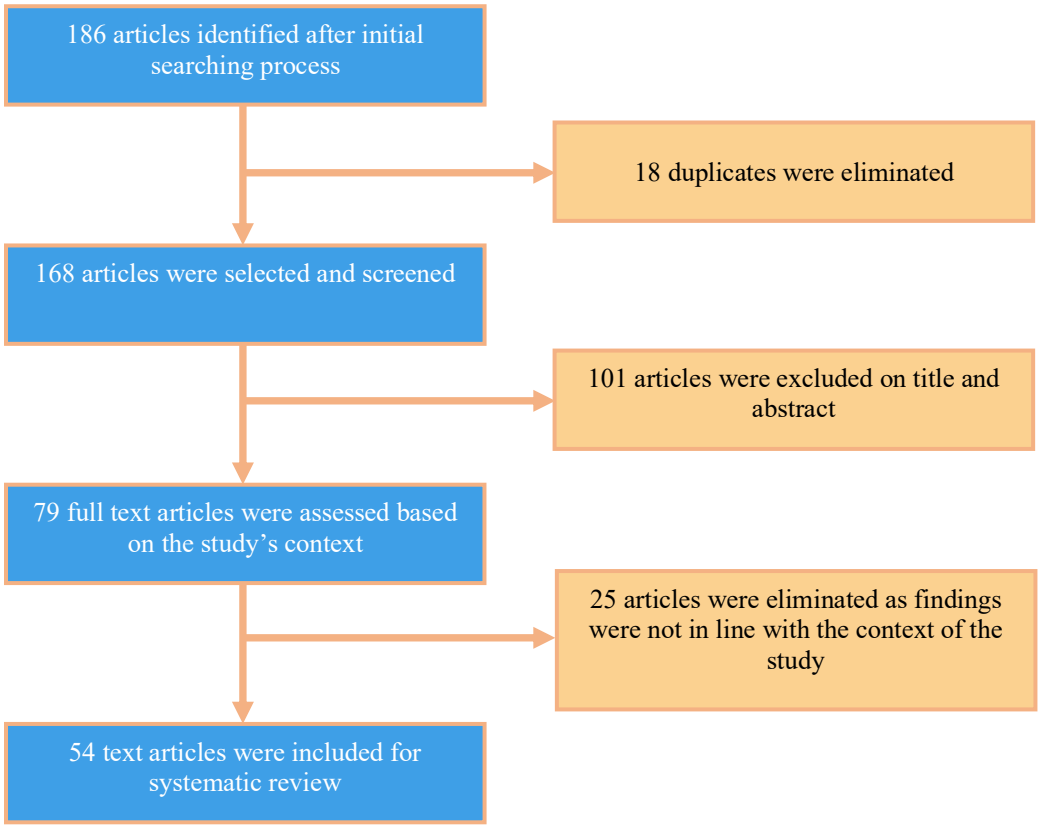


Figure 2 Article selection process.

RESULTS AND DISCUSSION

ISSUES AND CHALLENGES OF HALAL FOOD SMES IN MALAYSIA

The Halal food sector is experiencing swift growth within Malaysia and globally. However, several challenges hinder Malaysian SMEs from fully capitalizing on this burgeoning industry. This section delves into the primary obstacles encumbering these SMEs, focusing on Halal standards, certification, branding, financing, and marketing issues.

Ineffective Halal Standards and Certifications

The ambiguity surrounding Halal standards **[29]** is primarily because they are being developed by numerous government-affiliated organizations, private organizations, independent Halal certification bodies (HCBs), national standards bodies, regional bodies like the Association of Southeast Asian Nations (ASEAN), and the European Union (EU), and international organizations like the Standards and Metrology Institute for Islamic Countries (SMIIC) or Organisation of Islamic Cooperation (OIC) initiative. Finding a standard that would allow manufacturers access to the market is difficult, and exporters frequently need to acquire multiple certificates.

According to Tawil et al. **[30]**, while Muslim Malaysian entrepreneurs have a positive impression of Halal cuisine, Malaysian SMEs have a negative perception of Halal certification. Even though serving Muslim needs requires Halal certification **[31]**, the absence of an international agreement increases costs and potentially lowers value. Abdul-Talib and Abd-Razak **[32]** show that implementing an ineffective Halal system will immediately raise costs. Due to the need for universally recognized standards among certification bodies, Halal needs to improve international best practices. This frequently results in certification costs being duplicated and an increase in complexity. Malaysian Halal certification must adhere to general guidelines for producing, preparing, handling, and storing Halal foods that adhere to the most widely accepted and established standard to raise the bar further. Other requirements and provisions include the Malaysian Standard MS1500 GMP and GHP. Despite their contribution, SMEs are one of the business entities confronted with several regulatory obstacles, including Halal-standard regulations. Malaysia's SMEs will only be able to survive if Halal standards are upheld. SMEs can only obtain Halal certification if they uphold Halal standards. Zailani et al. **[33]** indicated that management should be crucial in ensuring that workers at all levels know Halal and how to comply with it. Businesses that comply with Halal requirements will produce products of higher quality than those that only adhere to conventional standards.

The increased interest in Halal certification and the Halal logo is a consequence of the rise in the sale of food and beverage items. JAKIM is the only recognized authority in Malaysia that has the authority to issue certain certificates; therefore, it is the only option. Although the validation term for the vast majority of Halal items is two years, many producers continue to use it after it has passed its expiration date. According to Majid et al. **[34]**, this situation occurred due to the lengthy and rigorous renewal procedure, which resulted in new costs. Halal governance and operational efficiency have been affected by the (late renewal) procedure **[35]**.

Another problem that JAKIM must deal with is the rapid pace at which the Halal logo is spread. This may be the case since JAKIM has yet to have a fully-fledged research and development department to test, evaluate, and conduct on-site inspections **[36]**. An external mediator enables the Halal application at a predetermined time. The Halal concept and traceability are closely related ideas; therefore, traceability promotes transparency, ensuring that information is accessible throughout the supply chain **[37]**. Malaysian authorities, for example, have conducted several enforcement programs since the Trade Description Act (TDA) passage in 2011. As a result, unrecognized Halal certificates have been discovered on the packaging of many food products and food manufacturers' properties. Halal certification is mandatory for SMEs that deal with Halal foods. Halal certification is required to earn the trust of Halal food consumers. For SMEs to be close enough to the worldwide Halal food market, they must also have Halal certification.

Moreover, the variations in Halal certification standards among different certifying bodies pose a considerable obstacle to the smooth operation of SMEs. For instance, while Malaysia follows its own standards, countries such as Indonesia **[38]** and Saudi Arabia have variations in permissible ingredients and processing methods. The impact of these inconsistencies becomes particularly evident when it comes to export-oriented SMEs seeking to enter global Halal markets. Different countries have their distinct Halal standards, which can pose difficulties for SMEs in Malaysia as they strive to comply with diverse and occasionally contradictory demands **[39]**, **[40]**. This not only places a strain on the financial resources of these businesses but also hinders their ability to expand their market reach to a global scale. For instance, if a Malaysian SME adheres strictly to the Halal standards established by the Malaysian authorities, it might face challenges when trying to gain acceptance in markets with varying interpretations in global markets. Furthermore, the need for a standardized and widely recognized approach to Halal certification potentially erodes consumer trust. Consumers, both domestically and internationally, may have concerns about the authenticity and reliability of Halal-certified products when confronted with varying standards

[41]. This uncertainty can result in a decline in consumer confidence, adversely impacting Malaysian SMEs' market position and competitiveness in the Halal food industry.

Therefore, the process of certifying products as Halal needs to be streamlined to take less time and be more reliable. JAKIM is the official authority to issue credentials, so they must be more precise in their work. Most Halal products only have two years to be validated, so JAKIM needs to ensure that they are not validating products that are no longer compliant with their standards. They also need to improve the efficiency of the renewal process, and, as a result, manufacturers should see lower expenses associated with the renewal process, and the acceleration of the shift in the infrastructure will benefit the industry **[42]**.

Substandard Packaging and Inadequate Branding

Small businesses also need help with packaging design, which is the primary obstacle in marketing small-capacity products. This is because small businesses are unaware of the impact of intellectual property protection. Hence, Omar **[43]** suggests that the aesthetics of a product's packaging play a significant role in determining whether it will pique the interest of potential buyers. Under intellectual property registration, operators can acquire information on these design elements. Packaging some low-quality goods is believed to demonstrate their superiority. However, poor packaging makes certain high-quality products less attractive to customers. Due to this, operators cannot expand the distribution of their products and compete with products already on the market.

Also, personal savings and family members' internal funds typically comprise the initial capital, which is usually very small. Lenders are unlikely to fund SMEs due to their high risk of failure. SMEs wishing to borrow money should expect to pay high-interest rates due to their higher risk profile **[44]**. Many people will only be able to qualify for the loan if lenders want collateral as a condition of approval. Because they cannot make monthly payments on their financing, some business owners may even be forced to give up and lose their businesses. According to Rao et al. **[45]**, the overall financial systems of both developed and developing nations are heterogeneous. The structure of financial institutions, the lending infrastructure, and the equity market infrastructure significantly impact the availability of funds for SMEs. Financial institutions are more likely to deny SMEs credit, making it harder for them to get money from outside sources. Giyanti et al. **[29]** observed that SMEs need access to external financing to lessen the impact of cash flow issues. SMEs need funding to start and expand their operations, create new products, and invest in new staff or production facilities. Most SMEs are funded internally (through contributions from owners, friends, and family). SME survival and expansion often depend on adequate internal financing. Inadequate capital structure or lack of resources are blamed for many SME failures. Furthermore, Malaysian SMEs, particularly food and beverage manufacturers, must be infrastructurally and technologically ready to exploit the lucrative international Halal market **[20]**, **[46]**. Due to the requirement for collateral, Islamic finance institutions need help to obtain loans.

Marketing Challenges and Innovative Capacity of Human Capital

Marketing challenges for SMEs could arise from the difficulty of tailoring promotional activities to foreign markets, offering technical and after-sales services, dealing with high transportation and insurance expenses, and delivering overseas inventories **[47]**. According to Measson and Campbell-Hunt **[48]**, another key challenge internationalized SMEs face when promoting their products or services is acquiring access to new clients and potential business partners in other countries.

In addition, Razak et al. **[49]** reported that SMEs in Malaysia need more information on the marketing channel, which prevents them from developing marketing networks. Furthermore, they discovered that the main challenges SMEs in Malaysia face when trying to enter the international market are a need for more knowledge about marketing strategies, exporting, branding, customer loyalty, and maintaining good contact with local and international businesses. The study claimed that the use of low-quality raw materials, a lack of quality control, labor skills, and a lack of skill in after-sales service are additional contributing factors.

The success or failure of a small or medium-sized enterprise (SME) is often tied to the peculiarities of its owners, and a lack of marketing expertise is a major contributor to the sector's instability and short lifespan **[50]**. Therefore, it is contended that if a small business owner lacks the requisite skills or information concerning marketing challenges and utilizes those skills, the enterprise may collapse or, at most, will not be as prosperous as it might become. While every component inside a business is crucial, only marketing directly contributes to the profitability and sustainability of the business. Moreover, most of the workers employed by small enterprises need formal education. Thus, they needed help to obtain well-paid positions, and just one possessed a certificate from a post-secondary institution **[51]**.

Lastly, one must possess diverse skills and knowledge relevant to the market's ever-changing characteristics to obtain and maintain a competitive advantage. This suggests that preparation for SMEs and other programs to support businesses should substantially impact leverage and boost competitive advantage by

supporting and promoting the efforts of the individuals involved. In short, the most important areas of expertise to develop include entrepreneurial marketing **[52]**, followed by innovation and technology **[53]**, leadership **[54]**, and production management **[55]**.

Structural Issues with the Financing of SMEs

There are structural issues with innovative SMEs' access to financing for three main reasons. First, innovation may be riskier to finance because of uncertain returns **[56]**. Only a small percentage of businesses typically experience significant growth following investments in innovative activity, and many products fail to be commercialized successfully or fail in the market **[57]**. There needs to be assurance that interest in innovative work (research and development) movement will lead effectively to new products. This problem of uncertainty may be especially acute for SMEs, who lack the scale to invest in multiple projects and thus risk "putting all their eggs in one basket".

Second, due to information asymmetries, banks may need help valuing innovative investments **[58]**. This is in part due to the uncertainties that were mentioned earlier. However, since innovative products are, by definition, brand-new, they necessitate specialized valuations like those provided by venture capitalists (VC). The abilities required to evaluate innovative investments can be highly sector-specific and distinct from those required for other SME lending and investment types. Compared to VC or other outside investors, banks are less interested in the value of the business and are less likely to finance innovation. A key criterion in the bank loan evaluation process is the judgment of "serviceability", or the capacity of an investment's cash flow stream to repay the capital and interest **[59]**.

Third, new advancements could be highly context-specific **[60]**. Research-generated intangible capital may not be useful collateral outside the company **[59]**. For instance, a brand-new process innovation might only be helpful within the company where it is implemented. These factors could make it more difficult for innovative small businesses to obtain financing or force banks to charge more.

Finally, the Malaysian SMEs operating in the Halal food industry encounter various challenges, with a notable emphasis on financial management. One significant hurdle these businesses face is the limited access to financial resources, impeding their growth and long-term sustainability. These SMEs consistently need help in securing financial support from conventional institutions. This restricted access to resources poses a barrier to making crucial investments in technology innovation and market expansion **[60]**.

Trade Barriers and Exchange Currency

Susanty et al. **[61]** highlight that trade barriers remain a formidable challenge for SMEs engaged in international operations. These barriers, often stemming from government-imposed restrictions on exporting and expanding into foreign markets, are collectively known as trade barriers. SMEs face a unique challenge in addressing these barriers, as they are frequently beyond their direct control **[56]**. Moreover, the vulnerability of SMEs to these obstacles is exacerbated by their limited economies of scale. This vulnerability manifests in a higher proportion of total exports being allocated to trade costs, further complicating their ability to operate in international markets effectively.

In addition to government restrictions, SMEs may face difficulties with currency exchange issues. For instance, when Malaysia's currency, the Ringgit Malaysia (RM), experiences weakness, SMEs find it more challenging to compete effectively in international markets **[20]**. The continuous process of globalization has undeniably made the global business landscape more accessible. However, despite this increased accessibility, many businesses, particularly those within the SME sector, lack the necessary resources and capabilities to successfully launch and sustain operations in highly competitive foreign markets.

Any impediment obstructing a company's efforts to establish, expand, or maintain operations abroad can be classified as an export barrier. These barriers can be broadly categorized into internal and external factors **[62]**. In the context of Malaysian SMEs engaged in the manufacturing and exporting Halal processed foods, the severity and prevalence of these export barriers pose significant challenges. Consequently, these SMEs need help to leverage their full export potential in the global market.

POTENTIAL STRATEGIES FOR A WAY FORWARD

To effectively address these challenges, it is necessary to implement a complete set of strategies that provide a road map for Malaysian SMEs operating in the Halal food market to navigate the complex landscape and unlock their growth potential. This assessment intends to give useful insights to stakeholders, policymakers, and industry participants who are invested in the sustainable growth of the Malaysian Halal food sector.

Strengthening Malaysia's Position as a Halal Food Hub

The government is strategically focused on expanding its portfolio of Halal products to enhance Malaysia's standing as an international Halal hub. Positioned at the forefront of the Halal food market, Malaysia is a moderate and progressive Islamic country boasting over 3,500 food companies, contributing to a total production value exceeding USD 9 billion **[63]**. In line with this ambition and recognizing the need for comprehensive infrastructure, Malaysia has established special economic zones known as Halal Malaysia Industrial Parks. These parks are integral to the nation's bid to become the world's leading centre for Halal food development. The Halal Malaysia Industrial Parks serve as dedicated zones designed to attract investments from both domestic and international sources. They offer specialized infrastructure, incentives, and support services tailored to businesses operating in the Halal industry. These initiatives aim to create a conducive environment for the growth and development of various Halal-related activities, encompassing Halal product manufacturing, processing, and distribution.

The government must lead additional initiatives to amplify the impact of these industrial parks. This includes establishing more Halal food parks, integrating Halal practices in slaughterhouses and transportation facilities, providing special import and export facilities, and instituting internationally accepted Halal laws. This integrated approach is important for Malaysia to foster a strong Halal ecosystem, ensuring sustained growth and prominence in the global Halal market. In addition, there are also opportunities to form strategic alliances with other businesses in the ASEAN region within the framework of cooperation in the ASEAN Free Trade Area (AFTA) **[64]**, **[65]**. Malaysian enterprises should actively seek partnerships with these countries to exploit Thailand and Vietnam's much-reduced labor costs and extensive expertise in production, logistics, and export market penetration. This is predicated on the idea that a central location (or "hub") might be used to mass-produce Halal products. The end products of these reverse investment ventures can be marketed in Malaysia, or even the host nation can export them back to Malaysia or elsewhere.

Access to Resources and Global Market

To address the challenges of limited credit and resources, exploring various financing mechanisms, such as peer-to-peer lending, venture capital, or Islamic financing options is crucial. Additionally, fostering collaboration between SMEs and financial institutions to develop tailored financial products that meet industry needs can help overcome capital limitations **[20]**. Promoting government initiatives and support programs to facilitate SMEs' access to financing in the Halal food sector is also essential. This will create a conducive financial environment for long-term and sustainable growth. By addressing these financial obstacles through focused strategies and collaborative efforts, Malaysian SMEs in the Halal food industry can navigate complexities and seize opportunities **[23]**.

Moreover, successfully entering international markets for Malaysian SMEs requires a well-thought-out strategy that considers different cultures, consumer preferences, and regulatory hurdles across multiple countries. Firstly, it is essential to grasp and adapt to a wide range of cultural subtleties **[66]**. Comprehensive market research is crucial for understanding the preferences, values, and communication styles of target audiences in various regions. Customizing products, marketing messages, and business practices to align with specific cultural norms and preferences is necessary. A versatile and customer-focused approach is crucial due to diverse consumer behaviors worldwide. Prioritizing market intelligence to deeply understand consumer behavior and preferences in different target markets **[42]**, **[60]** is vital. Customizing products and services to cater to these needs will boost market acceptance. Secondly, SMEs must stay updated on local regulations, trade policies, and compliance requirements in every target country by creating a well-defined legal and regulatory strategy. Working with local partners can also help in dealing with administrative obstacles.

Expansion of Branding and Modern Marketing

The global interest in Halal food has been growing rapidly in recent years, and this is not because of a rise in the world's Muslim population but rather because the world has come to accept Halal certification as a guarantee of sanitary conditions and a systematised approach to quality assurance **[67**]. Many Halal food businesses would prefer to invest in branding even though awareness and understanding of the products and services they want to sell to the user are the most important **[68]**.

Hence, SMEs must be prepared to embrace change challenges and adapt to modern and trending marketing strategies. Moreover, they should approach these strategies from various perspectives, extending beyond the purely technical aspects. The primary objective is to comprehend the goods and services SMEs aim to communicate to their customers. This can be effectively achieved through rebranding, which distinguishes a brand from its competitors in the market by cultivating a unique identity. Successful rebranding sets a brand apart but also aids SMEs in expanding their customer base **[69]**.

In today's competitive marketplace, generating buzz requires promotional efforts. The market for Halal products can be significantly influenced by advertising campaigns across various media platforms, including newspapers, television ads, magazines, brochures, and any form of social media **[70]**. For burgeoning Halal food companies, leveraging these avenues is crucial to their growth strategy. In the contemporary business landscape, it is more critical than ever for enterprises to capitalize on the myriad free promotional opportunities social media offers **[71]**.

In Malaysia's competitive Halal food business, SMEs must use successful branding strategies to create a unique and recognizable market position. A crucial strategy entails strongly emphasising authenticity and strict adherence to Islamic dietary guidelines, fostering consumer confidence. SMEs should strategically utilize digital platforms and social media to connect with their intended audience, demonstrating their dedication to maintaining high-quality standards and Halal purity. For instance, SimplySiti is a Malaysian cosmetics and skincare company that has effectively utilized social media platforms, especially Instagram and Facebook, to engage with its audience, showcase its products, and communicate its commitment to producing Halal and high-quality beauty products. They often share behind-the-scenes content, customer testimonials, and information about their Halal-certified products, creating a strong online presence and connection with their target audience **[72]**.

Engaging in partnerships with influential individuals (e.g., social media influencers) or respected figures within the local community can enhance the exposure and reputation of a business. In addition, developing a distinctive brand narrative that emphasizes the cultural importance and traditional origins of Halal products helps establish an emotional bond with customers **[67]**. The brand's reputation will be further enhanced by ensuring transparency in the supply chain and acquiring required certifications. By continuously implementing these tactics, SMEs in the Halal food business can establish a specialized market segment and cultivate enduring relationships with their client base.

A Strong Emphasis on E-Commerce

To propel Malaysia as a global digital communication hub for the Halal food industry, the government must establish a dedicated electronic commerce (e-commerce) platform. This strategic move is imperative in the contemporary landscape where e-commerce surpasses traditional in-person shopping. The envisioned e-commerce system should function as the primary information repository for the Malaysian Halal food sector, ensuring that all data is reliable, consistently updated, and easily accessible online. Alternatively, the Malaysian government can strategically establish an e-commerce platform for the Halal food industry by fostering collaboration with existing platforms such as Shopee and Lazada. This partnership would leverage the widespread reach and technological infrastructure of established e-commerce platforms, streamlining the process of creating a dedicated space for Halal products. For example, in 2019, the Indonesian government collaborated with Tokopedia, one of the leading e-commerce platforms in Indonesia, to launch the "Halal Navi" feature. This feature aimed to help Muslim consumers easily find and purchase Halal-certified products **[38]**.

To thrive in Malaysia's fiercely competitive Halal Food Industry, SMEs must strategically embrace e-commerce models to broaden their market reach and enhance overall business performance. Prioritizing investments in user-friendly and secure online platforms specifically designed to meet the unique requirements of the Halal consumer base is crucial. This involves integrating comprehensive product information, certification details, and seamless payment options. Furthermore, adopting a direct-to-consumer (DTC) e-commerce approach can empower SMEs to forge stronger connections with their customers and gather valuable insights for continuous improvements **[73]**. An exemplary case is MyBiz, a Malaysian B2B platform that links local food businesses with international buyers, experiencing significant growth in the Halal food sector. A strategic partnership with JAKIM ensures product listings adhere to Halal standards, attracting buyers from the Middle East and Southeast Asia. The success of MyBiz underscores the potency of B2B platforms in linking Malaysian SMEs with global Halal markets. Successful cases in related industries serve as examples, emphasizing the critical role of digital marketing strategies in enhancing visibility and effectively connecting with the desired audience **[74]**.

Participation in e-commerce is crucial for the growth and expansion of Halal food SMEs, as highlighted by Hidayat et al. **[75]**. The prevalence of e-commerce platforms enables buyers to evaluate and rate products, creating a transparent system for informed consumer choices. Customer feedback becomes a valuable tool for sellers to refine and enhance their offerings based on market preferences. This bidirectional communication fosters a symbiotic relationship, enhancing product quality and customer satisfaction. By embracing e-commerce, buyers and sellers contribute to the growth of the Halal SME business, cementing Malaysia's position as a digital pioneer in the Halal food industry.

Increase in Halal Food Export

According to a study by Abdul et al. **[76]**, globalization has facilitated companies' ease of entry into the global market. Given the substantial and expanding global Halal market, Malaysia needs to enhance its export presence by tapping into key Halal markets to meet the rising demand for Halal-certified products and services **[77]**. Giyanti et al. **[29]** reported that the Halal food and beverage business was valued at USD 1.173 trillion or 16.6 per cent of the global food and beverage sector in 2015. In 2021, its value surged to an estimated USD 1.914 trillion, constituting 18.3 per cent of the global food and beverage market. Furthermore, with approximately 16 million Muslims in Malaysia showing a growing interest in authentic Halal products, the country can boost its export potential and domestic demand. Capitalizing on the increasing spending power of consumers in other Muslim markets is key **[63]**. The demand for Halal products extends beyond the Muslim world, driven by factors such as the healthy lifestyles of non-Muslims and the emigration of Muslims to countries where they are not the majority.

Malaysia's Halal business has witnessed the emergence of new product categories, especially in non-Muslim nations, catering to the needs of Muslim consumers. The country's Halal industry benefits from membership in the OIC, where Halal certification is universally recognized. This positions Malaysia as a favorable first market for international exports, indicating promising prospects for the country's Halal business. Therefore, Malaysia must continue diversifying its Halal product offerings to meet the evolving preferences of Muslim consumers in non-Muslim nations **[78]**. This involves, among other strategies, research and development initiatives to identify market trends and consumer demands, investment in marketing and promotional campaigns, collaboration with international retailers and e-commerce platforms, and strengthening trade agreements and partnerships with non-Muslim nations to facilitate the export of Malaysian Halal products.

Stakeholder Collaboration and International Engagement

The Halal Food Industry in Malaysia thrives when different stakeholders work together, each playing a vital role in overcoming challenges. The government plays a crucial role in establishing and enforcing regulations that create a favorable business environment for SMEs, which includes regulations for Halal certification, financial incentives, and infrastructure support. Large industrial players serve a dual purpose in fostering SME growth by acting as catalysts for knowledge-sharing, providing networking opportunities, and advocating for shared interests **[79]**. Additionally, international organizations significantly contribute to global Halal regulations, fostering trade alliances and enhancing market entry opportunities for Malaysian SMEs.

The collaboration among these key stakeholders forms a comprehensive framework, ensuring the adaptability and success of SMEs in the dynamic environment of the Halal Food Industry in Malaysia. Notable industry bodies, such as the Malaysian Food Industry Council (MFIC) and the Halal Industry Development Corporation (HDC), are pivotal in facilitating collaboration, knowledge exchange, and collective issue resolution. These associations provide networking opportunities for SMEs, enabling them to leverage shared knowledge, enter new markets, and stay informed about industry developments. Furthermore, industry organizations actively promote sharing best practices among SMEs through regular discussions and joint initiatives. This support aids SMEs in enhancing competitiveness and overcoming common obstacles such as market access, technology adoption, and quality **[79]**, **[80]**.

For the growth and sustainability of SMEs in the Halal Food Industry, collaboration with global organizations and adherence to international standards are imperative. Malaysia actively collaborates with international bodies such as the World Halal Council and the OIC to establish consistent Halal standards, ensuring seamless market entry and recognition. Engaging in international trade fairs and agreements facilitates forming partnerships across borders, creating opportunities for SMEs to showcase their products and attract foreign investments **[38]**.

CONCLUSION

The Halal food business in Malaysia has excellent potential for growth in domestic and international markets. With the Muslim population increasing rapidly, the Halal food sector offers great potential for SMEs in Malaysia to expand. SMEs can reach their full potential by meeting customer demands, entering the international market from within the domestic market, and receiving government assistance and support. While experiencing rapid growth, Malaysia's Halal food sector faces challenges hindering the full potential of SMEs. Ineffective Halal standards, certification complexities, substandard packaging, and limited branding awareness pose significant hurdles. Streamlining Halal certification, emphasizing universally recognized standards, and supporting SMEs in branding initiatives are critical steps. Structural financing issues, trade barriers, and marketing challenges further impede SMEs. Addressing financial constraints, tackling trade barriers, and providing marketing education are essential for SMEs to thrive globally. Enhancing human capital with training in entrepreneurial marketing, innovation, and production management is crucial for SME success. Strategies are proposed to navigate these challenges and pave the way forward. Strengthening Malaysia's position as a Halal Food Hub involves expanding

the Halal product portfolio, creating dedicated industrial parks, and forming strategic alliances within the ASEAN region. Additionally, emphasizing branding, modern marketing, e-commerce, and increasing Halal food exports is crucial for Malaysia's sustained growth in the global Halal market. Malaysia has taken numerous branding, marketing, and other initiatives to promote its Halal products. The government effectively controls Halal development initiatives using entities such as Jabatan Kemajuan Islam Malaysia (JAKIM) and the Halal Development Corporation (HDC). The way Malaysia has developed its Halal food business is quite astounding. Malaysia has had great success in this field on the domestic front and is ready to take its expertise to new markets abroad. Policymakers should promote internationalization to inspire SMEs, as it provides opportunities for expansion into new markets and, ultimately, greater profits. Malaysia can dominate this industry if its SMEs are better equipped with knowledge. Preferential treatment for SMEs could be offered through market development and funding, attractive incentives to stimulate increased export volumes, and efforts to streamline export procedures.

REFERENCES

1. Harinderan, K. (2023). Commentary: Malaysia's Halal Food Industry's Exponential Growth Continues to Soar. In Business Today. Reach Publishing. Retrieved from https://www.businesstoday.com.my/2023/07/14/commentary-malaysias-Halal-food-industrys-exponential-growth-continues-to-soar/.
2. Suleman, S., Sibghatullah, A., & Azam, M. (2021). Religiosity, halal food consumption, and physical well-being: An extension of the TPB. In P. Foroudi (Ed.), Cogent Business & Management (Vol. 8, Issue 1). Informa UK Limited. https://doi.org/10.1080/23311975.2020.1860385
3. Al-Shami, H. A., & Abdullah, S. (2023). Halal food industry certification and operation challenges and manufacturing execution system opportunities. A review study from Malaysia. In Materials Today: Proceedings (Vol. 80, pp. 3607–3614). Elsevier BV. https://doi.org/10.1016/j.matpr.2021.07.331
4. Elseidi, R. I. (2018). Determinants of Halal purchasing intentions: evidences from UK. In Journal of Islamic Marketing (Vol. 9, Issue 1, pp. 167–190). Emerald. https://doi.org/10.1108/jima-02-2016-0013
5. Wan Sulaiman, W. S. H., Md Zaini, H. H., & Othman, R. (2023). Mini review on Halal Food Colorants and Potential Sources. In Halalpshere (Vol. 3, Issue 1, pp. 20–26). IIUM Press. https://doi.org/10.31436/hs.v3i1.59
6. Halal Development Corporation Berhad (HDC). (2023). Sustaining Malaysia as the Global Leader in the Halal Industry, HDC Official Portal. Retrieved from https://hdcglobal.com/news/2023/08/21/sustaining-malaysia-as-the-global-leader-in-the-Halal-industry/.
7. Parid, D. M., Amin, S. M. A., Adenan, H., & Dina, S. M. (2021). The challenges of a small medium entrepreneur in Melaka hub Halal to penetrate the external market. In Journal of Hospitality and Networks (Vol. 1, Issue 1, pp. 80–94). University Melaka.
8. Famiza, A. N., Samsinar, K. N., Kursimah, H., & Firdaus, R. B. R. (2017). Buying Halal pharmaceutical products: do consumers have imperfect knowledge? In Pertanika Journal of Social Sciences & Humanities (Vol. 25, Issue 8, pp. 153–164). Universiti Putra Malaysia.
9. Ismail, M. M., Islam, M. S., & Bakar, W. M. M. W. (2013). Export barriers to Halal food processing small and medium enterprises (SMEs) in Malaysia. In Pertanika Journal of Social Sciences and Humanities (Vol. 21, pp. 31–46). Universiti Putra Malaysia.
10. Randeree, K. (2019). Challenges in Halal food ecosystems: the case of the United Arab Emirates. In British Food Journal (Vol. 121, Issue 5, pp. 1154–1167). Emerald. https://doi.org/10.1108/bfj-08-2018-0515
11. Azam, M. S. E., & Abdullah, M. A. (2020). Halal industry: realities and opportunities. In International Journal of Islamic Business Ethics (Vol. 5, Issue 1, p. 47). Universitas Islam Sultan Agung. https://doi.org/10.30659/ijibe.5.1.47-59
12. Adekunle, B., & Filson, G. (2020). Understanding Halal food market: Resolving asymmetric information. In Food Ethics (Vol. 5, Issues 1–2). Springer Science and Business Media LLC. https://doi.org/10.1007/s41055-020-00072-7
13. Okpala, C. O. R., & Korzeniowska, M. (2021). Understanding the relevance of quality management in agro-food product industry: from ethical considerations to assuring food hygiene quality safety standards and its associated processes. In Food Reviews International (Vol. 39, Issue 4, pp. 1879–1952). Informa UK Limited. https://doi.org/10.1080/87559129.2021.1938600
14. Ziegler, Y., Uli, V., & Tatari, M. (2022). Implementing Halal logistics in a non-Muslim-dominant environment: a proposal for reengineering the business processes in two stages. In Business Process Management Journal (Vol. 28, Issue 8, pp. 48–65). Emerald. https://doi.org/10.1108/bpmj-12-2020-0593

15. Mohd Sulaiman, M. Z., Noordin, N., Md Noor, N. L., Suhaimi, A. I. H., & Wan Mohd Isa, W. A. R. (2019). Halal virtual inspection critical control point. In International Journal on Perceptive and Cognitive Computing (Vol. 5, Issue 2, pp. 87–96). IIUM Press. https://doi.org/10.31436/ijpcc.v5i2.96
16. Soon, J. M., Chandia, M., & Regenstein, J. M. (2017). Halal integrity in the food supply chain. In British Food Journal (Vol. 119, Issue 1, pp. 39–51). Emerald. https://doi.org/10.1108/bfj-04-2016-0150
17. Department Islamic Development Malaysia. (2023). The Recognised Foreign Halal Certification Bodies & Authorities. In Halal Hub Division (pp. 1–54). Retrieved from https://www.islam.gov.my/images/documents/CB_List_Januari_2018.pdf.
18. Al-Teinaz, Y. R., Spear, S., & Abd El-Rahim, I. H. A. (Eds.). (2020). The Halal Food Handbook. Wiley. https://doi.org/10.1002/9781118823026
19. Wilson, J. A. J. (2014). The Halal phenomenon: An extension or a new paradigm? In Social Business (Vol. 4, Issue 3, pp. 255–271). Westburn Publishers. https://doi.org/10.1362/204440814x14103454934294
20. Mahidin, N., Saifudin, A. M., & Othman, S. N. (2017). Halal food logistics: The challenges among food & beverages small and medium sizes manufacturers. In International Journal of Supply Chain Management (Vol. 6, Issues 3, pp. 337–346). ExcelingTech Publishers.
21. Department of Statistics Malaysia (DOSM). (2023). Halal Statistics, DOSM Official Portal. Retrieved from https://v1.dosm.gov.my/v1/index.php?r=column/cone&menu_id=LzRkYXlmRElhQVF2cEdhNWZLSXVhdz09.
22. Vanninen, H., Keränen, J., & Kuivalainen, O. (2022). Becoming a small multinational enterprise: Four multinationalization strategies for SMEs. In International Business Review (Vol. 31, Issue 1, p. 101917). Elsevier BV. https://doi.org/10.1016/j.ibusrev.2021.101917
23. Pahnke, A., Welter, F., & Audretsch, D. B. (2022). In the eye of the beholder? Differentiating between SMEs and Mittelstand. In Small Business Economics (Vol. 60, Issue 2, pp. 729–743). Springer Science and Business Media LLC. https://doi.org/10.1007/s11187-022-00612-x
24. Organisation for Economic Co-operation and Development (OECD). (2022). Financing SMEs and Entrepreneurs 2022: An OECD Scoreboard. OECD Publishing.
25. Hasan, H., Othman, I. W., Topimin, S., Ahmad, S. N. B., & Buncha, M. R. (2022). Production of Halal products: Fardhu kifayah among Muslim SMES, contribution to Muslim communities. In International Journal of Accounting (Vol. 7, Issues 5, pp. 259–268). Association for Academic Research and Innovation.
26. Kim-Soon, N., Mostafa, S. A., Nurunnabi, M., Chin, L. H., Kumar, N. M., Ali, R. R., & Subramaniam, U. (2020). Quality management practices of food manufacturers: a comparative study between small, medium, and large companies in Malaysia. In Sustainability (Vol. 12, Issue 18, p. 7725). MDPI AG. https://doi.org/10.3390/su12187725.
27. Mohamad, A., Mustapa, A. N., & Razak, H. A. (2021). An overview of Malaysian small and medium enterprises: contributions, issues, and challenges. in modelling economic growth in contemporary Malaysia. In Modelling Economic Growth in Contemporary Malaysia (pp. 31–42). Emerald Publishing Limited. https://doi.org/10.1108/978-1-80043-806-420211004
28. SME Corporation Malaysia. (2020). SME Corp. Malaysia Annual Report 2019. Retrieved from https://www.smecorp.gov.my/images/AnnualReport/2019/SMECorpMsiaAR_2019.pdf.
29. Giyanti, I., Indrasari, A., Sutopo, W., & Liquiddanu, E. (2020). Halal standard implementation in food manufacturing SMEs: its drivers and impact on performance. In Journal of Islamic Marketing (Vol. 12, Issue 8, pp. 1577–1602). Emerald. https://doi.org/10.1108/jima-11-2019-0243
30. Tawil, N. M., Ramlee, S., Jaafar, J., & Saat, F. M. (2015). An overview of foodpreneur awareness among small and medium-sized enterprises (SME) of Halal certification. In Asian Social Science (Vol. 11, Issue 21). Canadian Center of Science and Education. https://doi.org/10.5539/ass.v11n21p91
31. Ab Talib, M. S., Md. Sawari, S. S., Abdul Hamid, A. B., & Ai Chin, T. (2016). Emerging Halal food market: an institutional theory of Halal certificate implementation. In Management Research Review (Vol. 39, Issue 9, pp. 987–997). Emerald. https://doi.org/10.1108/mrr-06-2015-0147
32. Abdul-Talib, A., & Abd-Razak, I. (2013). Cultivating export market oriented behavior in Halal marketing. In Journal of Islamic Marketing (Vol. 4, Issue 2, pp. 187–197). Emerald. https://doi.org/10.1108/17590831311329304
33. Zailani, S., Iranmanesh, M., Jafarzadeh, S., & Foroughi, B. (2019). The influence of Halal orientation strategy on financial performance of Halal food firms. In Journal of Islamic Marketing (Vol. 11, Issue 1, pp. 31–49). Emerald. https://doi.org/10.1108/jima-01-2018-0023
34. Majid, M. A. A., Abidin, I. H. Z., Majid, H. A. M. A., & Chik, C. T. (2015). Issues of Halal food implementation in Malaysia. In Journal of Applied Environmental and Biological Sciences (Vol. 5, Issues 6, pp. 50–56). Textroad Publishing Co.

35. Firdaus, A., Azmi, M. N., Farahanim, N., Radzi, A., & Samicho, Z. (2016). Halal food warehouse: challenges and solutions. In Journal Applied Environmental Biology Science (Vol. 6, Issue 11S, pp. 80–85). Dr. Steph O. Jhanathin USA.
36. Ihwan, D. (2021). Implementation Halal food issues in Malaysia. In International Journal Law and Legal Ethics (IJLLE) (Vol. 2, Issue 2, pp. 69–80). Universitas Duta Bangsa.
37. Ab Rashid, N., & Bojei, J. (2019). The relationship between Halal traceability system adoption and environmental factors on Halal food supply chain integrity in Malaysia. In Journal of Islamic Marketing (Vol. 11, Issue 1, pp. 117–142). Emerald. https://doi.org/10.1108/jima-01-2018-0016
38. Giyanti, I., & Indriastiningsih, E. (2019). Impact of Halal certification on the performance of food small and medium enterprises. In Jurnal Ilmiah Teknik Industri (Vol. 18, Issue 2, pp. 116–123). Universitas Muhammadiyah Surakarta. https://doi.org/10.23917/jiti.v18i2.7242
39. Abdallah, A., Rahem, M. A., & Pasqualone, A. (2021). The multiplicity of Halal standards: a case study of application to slaughterhouses. In Journal of Ethnic Foods (Vol. 8, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s42779-021-00084-6
40. Man, S., & Pauzi, N. (2017). The Implication of Differences in Halal Standard of Malaysia, Indonesia, Brunei and Singapore. In The Journal of Muamalat and Islamic Finance Research (Vol. 14, Issue 2, pp. 157–170). Universiti Sains Islam Malaysia. https://doi.org/10.12816/0045784
41. Zakaria, Z., Abdul Majid, M. D., Ahmad, Z., Jusoh, Z., & Zakaria, N. Z. (2018). Influence of Halal certification on customers' purchase intention. In Journal of Fundamental and Applied Sciences (Vol. 9, Issue 5S, p. 772). African Journals Online (AJOL). https://doi.org/10.4314/jfas.v9i5s.55
42. Abdul Mokti, H., Kamri, N. A., & Mohd Balwi, M. A. W. F. (2023). Tayyiban in halal food production: a systematic literature review. In Journal of Islamic Marketing (Vol. 15, Issue 2, pp. 397–417). Emerald. https://doi.org/10.1108/jima-03-2022-0098
43. Omar, C. M. Z. C. (2013). Challenges and marketing strategies of Halal products in Malaysia. In Interdisciplinary Journal of Research in Business (Vol. 3, Issue 2, pp. 11–17). Center for Research Promotion.
44. Hidayah, K., Daud, D., Zainal, N., & Ibrahim, S. S. (2021). Effectiveness of Musharakah Financing in Empowering Small Micro Enterprises. In Studies of Applied Economics (Vol. 39, Issue 10). Editorial Universidad de Almeria. https://doi.org/10.25115/eea.v39i10.5847
45. Rao, P., Kumar, S., Chavan, M., & Lim, W. M. (2021). A systematic literature review on SME financing: Trends and future directions. In Journal of Small Business Management (Vol. 61, Issue 3, pp. 1247–1277). Informa UK Limited. https://doi.org/10.1080/00472778.2021.1955123
46. Ab Talib, M. S., & Zulfakar, M. H. (2023). Sustainable Halal food supply chain management in a small rentier Halal market. In Arab Gulf Journal of Scientific Research. Emerald. https://doi.org/10.1108/agjsr-11-2022-0251
47. Naradda Gamage, S. K., Ekanayake, E., Abeyrathne, G., Prasanna, R., Jayasundara, J., & Rajapakshe, P. (2020). A review of global challenges and survival strategies of small and medium enterprises (SMEs). In Economies (Vol. 8, Issue 4, p. 79). MDPI AG. https://doi.org/10.3390/economies8040079
48. Measson, N., & Campbell-Hunt, C. (2015). How SMEs use trade shows to enter global value chains. In Journal of Small Business and Enterprise Development (Vol. 22, Issue 1, pp. 99–126). Emerald. https://doi.org/10.1108/jsbed-09-2011-0025
49. Razak, D. A., Abdullah, M. A., & Ersoy, A. (2018). Small medium enterprises (SMEs) in Turkey and Malaysia: a comparative discussion on issues and challenges. In International Journal of Business, Economics, and Law (Vol. 10, Issue 49, pp. 1–10). ZES ROKMAN RESOURCES.
50. Lazaris, M., & Freeman, S. (2018). An examination of global mindset and international market opportunities among SMEs. In International Studies of Management and Organization (Vol. 48, Issue 2, pp. 181–203). Informa UK Limited. https://doi.org/10.1080/00208825.2018.1443739
51. Hendijani Fard, M., & Seyyed Amiri, N. (2018). The effect of entrepreneurial marketing on Halal food SMEs performance. In Journal of Islamic Marketing (Vol. 9, Issue 3, pp. 598–620). Emerald. https://doi.org/10.1108/jima-12-2016-0097
52. Kamarulzaman, N. H., Muhamad, N. A., & Mohd Nawi, N. (2021). An investigation of adoption intention of Halal traceability system among food SMEs. In Journal of Islamic Marketing (Vol. 13, Issue 9, pp. 1872–1900). Emerald. https://doi.org/10.1108/jima-11-2020-0349
53. Ismail, M., Mohamad, N., & Ahamat, A. (2020). Top management capabilities and performance of Halal product. In Innovation & Management Review (Vol. 17, Issue 4, pp. 431–446). Emerald Publishing.

54. Ali, M. H., & Suleiman, N. (2016). Sustainable food production: Insights of Malaysian Halal small and medium sized enterprises. In International Journal of Production Economics (Vol. 181, pp. 303–314). Elsevier BV. https://doi.org/10.1016/j.ijpe.2016.06.003

55. Nor, N. G. M., Bhuiyan, A. B., Said, J., & Alam, S. S. (2016). Innovation barriers and risks for food processing SMEs in Malaysia: A logistic regression analysis. In Geografia: Malaysian Journal of Society and Space (Vol. 1, Issue 2, pp. 167–178). Universiti Kebangsaan Malaysia.

56. Ahmad-Fauzi, N. R. H., & Md Saad, N. (2023). SMEs' intangible resources and their effects on export performance: a study on Malaysian halal F&B sector. In Journal of Islamic Marketing (Vol. 15, Issue 2, pp. 595–612). Emerald. https://doi.org/10.1108/jima-01-2023-0021

57. Suat, N., Mohd, T., & Yaacob, H. (2021). Information asymmetry and industry 4.0 among small and medium enterprise (SME) in Malaysian Halal industry. (2021). In Jurnal Pengurusan (Vol. 63). Penerbit Universiti Kebangsaan Malaysia (UKM Press). https://doi.org/10.17576/pengurusan-2021-63-03

58. Mina, A., Lahr, H., & Hughes, A. (2013). The demand and supply of external finance for innovative firms. In Industrial and Corporate Change (Vol. 22, Issue 4, pp. 869–901). Oxford University Press (OUP). https://doi.org/10.1093/icc/dtt020

59. Jafari-Sadeghi, V., Amoozad Mahdiraji, H., Bresciani, S., & Pellicelli, A. C. (2021). Context-specific micro-foundations and successful SME internationalisation in emerging markets: A mixed-method analysis of managerial resources and dynamic capabilities. In Journal of Business Research (Vol. 134, pp. 352–364). Elsevier BV. https://doi.org/10.1016/j.jbusres.2021.05.027

60. Umar, U. H., & Al-Faryan, M. A. S. (2023). The impact of working capital management on the profitability of listed halal food and beverage companies. In Managerial Finance (Vol. 50, Issue 3, pp. 534–557). Emerald. https://doi.org/10.1108/mf-12-2022-0606

61. Susanty, A., Puspitasari, N. B., Caterina, A. D., & Jati, S. (2020). Mapping the barriers for implementing Halal logistics in Indonesian food, beverage and ingredient companies. In Journal of Islamic Marketing (Vol. 12, Issue 4, pp. 649–669). Emerald. https://doi.org/10.1108/jima-11-2019-0244

62. Mohamad, A., Mohd Rizal, A., Kamarudin, S., & Sahimi, M. (2022). Exploring the co-creation of small and medium enterprises, and service providers enabled by digital interactive platforms for internationalization: a case study in Malaysia. In Sustainability (Vol. 14, Issue 23, p. 16119). MDPI AG. https://doi.org/10.3390/su142316119

63. Mohd Fauzi, N., Hashim, N. I., Ab Rahman, M. F., Wan Hassin, W. S., & Shah Shahar, W. S. (2022). Halal economic: challenges and emerging opportunities in Malaysia. In Journal of Islamic Philanthropy and Social Finance (Vol. 1, Issue 1, pp. 29–37). UiTM Press, Universiti Teknologi MARA. https://doi.org/10.24191/jipsf/v1n12020_29-37

64. Abdullah, M. A., & Azam, M. S. E. (2020). Halal Industry in ASEAN. In Economics, Business, and Islamic Finance in ASEAN Economics Community (pp. 77–104). IGI Global. https://doi.org/10.4018/978-1-7998-2257-8.ch005

65. Robbani, H. (2021). Development of the ASEAN qualification recognition framework (AQRF) on Halal management standardization as a success strategy for ASEAN free trade area (AFTA). In Literatus (Vol. 3, Issue 2, pp. 180–190). Neolectura. https://doi.org/10.37010/lit.v3i2.404

66. Othman, B., Shaarani, S. M., & Bahron, A. (2016). Evaluation of knowledge, Halal quality assurance practices and commitment among food industries in Malaysia. In British Food Journal (Vol. 118, Issue 8, pp. 2033–2052). Emerald. https://doi.org/10.1108/bfj-12-2015-0496

67. Secinaro, S., Radwan, M., Calandra, D., & Biancone, P. (2021). Halal certification impact on firms' corporate social responsibility disclosure: Evidence from the food & beverage sector in Italy. In Corporate Social Responsibility and Environmental Management (Vol. 28, Issue 4, pp. 1376–1385). Wiley. https://doi.org/10.1002/csr.2161

68. HANIM, A., ABDUL, S., & SALWA, U. (2015). Issues and Challenges of Halal Implementation in Food Industry in Malaysia. In Third International Conference on Advances in Economics, Management and Social Study - EMS 2015. Third International Conference on Advances in Economics, Management and Social Study - EMS 2015. Institute of Research Engineers and Doctors. https://doi.org/10.15224/978-1-63248-058-3-53

69. Demong, N. A. R., Kassim, E. S., Noor, N. A., Yunus, M., Shahrom, M., & Jailani, S. F. A. K. (2021). Issues and challenges in rebranding of Malaysian street markets. In International Journal of Business and Globalisation (Vol. 28, Issue 1/2, p. 206). Inderscience Publishers. https://doi.org/10.1504/ijbg.2021.115305

70. Ab. Wahab, N., & Kamarubahrin, A. F. (2019). Halal Industry: Are The Businesses Fully Awake? In Journal of Fatwa Management and Research (pp. 21–35). Universiti Sains Islam Malaysia. https://doi.org/10.33102/jfatwa.vol16no1.216

71. Aziz, A. A., Md Rahin, N., & Mohamed Asri, N. (2019). Halal advertising on Facebook: a case study of health and beauty products in Malaysia. In Management for Professionals. (pp. 177–192). Springer International Publishing. https://doi.org/10.1007/978-3-030-10907-3_16

72. Rosnan, H., & Roslin, R. M. (2014). Fighting the giants: the case of a new player. In South Asian Journal of Business and Management Cases (Vol. 3, Issue 2, pp. 129–138). SAGE Publications. https://doi.org/10.1177/2277977914548344

73. Hassan, S. H., Mohamed Haniba, N. M., & Ahmad, N. H. (2019). Social customer relationship management (s-CRM) among small- and medium-sized enterprises (SMEs) in Malaysia. In International Journal of Ethics and Systems (Vol. 35, Issue 2, pp. 284–302). Emerald. https://doi.org/10.1108/ijoes-11-2017-0192

74. Hassan, Z. (2007). Entrepreneur development for competitive SMEs: development strategy. In Asian Productivity Organization.

75. Hidayat, S. E., Rafiki, A., & Nasution, M. D. T. P. (2021). Halal industry's response to a current and post-COVID-19 landscape and lessons from the past. In Journal of Islamic Marketing (Vol. 13, Issue 9, pp. 1843–1857). Emerald. https://doi.org/10.1108/jima-06-2020-0180

76. Abdul, M., Ismail, H., Mustapha, M., & Kusuma, H. (2013). Indonesian small medium enterprises (SMEs) and perceptions on Halal food certification. In African Journal of Business Management (Vol. 7, Issue 16, pp. 1492–1500). Academic Journals.

77. Kabir, S., Shams, S., & Lawrey, R. (2020). Trade diversion risk for Halal food exports. In Journal of Islamic Marketing (Vol. 12, Issue 4, pp. 705–729). Emerald. https://doi.org/10.1108/jima-07-2019-0145

78. Mohd Nawawi, M. S. A., Abu-Hussin, M. F., Faid, M. S., Pauzi, N., Man, S., & Mohd Sabri, N. (2019). The emergence of Halal food industry in non-Muslim countries: a case study of Thailand. In Journal of Islamic Marketing (Vol. 11, Issue 4, pp. 917–931). Emerald. https://doi.org/10.1108/jima-05-2018-0082

79. Ariffin, N., Sundram, V. P. K., & Zulfakar, M. H. (2023). Halal Purchasing: A Qualitative In-depth Interview Towards Small and Medium Food Enterprises (SMFEs). In International Journal of Research in Business and Social Science (Vol. 11, Issues, 9, pp. 184–201). Atlantis Press International BV. https://doi.org/10.2991/978-94-6463-342-9_14

80. Hanim Yusuf, A., Abdul Shukor, S., & Salwa Ahmad Bustamam, U. (2016). Halal certification vs business growth of food industry in Malaysia. In Journal of Economics, Business and Management (Vol. 4, Issue 3, pp. 247–251). EJournal Publishing. https://doi.org/10.7763/joebm.2016.v4.399

Funds:

This research received external funding from the Ministry of Higher Education Malaysia for the Fundamental Research Grant Scheme. The project code is FRGS/1/2023/WAB04/USM/02/4.

Acknowledgements:

Acknowledgement to Ministry of Higher Education Malaysia for Fundamental Research Grant Scheme with Project Code: FRGS/1/2023/WAB04/USM/02/4 and Barokah Food Plt with Project Code: 6501398.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Sorna Umme Saima, Universiti Sains Malaysia, School of Social Sciences, Department of Development Planning and Managemnet, 11800 USM, Gelugor, Pulau Pinang, Malaysia,
Tel.: +60168554593
E-mail: saima.sorna@student.usm.my
ORCID: https://orcid.org/0009-0008-3284-6621

***Radin Badaruddin Radin Firdaus**, Universiti Sains Malaysia, School of Social Sciences, Department of Development Planning and Management, 11800 USM, Gelugor, Pulau Pinang, Malaysia,
Tel.: +60193874182
E-mail: radin@usm.my
ORCID: https://orcid.org/0000-0001-7022-0576

Sarjiyanto, Universitas Sebelas Maret, Fakultas Ekonomi dan Bisnis, Surakarta, 57126, Indonesia,
Tel.: +6281393124000
E-mail: masyanto@staff.uns.ac.id
ORCID: https://orcid.org/0000-0002-3912-1331

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 15.12.2023
Revised: 6.2.2024
Accepted: 11.2.2024
Published: 28.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 239-250
https://doi.org/10.5219/1953
ISSN: 1337-0960 online
www.potravinarstvo.com

Improvement of the quality of pork meat during salting due to the use of starter bacterial cultures

Svitlana Danylenko, Liana Nedorizanyuk, Oksana Potemska, Tsvitana Korol, Anastasiia Ivaniuta, Yuliya Kryzhova, Valentyna Israelian, Nataliia Kovalenko

ABSTRACT

The influence of the starter cultures, such as *Lactobacillus rhamnosus, L. plantarum, Kocuria rosea, Staphylococcus carnosus, L. plantarum, L. rhamnosus* and *L. paracasei,* on the functional-technological and physicochemical characteristics of the pork meat during the salting *is* investigated in this paper. It has been proven that the use of these starter cultures in the technology of raw ba-lik products makes it possible to obtain finished products with improved quality indicators, which is promising in the food industry. It had been shown that in the pork meat samples with the starter cultures, the acidity from 5.74 pH units is more intensively decreased – to 5.52 pH units compared to the control sample (up to 5.64 pH units). Using the starter cultures based on nitrite-reducing microorganisms for the salting in 72 hours positively influenced the formation of the required colour characteristics. It had been established that the moisture-binding capacity and plasticity of the pork meat samples with the starter cultures are characterised by the increased indicators compared with the control sample – by 4.73% and 7.73% and by 2.19 cm^2.g, respectively. The difference in the volatile fatty acids content in the pork meat samples with the starter cultures compared with the control sample is 22 and 33%, respectively, in 72 hours of salting. The obtained results can be used in the enterprises of the meat processing industry to produce fermented meat products, particularly raw dried logs.

Keywords: salting, starter culture, meat fermentation, lactic-acid bacteria, coagulase-negative staphylococci

INTRODUCTION

Lactic acid bacteria and coagulase-negative staphylococci or micrococci are used in the composition of the starter cultures for the fermentation process in the technology of the meat products. The most common types of lactic-acid bacteria during the fermentation process, when the sausages are produced, are *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus plantarum* and *Lactobacillus casei* **[1]**, **[2]**, which, due to their fermentative, proteolytic and lipolytic activity, will improve the structures and consistencies of the meat products, the recovery of nitrates to nitrites, the formation of nitrosomyoglobin, the dehydration and inhibition of lipid oxidation **[3]**, **[4]**.

A traditional pleasant taste of fermented meat products is achieved due to a large content of lactic bacteria. Researchers associate the participation of catalase-positive cocci in the aroma development process with the high biochemical activity of these microorganisms **[5]**.

Volatile low-molecular fatty acids contribute to forming a pronounced taste and are formed under lipolytic activity **[6]**, **[7]**. Some scientists studied the technological criteria that must be included in selecting lactic acid bacteria for producing fermented meat **[8]**.

The aroma development in fermented sausages mainly occurs throughout the ripening process, and the influence of biochemical reactions on the aroma depends on microbial diversity, which is strongly influenced by the production conditions **[9]**.

During fermentation, the metabolites of lactic bacteria perform antibacterial and antioxidant functions and improve the physical and chemical qualities of the fermented meat products **[10]**.

Culler et al. **[11]** proved that using starter cultures based on *L. curvatus* and *S. xylosus* makes it possible to produce salami with a lower fat and salt content while providing satisfactory product quality.

Rodríguez-González et al. **[12]** studied the influence of the addition of two different autochthonous starter cultures, including the strain of *Lactobacillus sakei* and *Staphylococcus equorum* or *Staphylococcus saprophyticus* on the biochemical changes, which are occurred during the production of Galician chorizo sausage. They confirmed the influence of the quality improvement and safety of such products.

When the meat is salted, the salting substances are penetrated, distributed, and accumulated in the meat, as well as the chemical and fermentative processes are developed with the formation of the taste and aromatic substances. Adding starter cultures to the brine can increase the safety of fermented meat products due to the rapid acidulation of the matrix or the production of antimicrobial substances such as bacteriocins.

Therefore, the use of the starter cultures from the strains, which were recovered from spontaneous meat products, their influence on the microbiological, and physicochemical properties, and the safety of various types of fermented meat products is the highly topical issue of the study.

Scientific Hypothesis

The scientific hypothesis lies in the fact that the technology of the fermented meat products can be optimized by salting the meat raw materials with the addition of the starter cultures within 72 hours. Such changes will increase the functional-technological characteristics after the end of the complete fermentation process.

MATERIAL AND METHODOLOGY

Samples

The pork meat of Poltava meat breed - according to DSTU 7158:2010 **[13]**, the starter cultures: *Lactobacillus rhamnosus* (LLC "Chr. Hansen", Denmark), *L. plantarum*, *L. paracasei* ("MKS", Ukraine), *Staphylococcus carnosus* ("Van Hees", Germany), edible salt according to DSTU 3583 **[14]**, white sugar according to DSTU 4623, sodium nitrite.

The pork back muscle of Poltava meat breed aged 7 months (supplier "Agro Plus", Cherkasy region, Ukraine) was used for the salting.

Chemicals

Potassium hydroxide, KOH (brand A, analytic grade, LLC "Khimlaborreaktiv", Ukraine), acetone (brand A, LLC "Khimlaborreaktiv", Ukraine).

Animals, Plants and Biological Materials

The pork meat of Poltava meat breed, the starter cultures: *Lactobacillus rhamnosus* (LLC "Chr. Hansen", Denmark), *L. plantarum, L. paracasei* ("MKS", Ukraine), *Staphylococcus carnosus* ("Van Hees", Germany).

Instruments

pH-meter (MP 512 manufacturer (LLC Ulab", China), analyzer of water activity "Aqua Lab", series TE METER ("Group Inc", USA), spectrophotometer Unico S 2100 ("United products &instruments", USA), steam-distillation apparatus PSD 1 (LLC "Chimlaborreaktiv", Ukraine), Digital laboratory thermometer TH310 Milwaukee (LLC "SPECTRO LAB", Ukraine), laboratory scales AXIS BDM 3 (LLC "SPECTRO LAB", Ukraine), conical flask (CF-100, CF-150, CF-200, CF-250, CF-500, producer (Laboratory equipment) Limited Liability Company, Ukraine), burette for titration (producer (Laboratory equipment) Limited Liability Company, Ukraine), filters (producer (Laboratory equipment) Limited Liability Company, Ukraine).

Laboratory Methods

The pH value was measured by the potentiometric method (DSTU ISO 2917) **[15]** after mixing 10 g of the sample with 90 ml of distilled water. The meat samples' water activity (aw) was measured using the method **[16]**. The pressing method determined the moisture-binding capacity, which consists of extracting water from the test sample during pressing, sorbing the extracted water with filter paper, and determining the amount of separated moisture by the size of the spot left on the filter paper. Plasticity was determined by the area of the minced meat spot formed on an ashless filter under the action of a static load of 1 kg for 10 minutes **[17]**.

The amount of nitric oxide pigments was determined by extraction with the use of the aqueous solution of acetone; the color stability was determined based on the difference in the optical density of the extracts of nitric oxide pigments before and after exposure under light source at a wavelength of 540 nm **[16]**.

Volatile fatty acids were determined by recovering such volatile fatty acids that accumulated in the pork meat during its salting and by determining its amount by titrating the distillate with potassium hydroxide. Volatile fatty

acids were recovered with the use of a steam distillation device. The number of volatile fatty acids (X) in milligrams of potassium hydroxide per 100 g of the meat is calculated by the following formula:

$$X=(Y-Y_0)\times K\times 5.61\times 100/M$$

Where:
Y and Y_0 amount is 0.1 n. of potassium hydroxide solution, which was used for the titration of 200 ml of the distillate from the meat and the control sample, respectively, in ml; K is the correction of titer of 0.1 n. of potassium hydroxide solution; 5.61 is the amount of potassium hydroxide, which is contained in 1 ml of 0.1 n. of solution in ml; M is the weight of the weight in g.

The temperature of the studied brines was measured using a digital needle thermometer TH310 Milwaukee. The samples were weighed using laboratory technical scales AXIS BDM 3.

Description of the Experiment

Sample preparation: The balyk samples, produced according to the traditional technology, but with three different recipes of the injected brines, were used for the studies. The longest muscle from the dorsal and lumbar parts was isolated along the line of placement of the spinous processes of the spine from the fifth rib to the first sacral vertebra.
The studies were conducted at the biotechnology department of the Institute of Food Resources of National Academy of Agricultural Sciences (Ukraine).

Number of samples analyzed: The analyzed samples were developed according to the added starter culture: control sample without any starter culture, SC 1 sample (inoculated with *Lactobacillus rhamnosus*, *L. plantarum,* and *Kocuria rosea*), and SC 2 sample (*Staphylococcus carnosus, L. plantarum, L. rhamnosus, L. Paracasei)*.

Number of repeated analyses: The studies were carried out in triplicate, and the mathematical statistics methods processed the experimental data.

Number of experiment replication: Each study was carried out three times, the samples were six, consequently fifty-four repeated analyzes were carried out.

Design of the experiment: All strains were recovered from domestic dry-cured and raw-smoked meat products produced according to traditional technology. In these products, these strains belonged to the dominant bacteria. The number of viable lactic-acid bacteria and micrococci was 3.5×10^{10} CFU.g and 2.9×10^{8} CFU.g, respectively, per 1 g of SC 1, and the number of viable lactic-acid bacteria and staphylococci was 5.1×10^{10} CFU.g and 3.3×10^{8} CFU.g, respectively, per 1 g of SC 2.

The technological scheme for the production of raw-dried beam included the following processes: dividing half-carcasses, deboning the meat, veining the meat with the removal of rough films and tendons, injecting with brine (30% to the weight of the meat), massaging according to the described program, sedimentation in the chamber (temperature 8 – 10 °C, duration 72 hours), drying in a chamber until the moisture content reaches 28-38%.

To prepare the brine, dissolve the required amount of table salt and glucose, and, by the brine recipe, the starter culture in 1 litre of water, stirring until the components are completely dissolved (Table 1). The temperature of the brine should be from minus 2 °C to plus 2 °C. Pork meat was injected with previously prepared brine (Table 1) using an injector of 30% of the weight of the meat.
This meat was massaged within 4 hours according to the program: 15 min – rotation (3-4 rpm), 15 min – pause. The vacuum depth in the massager was at least 90%. After massaging, the meat was kept in a chamber at a temperature of 8 – 10 °C for 72 hours. Then, the meat was moved to the drying chamber; the drying process continued until the moisture content in the product reached 28-38%.

The use of starter cultures in the production of raw balyks, which were injected into the meat during injection as part of the brine, ensured not only high organoleptic characteristics of the finished product, such as taste, aroma, and stable colour, but also increased functional and technological properties - moisture-binding capacity and plasticity, which was also reflected in the improved performance of the finished product.

For further analysis at 0 (right after the salting), 4, 14, 24, 48, and 72 hours after the salting, the samples were taken from each replication of each batch.
It should be noted that the classic production technology of raw-smoked balyk includes the following processes: preparation of raw materials, injection with brine, and ageing in a refrigerator at a temperature of 2 ±2 °C for 5-6 days, smoking for 24-36 hours, drying at a temperature of 11 ±1 °C. The developed raw-dried log technology differs from the existing technology of smoked products by the shorter duration of the heat treatment of the log,

namely, the shorter period of exposure in the refrigerating chamber (72 hours instead of 5-6 days), the absence of the smoking process, the shorter duration of the technological process of manufacturing this product due to the used starter cultures to accelerate fermentation and ensure high organoleptic and functional-technological indicators.

Table 1 Recipe compositions of brines (per 1 liter) CFU/ml of bacteria in the brines samples.

Components	Sample		
	Control sample	SC 1	SC 2
Edible salt, g	100		
Glucose, g	15		
Lactobacillus rhamnosus, *L. plantarum* and *Kocuria rosea*, g	-	2.5	
Staphylococcus carnosus, L. plantarum, *L. rhamnosus, L. Paracasei*, g	-	-	2.5

Statistical Analysis

The STATISTICA Microsoft Excel editor processed experimental data using mathematical statistics methods. The accuracy of the obtained experimental data was determined using the Student'st-test with confidence coefficient $p \leq 0.05$ with many parallel definitions of at least 5 (confidence probability $p = 0.95$). Linear programming problems were solved using the MS Excel table processor's'Search for a solution setting (Excel Solver).

RESULTS AND DISCUSSION

Study of pH-value

The pH unit was more intensively decreased in the samples with the starter cultures than in the control sample (Figure 1). Thus, the pH-unit was decreased from 5.74 to 5.64 in the control sample in 72 hours, and the pH-unit was decreased from 5.73 to 5.61 in the sample SC 1 in 48 hours, and at the end of the study (in 72 hours) – 5.5. It was indicated that the pH-unit was slowly decreased for the sample SC 2 in the first 24 hours, and in 72 hours of the salting, it had been almost equaled with SC 1 and was 5.52 **[18]**, **[19]**. This course of the biochemical changes in the meat raw materials is explained by the activity of lactic-acid microbiota, which, in the process of vital action, ferments the meat carbohydrates with the formation of acids, including lactic ones, which leads to the decrease in the pH-unit of the meat medium **[20]**.

It is known that the pH range of the meat raw materials of 5.5-5.8 is the most desirable because of the partial denaturation of the proteins, the tenderization of the muscle tissue, and the formation of the substances that are responsible for the taste and aroma of the "mature" meat are occurred at such acidity **[21]**.

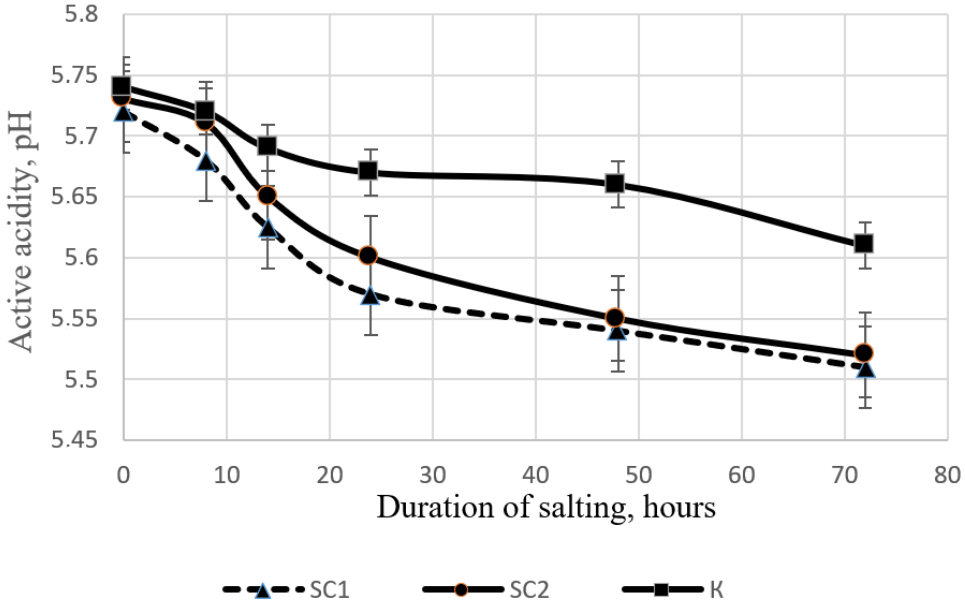


Figure 1 Dynamics of pH-unit changes in control and studied samples with bacterial drugs for salting.

The formation of lactic acid, particularly when the meat raw materials are fermented, prevents the development of putrefactive and pathogenic microbiota, thereby increasing the safety of the finished product **[22]**, **[23]**. It is known that the closer the pH value is to the isoelectric point of meat proteins (5.4 pH units), the lower its ability to bind moisture and, accordingly, the higher the drying speed **[24]**, **[25]**. A similar trend was observed when the bacterial drug "MKS" was used **[12]**.

Study of water activity

The content of free and weakly-bound water in the medium is an important factor for developing microorganisms. The water content available for microorganisms, and therefore, the stability of the product, can be estimated according to the parameter of water activity **[26]**, **[27]**.

A close relationship between the active acidity and water activity indicators was established for the samples SC 1 and SC 2 (Figure 2). It had been established that the reduction of a_w in the samples with the starter cultures more intensively than in the control sample, which may be a consequence of its active consumption during the active development of the starter-culture microbiota. In the first 24 hours, all samples' water activity was intensively decreased. The indicator for the sample SC 2 was 0.975 the control sample was 0.974, and the sample SC 1 was 0.972. Thus, at the 72nd hour of the salting, the water activity in the samples SC 1 and SC 2 was 0.965 and 0.963, respectively.

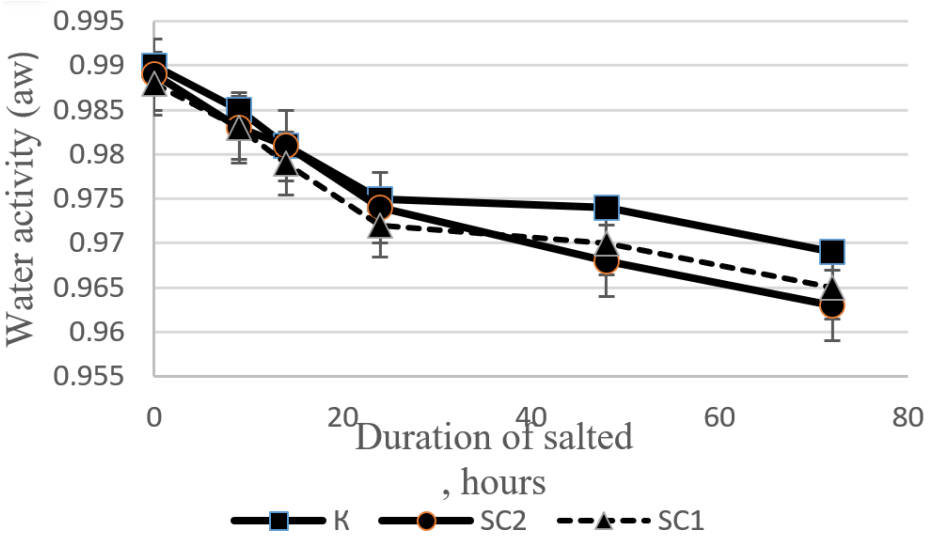


Figure 2 Change in water activity when control and studied samples are salted.

Therefore, adding the created compositions leads to the salting intensification of the meat products, which is desirable in production **[28]**.

Influence of culture starters on changes in the amount of nitric oxide pigments and colour stability of meat

In the production of meat products, one of the main food additives is sodium nitrite, which, when the meat products are salted, influences color formation and aroma development while providing preservative and antioxidant effects **[29]**, **[30], [31]**.

Improved color characteristics characterize the studied variants in comparison with the control ones; this is due to nitrite reductase, the producer of which is *Kocuria rosea*, as well as lactic bacteria, which actively reduced the pH-unit of the medium **[32]**, **[33]**. At 72 hours of the salting, the color stability of the studied samples exceeded the control ones by approximately 19%. Using the starter cultures, the studied samples are characterized by more stable and active color formation than the control ones, by 8-12% at 72 hours of the salting. The content of nitric oxide pigments in sample SC 1 was 6% higher than in SC 2, and 18% higher than in the control sample (Figure 3).

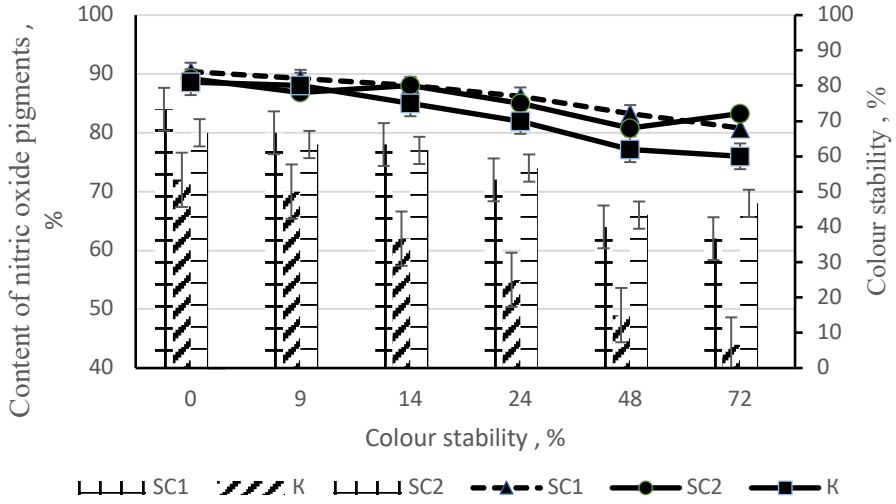


Figure 3 Dynamics of changes in the amount of nitric oxide pigments and color stability of control and studied samples while salting.

Thus, using bacterial drugs based on nitrite-reducing microorganisms for the salting positively influenced the formation of the required color characteristics.

Study of moisture-binding capacity and plasticity

An increase in the moisture-binding capacity of the meat raw materials is observed in the studied samples with the starter cultures (Figure 4). The studied variant, with the use of the starter cultures, was characterized by higher values of the moisture-binding capacity than the control sample and was 84-87 % at 72 hours and 84-87% at 72 hours of the salting.

At the end of the salting, this indicator for the control sample was 79.27%, 7.73% lower than for SC 2.

This influence is due to rapid glycolysis and the accumulation of the sour products of the metabolism of lactic bacteria, which more actively reduce the pH level, bringing it closer to the isoelectric point of the protein substances, which leads to structural changes in the protein **[34]**, **[35]**.

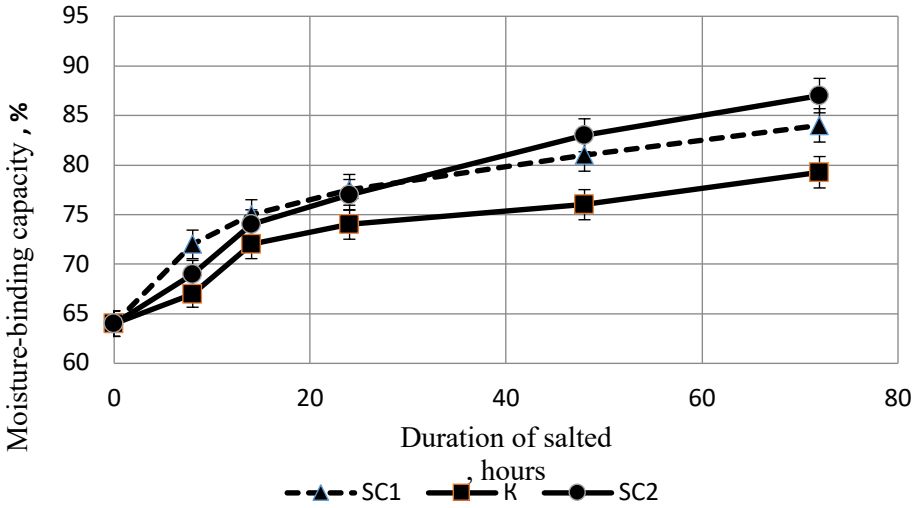


Figure 4 Dynamics of changes in the moisture-binding capacity of control and studied samples of meat raw materials while salting.

The plasticity of the studied samples is increased in direct proportion to the brine aging time (Figure 5). The plasticity of SC 1 varies from 7.78 cm^2.g to 17.23 cm^2.g, SC 2 – from 7.76 cm^2.g to 17.45 cm^2.g, and C – from 7.76 cm^2.g to 15.11 cm^2.g. This indicator for the studied samples SC 1 and SC 2 were at the same level and exceeded the control ones by 2.19 cm^2.g. Such a course of the biochemical changes of the studied samples can be explained by the proteolytic activity of lactic acid bacteria **[36]**, **[37]**.

An increase in plasticity can be considered a characteristic of the tenderization process of meat raw materials. An improvement in the plasticity of the studied samples is a positive result since the tenderness and, to a certain extent, the juiciness of the finished products depend on the plasticity of the meat raw materials **[38]**, **[39]**.

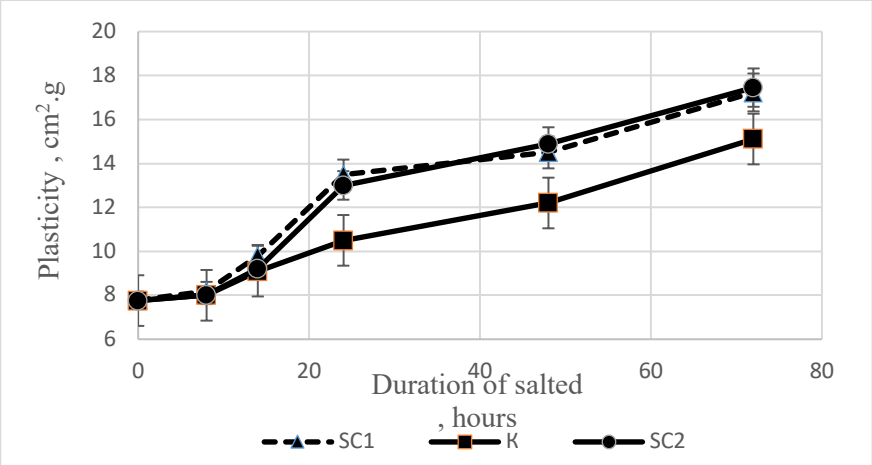


Figure 5 Dynamics of changes in control plasticity and studied samples of meat raw materials while salting.

Specific biochemical transformations occur when the meat's raw materials are salted, which determines the required organoleptic characteristics of the finished product **[40]**. Bacteria of the genus *Lactobacillus* are the pronounced producers of aroma and taste precursors and contribute to forming the specific organoleptic characteristics of the finished products **[41]**, **[42]**.

In the studied variants SC 1 and SC 2, a more intense accumulation of volatile fatty acids is observed compared to the control sample (Figure 6). Thus, the difference in the content of volatile fatty acids between SC 1 and the control sample was 22% at 72 hours of the salting. Because the formation process of volatile fatty acids is fermentative, this tendency is probably explained by the fact that the lipid hydrolysis has occurred under the action of not only tissue enzymes of cathepsins (as in the control ones) but also lipases, which were formed as a result of the vital activity of the bacterial compositions.

The content of volatile fatty acids is one of the most informative indicators for the taste-aromatic properties of the meat raw materials to be formed **[43]**.

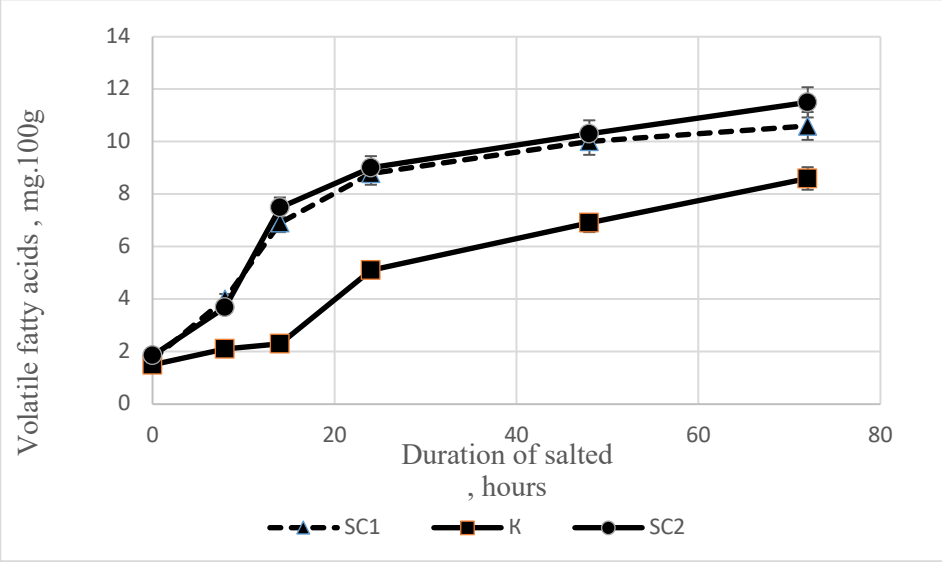


Figure 6 Accumulation dynamics of volatile fatty acids in studied variants while meat raw materials are salted.

The results of the final products of dry-cured balyks are shown in Figure 7.

Figure 7 Dry-cured balyks.

It should also be noted that the increase in the proteolytic activity of the enzymes depends on the medium acidity, which should be in the range of 5.4-5.6 pH units. As established earlier (Figure 1), the medium pH unit in the studied samples reaches the optimal values at 72 hours of the salting.

Thus, the results of our studies indicate that it is possible to use the starter cultures, which were created based on symbiotic compositions of strains of SC 1 *Lactobacillus rhamnosus*, *L. plantarum,* and Kocuria rosea and SC 2 *Staphylococcus carnosus, L. plantarum***,** *L. rhamnosus, L. paracasei* for the fermented meat products – dry-cured balyks to be produced.

CONCLUSION

The starter cultures of *Lactobacillus rhamnosus*, *L. plantarum Kocuria rosea,* and *Staphylococcus carnosus, L. plantarum***,** *L. rhamnosus, L. paracasei* were tested. At the same time, the pork meat is salted in the production of the dry-cured balyks. It had been established that in the samples with the starter cultures the acidity was more intensively decreased in 72 hours (from 5.74 pH units to 5.52 pH units) than in the control ones (from 5.74 pH units to 5.64 pH units). A close relationship was established between indicators of active acidity and water activity in SC 1 and SC 2 samples. At the 72nd hour of pickling, water activity in samples SC 1 and SC 2 was 0.965 and 0.963, respectively. At 72 hours of the salting, the color stability of the studied samples exceeded the control ones by approximately 19%. The moisture-binding capacity and plasticity of the pork meat samples with the starter cultures are characterised by the increased indicators compared to the control sample – by 4.73 and 7.73% and by 2.19 cm^2.g, respectively. The difference in the content of volatile fatty acids between SC 1, SC 2, and the control sample was 22% and 33%, respectively, at the 72 hours of the salting. The use of the starter cultures, such as *Lactobacillus rhamnosus*, *L. plantarum,* and *Kocuria rosea,* and *Staphylococcus carnosus, L. plantarum***,** *L. rhamnosus, L. paracasei*, when the pork meat is salted in the technology of the meat products makes it possible to obtain the finished products with the improved quality indicators, which is advanced in the food-processing industry.

REFERENCES

1. Shi, Q., Wang, X., Ju, Z., Liu, B., Lei, C., Wang, H., & Li, H. (2021). Technological and Safety Characterization of Kocuria rhizophila Isolates From Traditional Ethnic Dry-Cured Ham of Nuodeng, Southwest China. In Frontiers in Microbiology (Vol. 12). Frontiers Media SA. https://doi.org/10.3389/fmicb.2021.761019
2. Danylenko, S. G. (2014). Selection of microorganisms for fermentation of meat materials. In Biotechnologia Acta (Vol. 7, Issue 4, pp. 107–117). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/biotech7.04.107
3. Cullere, M., Novelli, E., & Dalle Zotte, A. (2020). Fat Inclusion Level, NaCl Content and LAB Starter Cultures in the Manufacturing of Italian-Type Ostrich Salami: Weight Loss and Nutritional Traits. In Foods (Vol. 9, Issue 4, p. 476). MDPI AG. https://doi.org/10.3390/foods9040476
4. Li, L., Perea-Sanz, L., Salvador, A., Belloch, C., & Flores, M. (2022). Understanding the impact of nitrogen and sulfur precursors on the aroma of dry fermented sausages. In Meat Science (Vol. 192, p. 108896). Elsevier BV. https://doi.org/10.1016/j.meatsci.2022.108896

5. Sallan, S., Yılmaz Oral, Z. F., & Kaya, M. (2023). A Review on the Role of Lactic Acid Bacteria in the Formation and Reduction of Volatile Nitrosamines in Fermented Sausages. In Foods (Vol. 12, Issue 4, p. 702). MDPI AG. https://doi.org/10.3390/foods12040702
6. Wang, J., Aziz, T., Bai, R., Zhang, X., Shahzad, M., Sameeh, M. Y., Khan, A. A., Dablool, A. S., & Zhu, Y. (2022). Dynamic change of bacterial diversity, metabolic pathways, and flavor during ripening of the Chinese fermented sausage. In Frontiers in Microbiology (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fmicb.2022.990606
7. Tatiyaborworntham, N., Oz, F., Richards, M. P., & Wu, H. (2022). Paradoxical effects of lipolysis on the lipid oxidation in meat and meat products. In Food Chemistry: X (Vol. 14, p. 100317). Elsevier BV. https://doi.org/10.1016/j.fochx.2022.100317
8. Todorov, S. D., Stojanovski, S., Iliev, I., Moncheva, P., Nero, L. A., & Ivanova, I. V. (2017). Technology and safety assessment for lactic acid bacteria isolated from traditional Bulgarian fermented meat product "lukanka." In Brazilian Journal of Microbiology (Vol. 48, Issue 3, pp. 576–586). Springer Science and Business Media LLC. https://doi.org/10.1016/j.bjm.2017.02.005
9. Wang, J., Aziz, T., Bai, R., Zhang, X., Shahzad, M., Sameeh, M. Y., Khan, A. A., Dablool, A. S., & Zhu, Y. (2022). Dynamic change of bacterial diversity, metabolic pathways, and flavor during ripening of the Chinese fermented sausage. In Frontiers in Microbiology (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fmicb.2022.990606
10. Afifah, D. N., Dewi, U. T. S., Anggraeni, R. D., Tsani, A. F. A., Widyastuti, N., Fulyani, F., & Anjani, G. (2022). Antioxidant Activity, Microbiological Quality, and Acceptability of Spontaneously Fermented Shrimp Sausage (Litopenaeus vannamei). In T. Zotta (Ed.), Journal of Food Quality (Vol. 2022, pp. 1–8). Hindawi Limited. https://doi.org/10.1155/2022/5553432
11. Bal-Prylypko, L., Yancheva, M., Paska, M., Ryabovol, M., Nikolaenko, M., Israelian, V., Pylypchuk, O., Tverezovska, N., Kushnir, Y., & Nazarenko, M. (2022). The study of the intensification of technological parameters of the sausage production process. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 27–41). HACCP Consulting. https://doi.org/10.5219/1712
12. Shevchenko, L. (2023). The effect of colloidal silver on clinical, morphological parameters and mineral composition of blood of calves. In Acta fytotechnica et zootechnica (Vol. 16, Issue 1, pp. 8–14). Slovak University of Agriculture in Nitra. https://doi.org/10.15414/afz.2023.26.01.8-14
13. DSTU 7158:2010 Meat. Pork carcasses and half carcasses. Specifications. General specifications. Quality management systems –Requirements.
14. DSTU 3583:2015 Table salt. Specifications. General specifications. Quality management systems – Requirements.
15. DSTU ISO 2917 (2001). Meat and meat products. Measurement of pH (Control method). State Committee of Ukraine on Technical Regulation and Consumer Policy.
16. ISO 18787 (2017). Foodstuffs - Determination of water activity. Vernier, Geneva, Switzerland.
17. Grau, R.(1960). Fleisch und Fleischwaren. 1.ed., Berlin Verlag A.W. Hayn´s Erben. p. 240.
18. Yang, P., Zhong, G., Yang, J., Zhao, L., Sun, D., Tian, Y., Li, R., & Rong, L. (2022). Metagenomic and metabolomic profiling reveals the correlation between the microbiota and flavor compounds and nutrients in fermented sausages. In Food Chemistry (Vol. 375, p. 131645). Elsevier BV. https://doi.org/10.1016/j.foodchem.2021.131645
19. Hu, Y., Chen, Q., Wen, R., Wang, Y., Qin, L., & Kong, B. (2019). Quality characteristics and flavor profile of Harbin dry sausages inoculated with lactic acid bacteria and Staphylococcus xylosus. In LWT (Vol. 114, p. 108392). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.108392
20. Mei, L., Pan, D., Guo, T., Ren, H., & Wang, L. (2022). Role of Lactobacillus plantarum with antioxidation properties on Chinese sausages. In LWT (Vol. 162, p. 113427). Elsevier BV. https://doi.org/10.1016/j.lwt.2022.113427
21. Sallan, S., Yılmaz Oral, Z. F., & Kaya, M. (2023). A Review on the Role of Lactic Acid Bacteria in the Formation and Reduction of Volatile Nitrosamines in Fermented Sausages. In Foods (Vol. 12, Issue 4, p. 702). MDPI AG. https://doi.org/10.3390/foods12040702
22. Stegmayer, M. Á., Sirini, N. E., Ruiz, M. J., Soto, L. P., Zbrun, M. V., Lorenzo, J. M., Signorini, M. L., & Frizzo, L. S. (2023). Effects of lactic acid bacteria and coagulase-negative staphylococci on dry-fermented sausage quality and safety: Systematic review and meta-analysis. In Meat Science (Vol. 206, p. 109337). Elsevier BV. https://doi.org/10.1016/j.meatsci.2023.109337
23. Bis-Souza, C. V., Penna, A. L. B., & da Silva Barretto, A. C. (2020). Applicability of potentially probiotic Lactobacillus casei in low-fat Italian type salami with added fructooligosaccharides: in vitro screening and

technological evaluation. In Meat Science (Vol. 168, p. 108186). Elsevier BV. https://doi.org/10.1016/j.meatsci.2020.108186

24. Danylenko, S., Naumenko, O., Yemtsev, V., Kryzhska, T., Potemska, O., Tolok, G., Kanishchev, O., Ochkolyas, O., Prokopenko, N., & Omelian, A. (2023). Justification and microbiota compositions development for the fermentation of raw meat. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 405–418). HACCP Consulting. https://doi.org/10.5219/1874

25. Wang, J., Aziz, T., Bai, R., Zhang, X., Shahzad, M., Sameeh, M. Y., Khan, A. A., Dablool, A. S., & Zhu, Y. (2022). Dynamic change of bacterial diversity, metabolic pathways, and flavor during ripening of the Chinese fermented sausage. In Frontiers in Microbiology (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fmicb.2022.990606

26. Latoch, A., & Libera, J. (2019). Quality and Safety of Pork Steak Marinated in Fermented Dairy Products and Sous-Vide Cooked. In Sustainability (Vol. 11, Issue 20, p. 5644). MDPI AG. https://doi.org/10.3390/su11205644

27. Sindelar, J. J., & Milkowski, A. L. (2012). Human safety controversies surrounding nitrate and nitrite in the diet. In Nitric Oxide (Vol. 26, Issue 4, pp. 259–266). Elsevier BV. https://doi.org/10.1016/j.niox.2012.03.011

28. Bal-Prylypko, L. V., Leonova B. I., & Ryabovol, M. V. (2019). Study of biotechnological changes in raw meat during salting process. In Chemical technology. control and managment (Vol. 2, Issue 86, pp. 16−22). Tashkent State Technical University Named after Islam Karimov. https://doi.org/10.34920/2019.2.16-22

29. Riabovol, M., & Bal-Prylypko, L. V. (2021). Justification and development of sausage technology with health properties. In Animal Science and Food Technology (Volume 12, Issue 1, pp. 39–47). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.01.039

30. Nikolaenko, M. S. (2021). Optimization of the technology of production of functional semi-finished meat products covered in pastry. In Animal Science and Food Technology (Vol. 12, Issue 1, pp. 19–27). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.01.019

31. Ma, Y., Gao, Y., Xu, Y., Zhou, H., Zhou, K., Li, C., & Xu, B. (2023). Microbiota dynamics and volatile metabolite generation during sausage fermentation. In Food Chemistry (Vol. 423, p. 136297). Elsevier BV. https://doi.org/10.1016/j.foodchem.2023.136297

32. Bal-Prylypko, L., Nikolaenko, M., Stepasyuk, L., Cherednichenko, O., & Lialyk, A. (2022). Forecasting the sale price of pork in agricultural enterprises In Agricultural and Resource Economics: International Scientific E-Journal (Vol. 8, Issue 4, pp. 170–187). Institute of Eastern European Research and Consulting. https://doi.org/10.51599/are.2022.08.04.08

33. Ren, H., Deng, Y., & Wang, X. (2022). Effect of a compound starter cultures inoculation on bacterial profile and biogenic amine accumulation in Chinese Sichuan sausages. In Food Science and Human Wellness (Vol. 11, Issue 2, pp. 341–348). Tsinghua University Press. https://doi.org/10.1016/j.fshw.2021.11.009

34. Stadnyk, I., Bodnarchuk, O., Kopylova, K., Petrov, P., Bal-Prylypko, L., & Narizhnyy, S. (2021). Modification of the properties of milk-fat emulsions with the phase structure of “oil in water” in the dependence on the mass part of the lipoid and the stabilizing systems. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 741–748). HACCP Consulting. https://doi.org/10.5219/1389

35. Shi, Q., Wang, X., Ju, Z., Liu, B., Lei, C., Wang, H., & Li, H. (2021). Technological and Safety Characterization of Kocuria rhizophila Isolates From Traditional Ethnic Dry-Cured Ham of Nuodeng, Southwest China. In Frontiers in Microbiology (Vol. 12). Frontiers Media SA. https://doi.org/10.3389/fmicb.2021.761019

36. Zhao, X., Zhang, Z., Cui, Z., Manoli, T., Yan, H., Zhang, H., Shlapak, G., Menchynska, A., Ivaniuta, A., & Holembovska, N. (2022). Quality changes of sous-vide cooked and blue light sterilized Argentine squid (Illex argentinus). In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 175–186). HACCP Consulting. https://doi.org/10.5219/1731 DOI: https://doi.org/10.5219/1731

37. Rodionova, K., Khimych M., & Paliy, A. (2021). Veterinary and sanitary assessment and disinfection of refrigerator chambers of meat processing enterprises. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 616–626). HACCP Consulting. https://doi.org/10.5219/1628

38. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/171

39. Zheplinska, M., Mushtruk, M., Vasyliv, V., Slobodyanyuk, N., & Boyko, Y. (2021). The Main Parameters of the Physalis Convection Drying Process. In Lecture Notes in Mechanical Engineering (pp. 306–315). Springer International Publishing. https://doi.org/10.1007/978-3-030-77823-1_31

40. Kondratiuk, V., Slobodyanyuk, N., & Ivaniuta, A. (2021). Effect of feeding conditions on the quality traits of rainbow trout. In Acta fytotechn zootechn (Vol. 24, Issue 3, pp. 256–264). Elsevier BV. https://10.15414/afz.2021.24.03.256-264

41. Ugnivenko, A., Nosevych, D., Antoniuk, T., Chumachenko, I., Ivaniuta, A., Slobodyanyuk, N., Kryzhova, Y., Rozbytska, T., Gruntovskyi, M., & Marchyshyna, Y. (2022). Manifestation of living and post-slaughter traits of productivity in inbred and outbred bull calves of Ukrainian meat cattle breed. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 356–366). HACCP Consulting. https://doi.org/10.5219/1769

42. Israelian, V., Palamarchuk, I., Sevin, S., Holembovska, N., Prokopenko, N., Ivaniuta, A., Shynkaruk, O., Rudyk, Y., Nosevych, D., & Tverezovska, N. (2022). The effect of vibration massage on the salting process of ostrich meat. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 530–544). HACCP Consulting. https://doi.org/10.5219/1775 DOI: https://doi.org/10.5219/1775

43. Zheplinska, M., Mushtruk, M., Vasyliv, V., Kuts, A., Slobodyanyuk, N., Bal-Prylypko, L., Nikolaenko, M., Kokhan, O., Reznichenko, Y., & Salavor, O. (2021). The micronutrient profile of medicinal plant extracts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 528–535). HACCP Consulting. https://doi.org/10.5219/1553

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Dr. For Svitlana Danylenko

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Svitlana Danylenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technologies of Meat, Fish and Seafood and Envi-ronmental Sciences of Ukraine, Heroiv Oborony Str., 15, 03040, Kyiv, Ukraine,
E-mail: svet1973@gmail.com
ORCID: http://orcid.org/0000-0003-4470-4643

Liana Nedorizanyuk, Institute of Food Resources of NAAS, Department of technology of meat products Yevhen Sverstiuk str., 4-A, 02002, Kyiv, Ukraine,
E-mail: nedliana4@gmail.com
ORCID: https://orcid.org/0000-0002-2190-5648

Oksana Potemska, Institute of Food Resources of NAAS, Department of Biotechnolog Yevhen Sverstiuk str., 4-A building, 02002, Kyiv, Ukraine,
E-mail: potemskaok@gmail.com
ORCID: http://orcid.org/0000-0003-4725-5847

Tsvitana Korol, Institute of Postgraduate Education of the National University of Food Technologies, Department of Meat and Milk Processing, Estonska str., 8A, 03190, Kyiv, Ukraine,
E-mail: tsvetana.korol@ukr.net
ORCID: http://orcid.org/0000-0001-9827-463X

***Anastasiia Ivaniuta**, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technology of meat, fish and marine products, Polkovnika Potekhina Str., 16, 03040, Kyiv, Ukraine,
E-mail: ivanyta07@gmail.com
ORCID: https://orcid.org/0000-0002-1770-5774

Yuliya Kryzhova, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technology of meat, fish and marine products, Polkovnika Potekhina Str., 16, 03040, Kyiv, Ukraine,

E-mail: yuliya.kryzhova@ukr.net
ORCID: https://orcid.org/0000-0003-1165-8898

Valentyna Israelian, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technology of meat, fish and marine products, Polkovnika Potekhina Str., 16, 03040, Kyiv, Ukraine,
E-mail: vs88@ukr.net
ORCID: https://orcid.org/0000-0002-7242-3227

Nataliia Kovalenko, Odesa National University of Technology, Faculty of innovative food technologies and restaurant, hotel business, Hotel and Restaurant Business Departments, 112 Kanatna street, 65039, Odesa, Ukraine,
E-mail: kovalenkonatalia241@gmail.com
ORCID: https://orcid.org/0000-0003-4167-5779

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 10.1.2024
Revised: 26.2.2024
Accepted: 28.2.2024
Published: 29.2.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 251-267
https://doi.org/10.5219/1951
ISSN: 1337-0960 online
www.potravinarstvo.com

Innovative thermodynamic modeling for enhanced yeast dough mixing: energy perspectives and applications

Volodymyr Piddubnyi, Anna Sabadosh, Mikhailo Mushtruk, Andrii Chahaida, Viktor Fedorov, Khrystyna Kravcheniuk, Svitlana Krasnozhon, Iurii Radchenko

ABSTRACT

A thermodynamic model for the calculation of energy exchange in the chamber of a new mixer with effective use of structural and technological parameters of the mixing process without the necessary introduction of experimental data correlations in the distribution of fluid velocities is proposed, which determines the relevance of this direction of calculation with the perspective of its development. The purpose of the presented work is to determine the specific power by substantiating the effective mode parameters of the preparation of the mixture (dough) as a result of evaluating the thermodynamic energy parameters of the kneading process. The assessment was carried out by developing a methodology for determining specific costs for creating a viscous medium when mixing components, which allows you to establish the required power depending on the design and technological parameters of the new mixer. The considered principle of the proposed open-type thermodynamic system of the description of the working process of mixing made it possible to reveal and determine the ways of converting energy into useful work of interphase heat and mass transfer of a heterogeneous medium. In the conditions of circulation mixing with multiple mechanical effects on the mixture of components in the closed circuit of the cylindrical working chamber, which is an effective way to achieve homogeneity of the environment, it was possible to obtain an analytical determination of the specific work and power of the drive in the absence of a clear description of the model of the interconnection of components. The proposed thermodynamic description of the system's energy balance allows to perform only a few experiments. In general, the practical value of the given calculations is of practical importance for improving productivity and efficiency and minimizing energy consumption for the process while reducing the dynamic loads of the designed mixer.

Keywords: components, financing, specific costs, specific identity, thermodynamic model, energy balance, investment

INTRODUCTION

High-quality forming mixtures during mixing in the sectors of the national economy is an extremely important task, as it can affect the safety and efficiency of products and the overall success of enterprises in these sectors. After all, in the conditions of a market economy, using modern equipment with accurate regulation and control systems can significantly reduce losses and improve mixing quality. This may include measuring temperature, humidity, pH, and concentration of ingredients to establish the correct mixing parameters. It is important to choose the right parameters, such as speed, time, temperature, and pressure. These directions can be used in the food and pharmaceutical industries to improve energy consumption in mixing processes and ensure high product quality.

According to conventional fuel, the energy intensity of Ukraine is 2.6 times higher than a relatively similar indicator of the industrial development of the world's countries. The data are reflected in the National Development of Industry program **[1]**, **[2]**. Priority areas include the introduction of resource- and energy-saving technologies. In addition, there remains an important direction of mastering the production of the latest

technological equipment. Thus, there is practicality in continuing the search and innovation in implementing the mentioned State Program.

One of the ways to reduce production costs is the economical use of thermal and electrical energy per unit of finished products. To achieve a reduction in energy consumption, it is necessary at each stage of the production process to study how much energy the material flows can provide, how much energy is needed to make the necessary changes in these material flows, and how to minimize the loss of thermal energy and energy associated with chemical processes.

An analytical review of scientific works confirms the existence of ways to increase the efficiency of producing quality products and the main laws of improving technological processes **[3]**. This may result from Ukrainian and foreign researchers' work in production and product quality. Mechanical mixing is an important and reliable process that plays a vital role in food production due to its simplicity and versatility.

In addition, it is important to conduct a market analysis and compare different equipment models, considering their technical characteristics, cost, reliability, and warranty conditions. After that, you can make an informed choice that will meet the needs of the enterprise and help achieve the desired results. A wide selection of different types and designs of mixing devices allows you to choose the best option for a specific task and product. According to the authors **[4]**, these characteristics of working bodies make them universal tools for many processes in industry and research. They are used in various industries, including the food, pharmaceutical, chemical, and cosmetic industries, and in laboratory research to solve various tasks related to mixing and homogenization.

An analysis of publications on parameters and characteristics of mechanical mixing using vibratory mixers indicates the importance of further research in this direction. Today, there is no clear theoretical explanation for the required amount of energy to make the necessary changes in material flows in the connection between the main parameters and mixing characteristics **[5]**.

Therefore, creating discrete-impulse influence, pressure pulsations, and liquid flow rate for the development of turbulence in local volumes of flow in the working chamber of the machine is an interesting approach for the modernization and improvement of mixing processes. However, it is also important to consider this approach's potential challenges and limitations, including process stability, equipment costs, and quality control. Detailed studies and modeling may be useful to evaluate and optimize such a method before its implementation in practice, which was considered **[6]**. The approach points to the importance of scientific research and innovation in improving technologies and production processes to achieve quality products and optimize production.

Scientific Hypothesis

The specific power when kneading the components of the yeast dough depends on various factors, such as the kneading time, the temperature of the environment, and the properties of the ingredients used. The basis of the work is the determination of the specific power for the preparation of steam by substantiating the effective thermodynamic energy regime parameters for the analysis of a complex work process. Proposing the hypothesis, we assume that optimal kneading parameters will contribute to increasing the process's efficiency and improving the final product's quality.

MATERIAL AND METHODOLOGY

Samples

Experimental and theoretical research was conducted in the laboratories of Technological Equipment of the Ternopil National Technical University. According to the specified recipe **[7]**, wheat flour of the first grade with a moisture content of 13.6 ±0.2% and a raw gluten content of 26% was used. The materials came from the manufacturer "ZACHID-HLIB-ZBUT-2002" in Ternopil, Ukraine. Pressed yeast was produced according to the technical conditions of TU U 10.8-00383320-001 **[8]** by PrJSC "Company Enzym" in Lviv, st. Ukraine. Granulated sugar met the DSTU 4623:2023 **[9]**standard and was purchased in Rivne, Ukraine.

Chemicals

Water(chemical formula H_2O) was used to mix the components to prepare the paste. Water meets the national standard DSTU 7525:2014 **[10]**.

Animals, Plants, and Biological Materials

Flour of the first grade, made from winter wheat varieties ("*Myras*", "*Shestopalovskaya 28*", "*Flagman*"), grown in the west of Ukraine in the Ternopil region in the forest-steppe zone.

Pressed yeast TU U 10.8-00383320 **[8]**.

Instruments

DMK 30 digital electronic meter (implemented by TechnoSvit LLC, Ternopil, Ukraine).

Altinar-71 analog-digital frequency converter (implementer of TechnoSvit LLC, Ternopil, Ukraine).

Lenovo G 500 personal computer (implemented by TechnoSvit LLC, Ternopil, Ukraine).
Communication with a PC (implemented by TechnoSvit LLC, Ternopil, Ukraine).
Power Suite software version 2.3.0 (implemented by TechnoSvit LLC, Ternopil, Ukraine).

Laboratory Methods

Depending on the specific requirements and characteristics of the mixing process, there may be different combinations of these methods and approaches to achieve a successful result. In our study, to achieve the distribution of the components before the period when the absorption of the liquid components of the flour particles has passed, the main strategy is to speed up the mixing process and ensure the uniformity of the distribution of the components as much as possible. After all, the first mixing stage involves obtaining a mixture by combining flour and liquid ingredients in a thin layer. This is known as the "binding" or "wet mixing" stage.

This process is important in the good distribution of the liquid components in the flour to obtain a uniform mixture before the following operations. It is important to consider that the effectiveness of the "binding" stage can be achieved due to the correct combination of flour and liquid ingredients in a thin layer to create a quality mixture. The basis of the second stage of the process, where the components must be mixed in a weighted state in a continuous flow with the assistance of working bodies, creates conditions for intensive mixing to achieve the desired distribution of components in the mixture. In this case, the integration of working bodies and the continuous flow of components affect the achievement of high-quality structuring.

To perform experimental research, a physical model was developed, which is schematically presented in Figure 1. With the help of the developed mixer, the processes that take place during the circulation of the flow of the formed liquid mixture under the action of the plate-shaped working body and the suspended state on the other side are considered.

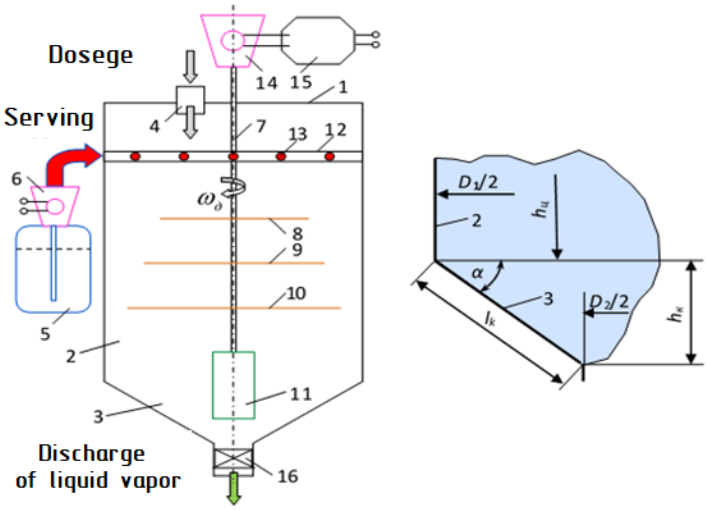


Figure 1 Scheme of the new mixer components: 1 – working chamber; 2, 3 – cylindrical and conical particles of the working chamber; 4 – loading neck; 5 – water container; 6 – water supply pump; 7 – drive shaft; 8, 9, 10 – disc-shaped working bodies; 11 – stirrer blade; 12 – water supply; 13 – water spraying devices; 14 – electric motor; 15 – control unit; 16 – drain tap.

Figure 1 does not show the flour vibrodispenser, which creates conditions for spraying flour over the entire volume of the cylindrical chamber. Thanks to this mixer design, uniform layer-by-layer mixing of the components is ensured, and the quality of the created environment is improved. The design parameters of the mixer are given in Table 1.

An important assumption for the thermodynamic model about region Y is that subsystem X receives energy from an external source. This assumption indicates the possibility of inputting or outputting energy to subsystem X from an independent source that is separated from other parts of the system and is important for regulating the work process, creating conditions for certain effects, or ensuring energy balance in the system.

Usually, to calculate the efficiency of a system or equipment, it is important to consider all factors, including the power consumption of the working body and the losses that may occur during power transmission through the shaft and bearing assembly. Minimizing such losses can lead to improved efficiency and equipment life. Therefore, the working body's consumed energy is spent without considering the mechanical loss of power in the shaft seals and the bearing assembly. The mechanical energy of subsystem X can perform technical work in subsystem Y. At the same time, there is a change in parameters: pressure and temperature, both of the gas

subsystem and the entire system. Such a change in system parameters aims to increase the efficiency of the system's functioning and achieve the target destination of the system flow.

Table 1 Design parameters of the mixer.

Mixer indicators	Value
Cylindrical chamber diameter, m	0.25
Total height of the chamber, m	0.55
Number of disc working bodies, pcs.	3
Diameters of the disc working body, m	0.23, 0.21, 0.19
The distance limit between plate-shaped r.o.m.,	0.1-0.15
Speed, rpm	160-200
second sender flour, kg/s	0-0,08
water-yeast mixture feeds, kg/s	0-0.05

The process of determining the components of the working mixture was considered according to the "black box" method in the form of an energy balance calculation scheme (Figure 2).

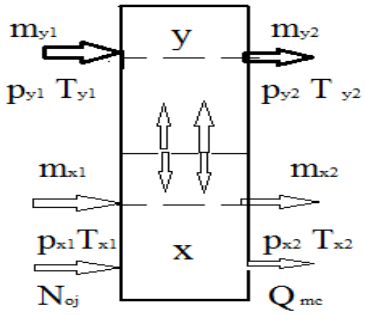


Figure 2 Scheme for calculating the energy balance of yeast dough during pulsed mixing of components: X is – a liquid subsystem; Y is a gas subsystem.

Studies were considered according to the developed methodology **[11]**. The nature of the mixing process in the developed laboratory unit allows us to reveal ways of determining the specific power when mixing components. The degree of chamber filling was determined using the Altinar-71 analog-to-digital converter. With the help of the converter, the impact on the scattering energy, mixing in the environment, and the actual volume that can be effectively used in the researched process were determined. The rate of oxygen absorption can be determined by equating the rate of oxygen dissolution to the rate of its consumption by yeast cells **[12]**. All the oxygen consumed by the cell is spent on the process during the mixing period.

Two different methods of influencing the environment in the working chamber of the mixer were used to achieve the desired result - the formation of a homogeneous mixture of components. The main key elements of the installation description are the chaotic behavior of the dosed components, where the interaction and mixing occur in the first stages. This behavior reveals the impulse effect, which is carried out with the help of a flour vibrator and a liquid mixture dispenser. Impulses in the dosing process can change their nature in intensity or time interval. In addition, the continuous impact of the large surface of the working body ensures constant impact, creating a stable and gradual change in temperature. The temperature in the environment has limits and a possible gradual increase in temperature is carried out within its limits. Such an approach using different exposure and temperature control methods creates a new approach to achieving the desired results in research on the proposed physical model of the mixer.

Description of the Experiment

Sample preparation: Opar samples were prepared by mixing first-grade wheat flour with water according to a specified recipe. The settling process was studied under certain temperature and humidity conditions for the development of enzymatic processes. Afterwards, samples were taken for further studies, including measurements of crude gluten content and other physico-mechanical and rheological parameters.

Number of samples analyzed: We analyzed 12 samples.

Number of repeated analyses: All measurements of instrument readings were performed two times.

Number of experiment replications: The number of repetitions of each experiment to determine one value was two times.

Design of the experiment: Passing the mixing process: Components are introduced into the working chamber of the machine in a pulse or discrete mode, that is, at certain moments of time or certain steps. At the

same time, components are loaded and selected through different camera ends, which is important for our process. After all, the finished mixture is submitted to the further technological process **[13]**. In this way, it is possible to change and improve the process of loading and mixing components in the working chamber if the process is carefully analyzed, and its advantages and disadvantages can be identified. Therefore, during the first minutes of their introduction into the working chamber, the dosing components behave chaotically with interaction and mixing. This approach allows you to use two ways to influence the working chamber's environment. Impulse exposure, on the one hand, indicates that a flour vibrator and a liquid mixture dispenser with variable intensity or time intervals carry out the exposure. continuous exposure to the large surface of the working body creates a stable and gradual change in temperature. At the same time, the temperature in the environment has its limits and can gradually increase to its limit.

A theoretical approach to the energy balance equation: Analytical determination of the specific work and power of the drive is quite challenging due to the lack of explicit dependencies of units that describe the models of the interconnection of components in the working chamber of the mixer under discrete-impulse influence. The limited study of these factors' influence on the system's energy balance during experiments affects the performance of many experiments.

A thermodynamic description of the mixing workflow in the proposed mixer reveals and provides important context for understanding the energetic and thermal aspects of the forming system. It emphasizes the main points of the description, focusing on aspects of thermodynamics, which studies the relationships between energy, heat, and mechanical work in a system.

Conducting a thermodynamic description of the work process is focused on aspects of thermodynamics, which studies the relationships between energy, heat, and mechanical work in a system. Our component interaction system is open and can exchange energy with the environment. It can involve heating or cooling and converting energy into work. It converts energy into work or vice versa, indicating that it is in a "quasi-steady equilibrium" state. This state of the system changes very slowly compared to other processes. This mode allows you to perform some simplified thermodynamic analyses.

Using a thermodynamic model to analyze a complex work process that includes two subsystems and energy exchange through an open boundary between them describes the presence of two subsystems in a working chamber where the working environment is in a two-phase state. This indicates the presence of different phases in the dough (liquid and gas) in the formed system and is important for the analysis and control of thermodynamic processes. This model requires complex mathematical and physical analysis to determine and optimize the work process according to the tasks. It can be useful for developing new technologies, increasing the efficiency of work processes, and solving complex engineering tasks.

Using "quasi-steady equilibrium" for simplified thermodynamic analyses is important because it allows one to focus on basic thermodynamic relationships while avoiding significant changes in the system.

Statistical Analysis

To establish the technological efficiency or to determine the energy costs of the discrete-pulse mixing of components during the preparation of liquid steam, experimental comparative studies of consumption power consumption were conducted depending on the established cycle of operation of the electric motor 14 (Figure 1).

Preparation of liquid foam took place in three ways, which differed from each other in the nature and sequence of the adopted cycle:

- "Cycle 1": the electric motor 14 (Figure 1) was turned on simultaneously with the start of loading the metered components of the mixture (metered flour and metered water-yeast mixture) into the working chamber of the mixer, while the time of operation of the disk disks 8, 9, 10 and the stirrer 11 was t_z = 2.8-3.05 min;
- "Cycle 2": the electric motor was turned on 1.2 min after the start of loading the dosed components of the mixture (dosed flour and dosed water-yeast mixture) into the working chamber of the mixer, while the time of operation of disc disks 8, 9, 10 and stirrer 11 was t_z = 2.8-3.3 min;
- "Cycle 3": the electric motor was turned on 2.2 min after the start of loading the dosed components of the mixture (dosed flour and dosed water-yeast mixture) into the working chamber of the mixer, while the total working time t_z = 2.9-3.4 min.

As a result of the statistical processing of the experimental data set, a regression equation was obtained in the form of a linear model, which is written as a function $P_i = a_0 + b_1 t + b_2 T$ and functions $k_{io} = a_0 + b_1 t + b_2 T$ that characterize the functional change of the optimization parameter for the three cycles of operation of the mixer electric motor:

- energy consumption P_1 ("cycle 1"), P_2 ("cycle 2"), P_3 ("cycle 3"):

$$\left.\begin{aligned} P_1 &= -2{,}86 + 1{,}31t - 0{,}014T; \\ P_2 &= 0{,}45 - 0{,}24t + 0{,}022T; \\ P_3 &= -0{,}91 + 0{,}4t + 0{,}006T \end{aligned}\right\};$$

Figure 3 shows the dependencies of the functional change in the consumption of power consumption P_i as a function of $P_i = f_P(t;T)$, Figure 4 – dependencies of functional change in power consumption P_i as a function of $P_i = f_P(t)$ for three cycles of operation of the discrete-pulse mixer motor.

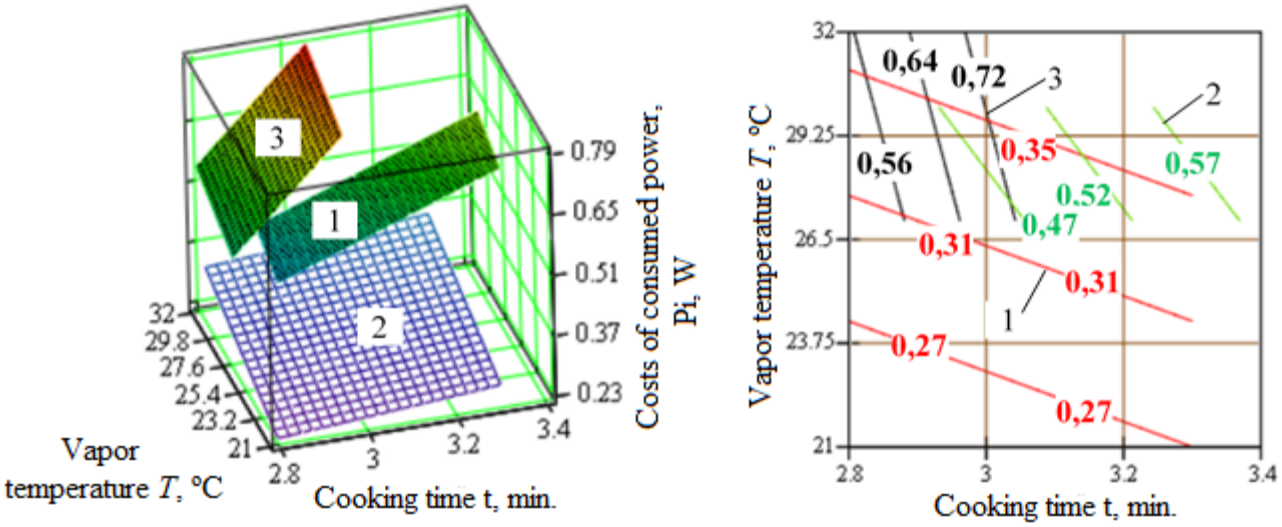
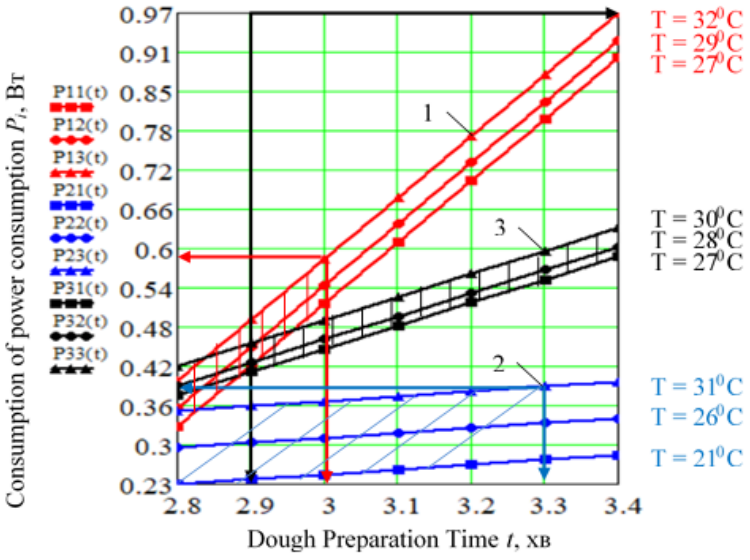


Figure 3 Dependence of the change in consumer power costs as a function $P_i = f_P(t;T)$: 1 – the electric motor. was turned on at the beginning of loading the dosed components of the mixture into the working chamber of the mixer, the working time t = 2.8-3.05 min; 2 – the electric motor was turned on 1.2 minutes after dosing the components into the working chamber, working time t = 2.8-3.05 minutes; 3 – the electric motor was turned on 2.2 minutes after dosing the components into the working chamber, working time t = 2.9-3.4 minutes.

Based on the analysis of figures 3 and 4, it was found that the lowest values of the power consumption P_i obtained with the variant of the electric motor operation for "cycle 2", and the indicators of the approximated values of the power consumption P_2 are in the range from 0.23 to 0.39 W.



$$P_2 = f_P(t_2;T_2);\ P_3 = f_P(t_3;T_3)$$

Figure 4 Dependence of power consumption P_i as a function: 1 – "cyc 1":2 – "cycle $P_i = f_P(t)$2"; 3 – "cycle 3".

This indicates that mixing the components in a suspended state has a positive effect on the duration of the process, or the preparation time of the liquid dough and the temperature of the dough, which are quite favorable and of good quality for satisfactory performance of the prepared liquid dough.

Switching on the electric motor of the discrete-pulse mixer during the discharge of the dough and partial mixing of dosed components ("cycle 1" and "cycle 2") leads to a reduction in the process time, but increases the consumption of consumer power. Therefore, the proposed dough preparation method allows you to optimize the process and is promising for its introduction into production to prepare bakery confectionery products.

RESULTS AND DISCUSSION

The difference in the approaches of different authors in the analysis and calculations of mixing machines reflects the complexity and multifaceted nature of this problem. Different parameters and influences are important in real mixing systems, and authors may use different methods and models to analyze them. Different approaches can be important to understand which aspects are being investigated and how they affect the analysis results. In such cases, it is important to clearly describe the conditions and assumptions on which the calculation is based and consider them in the context of the specific application of the mixing system.

Thus, the change in the total internal energy of the chemical bonds of the material flow is an important factor in the analysis of the process of transformation of the environment during the interaction of new working bodies. This is because, during any chemical or physical process in the system, there are changes in the energy of chemical bonds between atoms and molecules. These changes can be accompanied by the absorption or release of energy during the destruction of chemical bonds during mechanical impact **[14]**, **[15]**. This reaction can be endothermic or exothermic, and it is important to consider how energy interacts with the environment. In researching a new mixer, this means taking into account heat losses, convection heat transfer, and other effects that affect the energy distribution in the system. The energy balance equation allows you to consider all important sources and consumers of energy in the system, which helps to understand how energy is distributed during the process. Consider the work process in a working chamber with a homogeneous state of the working environment, in which gas bubbles are considered ideal and specific heat capacities remain constant during minor temperature changes during the action of working bodies (compression, crushing, stretching). Considering the ideal nature of the gas and the constancy of the specific heat capacities, we can obtain important data for designing and optimizing work processes in your system. This approach makes it possible to simplify calculations and modeling of work processes.

For effective design and management of energy exchange systems, it is important to consider hydraulic losses (energy dissipation), evaluate their impact on process efficiency, and, if necessary, take measures to reduce these losses. In energy performance calculations, the hydraulic loss coefficient is an important variable in hydraulic systems and fluid or gas transportation systems, and it helps ensure the efficient operation of these systems **[16]**, **[17]**. In our case, the processes of movement of the general system are complex and cannot be adequately described using standard correlation coefficients for determining energy consumption in the mixer. In general, the accurate determination of energy characteristics in complex systems requires research, numerical calculations, and experiments, as well as careful analysis and consideration of all factors affecting the processes in the system. Therefore, a thermodynamic energy exchange model was used, which does not require correlations based on experimental data to determine energy characteristics in mixing systems, especially when determining the distribution of fluid movement speeds. Sometimes, the accuracy of such correlations can be lower than temperature field measurements (Figure 5).

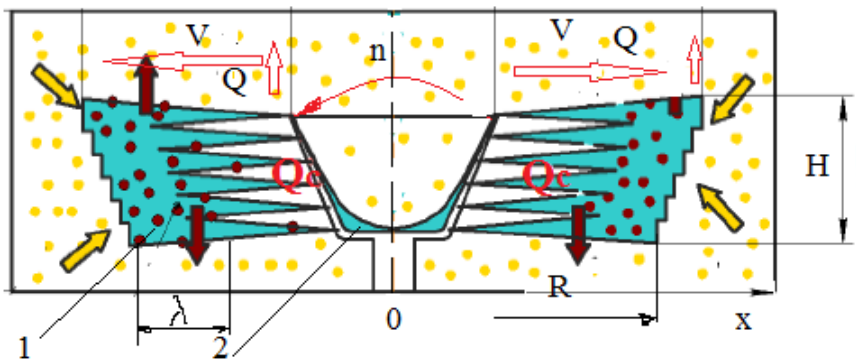


Figure 5 Scheme for calculating the interaction of components - distribution of the mixture at the outlet and from the surface of the disc working body; 2- disc working body within the radius R_{dgp} and time τ for the element of the dispersed gas phase during the existence time τ_0.

Taking into account specific aspects of the process of interacting components, key questions that may arise regarding their realism in the context of reality have been identified. Therefore, such as the heat balance equations of the gas-dispersed phase and the calculation of the specific power are important and correspond to the assumptions of real conditions. Considering that in the working chamber, the mixture is saturated with the gas phase and gradual fermentation, which constantly changes the value of the gas-dispersed phase, representative assumptions were made in the conditions of the real process. In the assumption, we did not take into account the change in the properties of the forming medium at different points; non-stationarity of mixing conditions and heat balance, especially in the case of changes in mixing volume or temperature; rheological properties, such as mixture viscosity, to more accurately reflect their behavior during mixing. The need for assumptions made regarding the properties of the resulting mixture and mixing conditions allows us to understand how the considered factors can affect the results of the study, how this can affect the accuracy of the model, and the relevance of the results obtained.

The heat balance of the gas-dispersed phase element is given by equation (1):

$$q_{\lim} = -\lambda\left(\frac{\partial t}{\partial}\right)_{\lim} = r\rho_{\lim}\frac{dR}{d\tau} \tag{1}$$

Where:
r is the specific heat of vaporization, R is the radius of the gas-dispersed phase; τ is time.

Integrating certain values of the radius of the element of the gas dispersed phase from 0 to R_{dgp}, and the time τ from o to τ_0 (τ_0 is the time of the gas dispersed phase) made it possible to obtain:

$$\int_0^{\tau_0} q_{\lim} d\tau = \int_0^{\tau_0} r\rho_{gas}\frac{dR}{d\tau}, \tag{2}$$

$$q_{\lim}\tau_0 = r\rho_{gas}R_{dgp}, \tag{3}$$

$$q_{\lim} = \frac{r\rho_{gas}R_{dgp}}{\tau_0}. \tag{4}$$

In general, the heat flow from the formed medium to the element of the gaseous dispersed phase is determined by the expression (1):

$$Q_{\lim} = N_{gen}q_{\lim} = \left(N + N_{mov}\right)\frac{r\rho_{gas}R_{dgp}}{\tau_0}. \tag{5}$$

Heat flux density, however, has the value of heat potential. It crossed the boundary of phase separation (liquid/gas phase) with the molecules of the evaporating liquid phase during the time τ0.

Assuming the absence of energy-mass exchange in the mixer with the external environment (leakage through the wall), the equation of the energy balance of the system has the form:

$$N_{oi} \pm \dot{Q}_{н.с} \pm \dot{Q}_m = \sum \Delta H. \tag{6}$$

Where:
$\sum \Delta H$ – the total change in the enthalpy working environment (between the intersection of a given system); N_{oi} – power supplied to the disc working body; $\pm\dot{Q}_{н.с}$ – heat flow between the formed medium and the external environment; $\pm\dot{Q}_m$ – heat flux in the generating medium (viscous friction).

Mostly, these flows are directed into the environment. Therefore, in the future, we consider it as a negative value in the energy balance equation.

As for the value $\sum \Delta H$, it is given according to the scheme (Figure 1) by the equation:

$$\sum \Delta H = \Delta H_Y + \Delta H_X. \tag{7}$$

$$\sum \Delta H = \left(m_{Y2}\cdot h_{Y2} - m_{Y1}\cdot h_{Y1}\right) + \left(m_{X2}\cdot h_{X2} - m_{X1}\cdot h_{X1}\right). \tag{8}$$

Where:

m_Y i m_X – Mass consumption of the working environment; h_Y, h_X – Specific enthalpy of media; Indices "1" and "2" of these values are the input and output states of the components of the working environment.

In some cases, the indexes of component parameters may differ from those specified in equation (8).

The proposed changes in the mass flow rate of gas and liquid at the moment of flow between the working bodies allow equation (6) with consideration of (8) to be given as specific values. Therefore, we denote the partial ratio of the mass flow rate of the input values of the environment B, since it is constantly changing:

$$g = \frac{m}{m_{Y1}} \tag{9}$$

So, we obtain from equation (6) the expression of the specific work of mixing

$$l_{oi} = (g_{Y2} \cdot h_{Y2} - h_{Y1}) + c_f \cdot (g_{X2} \cdot T_{X2} - g_{X1} \cdot T_{X1}) + \tilde{q}_{н.с.} \tag{10}$$

or

$$l_{oi} = l_Y + l_X + \tilde{q}_{н.с.} \tag{11}$$

Thus, the amount of specific mixing work and specific power can be determined based on the specific work of each subsystem with heat losses to the environment **[18]**, **[19]**.

Equation (11) indicates that the specific work is provided that the specific mass flow rate of the environment and the thermal parameters of each subsystem are determined. Typically, specific power is used when comparing energy efficiency

$$N_{num} = \frac{N_e}{\dot{V}_{Y1}}$$

or after transformations

$$N_{spec} = \frac{N_{oi} \cdot \rho_{Y1}}{\dot{m}_{Y1} \cdot \eta_{mech}} = l_{oi} \cdot \frac{p_{Y1}}{R_{Y1} \cdot T_{Y1} \cdot \eta_{mech}} \tag{12}$$

Where:

N_e – efficient power on the mixer shaft; $\dot{V}_{Y1}$ – volumetric capacity of the mixer; η_{mech} – mechanical efficiency of the mixer; ρ_{Y1} – density of gaseous medium *Y* at the entrance of the working bodies; R_{Y1} – gas became; $\tilde{q}_{н.с} = \dot{Q}_{н.с}/\dot{m}_{Y1}$ – specific heat flow to the environment.

When kneading dough (homogeneous liquid medium), the specific power equation:

$$N_{spec} = \frac{p_{Y1}}{R_{Y1} \cdot T_{Y1} \cdot \eta_{mech}} \left[(g_{Y2} \cdot h_{Y2} - h_{Y1}) + c_f \cdot (g_{X2} \cdot T_{X2} - g_{X1} \cdot T_{X1}) + \tilde{q}_{н.с.} \right]. \tag{13}$$

In this case, we consider:

- ideal, one-component gas;
- homogeneous medium and not compressible;
- gas solubility, condensation, and evaporation of liquid are absent.

Based on what has been said, expression (13) is simplified, since $\dot{m}_{Y2} = \dot{m}_{Y1}, g_{Y2} = 1$ $\dot{m}_{X2} = \dot{m}_{X1}$;

$$h_{Y2} - h_{Y1} = c_p \cdot \left(T_{Y2} - T_{Y1}\right) = c_p \cdot T_{Y1} \cdot \left(\pi^{\frac{n-1}{n}} - 1 \right).$$

Given the simplification of expression (13), the specific power would be:

$$N_{spec} = \frac{p_{Y1}}{R_{Y1} \cdot T_{Y1} \cdot \eta_{mech}} \cdot \left[c_p \left(\pi^{\frac{n-1}{n}} - 1 \right) + g_{X1} \cdot c_f \cdot \frac{\Delta T_X}{T_{Y1}} + \frac{q_{н.с}}{T_{Y1}} \right] \tag{14}$$

Where:

c_p – isobaric heat capacity of the specific gas component of the dough; $\pi = p_{Y2} / p_{Y1}$ – compression level of the gas component of the dough; n – showing the compression polytrope; c_f – specific heat capacity of liquid; $\Delta T_X = T_{X2} - T_{X1}$ – changing the heating in the system.

Thus, equation (14) makes it possible to determine the specific power.

The thermal gas flow formed during compression goes not only inward to the formed medium but also to the adjacent working surfaces of the mixer. We assume that surfaces are thermal only to the liquid and do not consider the heat flow from the gas to the environment, that is, heat exchange occurs between the liquid and the surface, but there is no heat loss in the form of radiation to the atmosphere. Component Definitions l_X i l_Y and Thermal Communication $\Delta T_X = f\left(g_{X1}, n\right)$ In this case, it requires considering the energy balance of the subsystem. Since they are interconnected, it is enough to determine the desired parameter l_X and its associated quantities.

Consider the energy balance of the subsystem, the diagram in Figure 6.

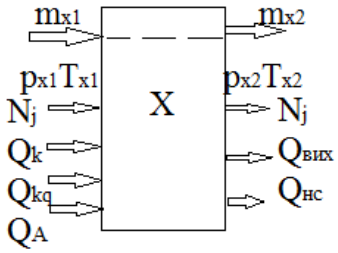


Figure 6 Diagram of the fluid subsystem of energy balance.

The equation for the conservation of energy of the subsystem is as follows:

$$Q_k + Q_{kd} + Q_A - Q_{ev} - Q_{н.с} + N_{oi} - N_i = \Delta H_X \tag{15}$$

Where:

convective heat flow removal from the compressible medium; – heat fluxes of condensation and liquid evaporation processes; Q_A – heat flux is due to the absorption of gas in a liquid; N_i – polytropic compressive power; N_{oi} – shaft power.

This equation shows the dependence of the heating of the liquid in the process of mixing the components since it is proportional. ΔH_X ΔT_X

The given specific values of expression (15) are transformed as:

$$c_f\left(g_{X2} \cdot T_{X2} - g_{X1} \cdot T_{X1}\right) q_k + q_{kd} + q_A + q_{ev} + q_{н.с} + l_{hyd} \tag{15}$$

Where:

$l_{hyd} = \left(N_{oi} - N_i\right) / m_{Y1}$ – specific work is spent on overcoming hydraulic resistances in the medium (dissipation of energy consumption during fluid movement). It is determined from experimental studies.

The solution of equation (16) is carried out concerning the parameter T_{X2}

$$T_{X2} = \frac{g_{X1}}{g_{X2}} \cdot T_{X1} + \frac{q_k + q_{kd} + q_A - q_{ev} - q_{н.с} + l_{hyd}}{c_X \cdot g_{X2}}. \tag{17}$$

Its dependence is established by: the specific heat flux of convective heat transfer between gas and liquid $q_к$, the process of condensation of the vapor phase of the liquid q_{kd}, absorption of gas into the liquid q_A, evaporation of a liquid into a compressible gas q_{ev}, heat exchange with the environment $q_{н.с}$ and the specific operation of

hydrodynamic flow rates in the mixer chamber. These values can be determined based on generally accepted laws and regularities of heat and mass transfer.

Kneading temperature readings refer to microbiological, physical, and chemical influences. They can approach the optimal and stable temperature due to heat transfer. Therefore, the disadvantage, in our opinion, is the accumulation of heat in the medium. We do not take into account the condensation of the vapor phase of the liquid q_{kd}, absorption of gas components into the working fluid q_A, or Evaporation of a liquid into a compressible gas q_{ev}, because they have a fairly limited period of existence. In addition, they can be compensated for by heat transfer by mass transfer in the forming dough. Based on this:

$$T_{X2} = \frac{g_{X1}}{g_{X2}} \cdot T_{X1} + \frac{q_k - q_{н.с} + l_{hyd}}{c_X \cdot g_{X2}}.$$

Hydrodynamic losses (specific work) in the mixer can be defined by the expression:

$$l_{hid.mov} = \frac{k_{mov} \cdot \rho_X \cdot \omega^3 \cdot r_2^5}{m_{Y1}} \quad (18)$$

Where:

ρ_X – density of the formed medium; ω – Shaft speed; k_{mov} – power factors determined experimentally; r – the radius of action of the working body on the mass of the medium. Its values depend on the geometry and Reynolds numbers Re_{mov} Defined by the expression:

$$\mathrm{Re}_{mov} = \frac{2 \cdot \omega \cdot r_2^2}{\nu_X}. \quad (19)$$

Where:

ν_X – kinematic viscosity of the medium at average temperature.

Effects of working environment temperature are determined by the value of kinematic viscosity ν_X.

Experimental and theoretical studies of the specific power Npyt during mixing and operation of a new mixer are important for determining its efficiency in various conditions of the interaction of components and the influence of the mechanical action of working bodies on them. Considering the context of mixing efficiency in determining energy consumption per process allows different mixing methods or equipment to be compared in terms of efficiency. It remains quite relevant how the change of thermodynamic parameters in expression (14) will affect the value of specific power. After all, from the above, it will be kneading without considering the constructive parameters.

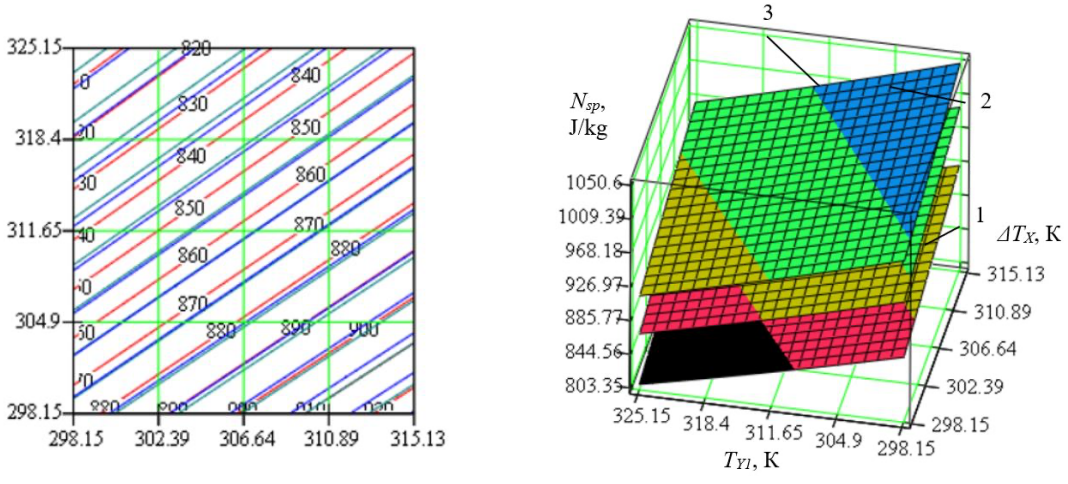


Figure 7 Dependence of specific power on changes in temperature indicators at the stages of the process: 1 – p_{Y1} = 208 Pa; 2 – p_{Y1} = 224 Pa; 3 – p_{Y1} = 236 Pa.

Based on equation (14), the thermodynamic data of the process were established. Dependencies in Figure 7 show that the specific power has an increase in its values relative to the passage of the structure formation of the dough mass.

So, in the first minutes of dosing (suspended state), we have low indicators of the created pressure in the system. These data change accordingly at the other two stages of dough mass formation, as there is a slight increase in the mechanical action of the working organs. Therefore, from the initial compression at 208 Pa of the first stage, it gradually increases in the other two. Increasing the influence of the mechanical action of the working bodies on forming the mass structure (plasticization) leads to a change in pressure from 224 Pa to 236 Pa. The fact that the specific power during its transition at each process stage has its smoothly interconnected values relative to thermodynamic processes is also worth noting.

Comparing model predictions with experimental data is an important step in validating and confirming the relevance and accuracy of the model. Such an analysis helps to determine how well the model reproduces real conditions and whether its predictions can be trusted in various scenarios of the real process. Therefore, equation (14) helps to determine whether the acquired knowledge is transferred from the model to the reality of the process or whether the model needs adjustment. The heat balance equation of the gas-dispersed phase and the calculation of specific power are key elements of modeling mixing processes in various technical applications. These mathematical models and formulas were used in the practical construction of the design parameters and process of the new mixer. They establish the impact on the design of mixing equipment and the optimization of mixing processes. Thus, the heat balance equation of the gas-dispersed phase allows us to determine how heat is transferred between different phases in the system during mixing. This is important for understanding energy losses and process efficiency. Changing parameters in the heat balance equation may affect the need to simplify the process or other aspects of heat exchange. The specific power calculation equation allows you to determine how much energy is consumed or generated during mixing per unit volume or mass of components. Changing the parameters in the equations affects the amount of power needed to achieve a specific degree of homogeneity when mixing components.

In each specific case, the effect of temperature on the specific power may be different, and to accurately determine this effect, you need to consider the specific conditions and properties of the system. Note that the effect of temperature change on specific power in a thermodynamic (isentropic) process also plays an important role in the gas law, which describes the dependence of gas pressure, volume, and temperature during kneading of yeast dough. Summarizing the results of experimental studies (Figure 5), it can be seen that the specific power in the isentropic process depends on the temperature change between the initial and final states of the mixing process. Indicators of statistical processing of experimental data of consumer power consumption for steam preparation and substantiation of effective thermodynamic energy regime parameters of specific power noted their dependence on changes in temperature indicators at the stages of the process.

The problems of the modern trend in the design of mixing working bodies in machines of various classes for performing the work processes of mixing, injection, kneading, and transportation, focused on improving productivity, efficiency, and reducing energy consumption, are presented. This makes it possible to predict the improvement of the geometry of the working bodies to reduce the resistance from the mixture of components and the formed viscous medium and avoid unforeseen consumption of energy resources. These directions in the design of mixing working bodies are also aimed at achieving greater productivity, quality, and stability in machines of various classes that perform various work processes. Inventors **[20]**, **[21]** often directed design calculations toward profile efficiency and optimization in precast applications. Circulation mixing, carried out by repeated mechanical effects on the liquid in a closed circuit of the working chamber, can be a very effective method for achieving a homogeneous environment. This process consists of the liquid circulating the circuit and undergoing mechanical effects such as displacement, turbulence, and pulsations.

In the works of the authors **[22]**, **[23]** it was noted that circulation mixing with multiple mechanical effects on the components in the closed circuit of the working chamber is an effective way to achieve homogeneity of the medium. Thanks to the repeated mechanical impact, large contact surfaces between the bulk components and the liquid are achieved, This creates the possibility of changing the mixing parameters, such as speed, time, and others, which allows you to adjust the process to increase efficiency for specific requirements and production tasks. This is important in many manufacturing processes where product accuracy and uniformity are critical.

In general, as noted by the authors **[24]**, **[25]** circulation mixing is an essential technology for achieving uniformity and efficiency in various production processes where the mixing of liquid materials is required. It can produce products, chemical reactions, biological processes, and many other applications.

Methods for determining specific costs for forming a viscous medium when mixing components are an important tool for developing optimal design parameters of machines and equipment **[26]**, **[27]**. These techniques make it possible to establish the necessary capacity required to ensure the appropriate quality and productivity of

the process. Research and tasks in mixing and thermophysical processes are of great practical importance. They can contribute to improving production processes and the efficiency of resource use. Some key aspects and benefits of these tasks are:

1. A detailed analysis of thermophysical processes helps to understand how heat and other physical parameters affect the mixing process and how this affects quality and performance **[28]**, **[29]**.
2. Evaluating and optimizing energy use is key to reducing costs and improving process sustainability **[30]**.
3. Optimizing the hardware implementation of the mixing technology allows for improving the accuracy and efficiency of the process **[31]**, **[32]**.
4. Understanding the factors that affect temperature flows allows you to manage these processes and achieve the desired results **[33]**.
5. Combining theoretical research and experiments allows you to build and test reliable models in practice **[34]**.

At the same time, there is a possibility of dosing components in a state of vibrating boiling with a corresponding decrease in the technological resistance of the medium. In addition, creating a vibration field reduces the influence of some factors. These factors, such as the friction of the components on the walls of the working chamber, the geometrical parameters of the local processing area, and the physical and mechanical properties of the components **[35]**, **[36]**, prevent the uniform distribution of the components in the volume of the mixer. The authors of the work **[37]**, **[38]** reveal the fundamental advantages of vibration action. Yes, this action allows you to effectively solve technological problems in preparing compound feeds, premixes, protein-vitamin supplements, and others.

The analysis of theoretical studies showed **[39]**, **[40]** that using rheological dependencies during the work processes of mixing the medium depends on the specific situation and process requirements. There are situations when considering rheological properties is important, but there are also cases when it is unnecessary or far from always appropriate. Deformation processes that occur when the temperature changes in the working chamber can be very complex and challenging to measure due to many factors that affect these processes. However, this complexity can also open up new research opportunities. Alternative studies can lead to developing new methods and technologies for controlling and optimizing deformation processes at a temperature change. To succeed in such studies, combining theoretical approaches with experimental methods, such as temperature field measurements and strain analysis, is important.

These research areas can significantly contribute to the industry's development and help solve problems related to mixing and heat balance in real production scenarios.

CONCLUSION

In general, the specific power of the mixer depends on the specific mixer and the properties of the liquid being mixed. Considering these parameters and performing the appropriate calculations carefully is important to optimise the mixing process. The considered dependencies are correlated with the expressed design parameters of the mixer in compliance with the technological regime. The directed movement of temperature flows in the improved heat exchange processes of the working chamber of the mixer made it possible to conduct a thermodynamic description based on an open thermomechanical system. The mixing energy balance calculation schemes using the "black box" method allow us to consider the process as two subsystems with a two-phase state of the working medium and energy exchange through an open boundary separating these subsystems. The set of theoretical calculations for determining the influence of the parameters of the hydrodynamic mixing process on the properties of the resulting dough with established specific costs considered the nature of the directed influence of the energy balance on the structural properties of the system. It is possible to judge the effective scheme of the developed technological equipment for carrying out mixing processes according to the established directions for determining specific costs. Based on the analysis, it was established that the lowest values of power consumption Pi were obtained with the option of operation of the electric motor-reducer for "cycle 2", and the indicators of the approximate values of power consumption P2 are in the range from 0.23 to 0.39 W. Any change in the mixing parameters leads to a change in the properties of the medium. Changing the state parameters of gases (vapors) and liquids is a gradual transition of matter from one state to another. The considered parameters are important for kneading from analysing the results of determining the specific power. The results of the presented rationale for determining the specific power in the mixer range from 844.56 J/kg to 1050 J/kg. Therefore, in comparison with conventional calculation methods, the proposed method allows directed control of the process of dispersion and homogenization. The developed specific surface of our system allows us to ensure the sorption activity of the components without increasing costs, maintaining the temperature parameters of the process, and undoubtedly obtaining a high-quality mixing process and kneading components while reducing specific costs. Understanding the heat balance and specific power allows you to optimize the efficiency of mixing processes, considering the energy costs and needs of the system. Modeling these processes can serve as a basis

for developing optimization strategies, for example, the selection of optimal equipment turnover or the location of dosing equipment. In summary, heat balance and specific power calculations influence mixing equipment design and can be key to optimizing mixing processes in various industries. Consequently, comparing the models shows a high correspondence to the experimental data, which allowed us to determine how well they are adapted to different scenarios and variable conditions, where the conditions can vary.

REFERENCES

1. Doğan, B., Driha, O. M., Balsalobre Lorente, D., & Shahzad, U. (2020). The mitigating effects of economic complexity and renewable energy on carbon emissions in developed countries. In Sustainable Development (Vol. 29, Issue 1, pp. 1–12). Wiley. https://doi.org/10.1002/sd.2125
2. Nižetić, S., Djilali, N., Papadopoulos, A., & Rodrigues, J. J. P. C. (2019). Smart technologies for the promotion of energy efficiency, utilization of sustainable resources, and waste management. In Journal of Cleaner Production (Vol. 231, pp. 565–591). Elsevier BV. https://doi.org/10.1016/j.jclepro.2019.04.397
3. Palamarchuk, I. P., Slobodianuk, N. M., Zozuliak, O. A., & Ivolga, A. R. (2020). Valuation of vibration and electric technical parameters of the draining process in the high-damp and liquid heterogeneous systems of processing. In Animal Science and Food Technology (Vol. 11, Issue 1, pp. 67–76). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2020.01.067
4. Stadnyk, I., Piddubnyi, V., Kravchenko, M., Rybchuk, L., Kolomiiets, O., & Danylo, S. (2020). Adhesion of marzipan pastes based on dry demineralized whey. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 1149–1160). HACCP Consulting. https://doi.org/10.5219/1351
5. Dolinskiy, A. A., Obodovich, A. N., & Sydorenko, V. V. (2018). Intensification of aeration and mass transfer in wastewater treatment by discrete-pulse energy input. In Thermophysics and Aeromechanics (Vol. 25, Issue 4, pp. 623–630). Pleiades Publishing Ltd. https://doi.org/10.1134/s0869864318040145
6. Wang, J., Geng, L., Ding, L., Zhu, H., & Yurchenko, D. (2020). The state-of-the-art review on energy harvesting from flow-induced vibrations. In Applied Energy (Vol. 267, p. 114902). Elsevier BV. https://doi.org/10.1016/j.apenergy.2020.114902
7. Stadnyk, I. Y., Pankiv, J., Havrylko, P., & Karpyk, H. (2019). Researching of the concentration distribution of soluble layers when mixed in the weight condition. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 13, Issue 1, pp. 581–592). HACCP Consulting. https://doi.org/10.5219/1129
8. TU U 10.8-00383320-001. Pressed baker's yeast Lviv yeast m/u 100g. General specifications. Quality management systems –Requirements.
9. DSTU 4623:2023. Sugar. Specifications. General specifications. Quality management systems – Requirements.
10. DSTU 7525:2014. The water is drinkable. Requirements and methods of quality control. General specifications. Quality management systems –Requirements.
11. Qiu, Q., Ding, G., Zhang, Z., Fu, C., & Oeser, M. (2021). Investigation on bitumen-epoxy interface in cold mixed epoxy bitumen using experimental observation and molecular dynamics simulation. In Construction and Building Materials (Vol. 303, p. 124490). Elsevier BV. https://doi.org/10.1016/j.conbuildmat.2021.124490
12. Kraievska, S., Zheplinska, M., Mushtruk, M., Piddubnyi, V., Stadnyk, I., & Vasyliv, V. (2022). Determination of rational parameters of pseudomixing by complex modelling of work processes. In Animal Science and Food Technology (Vol. 13, Issue 1, pp. 7–15). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(1).2022.7-15
13. Harrison, S. T. L., Kotsiopoulos, A., Stevenson, R., & Cilliers, J. J. (2020). Mixing indices allow scale-up of stirred tank slurry reactor conditions for equivalent homogeneity. In Chemical Engineering Research and Design (Vol. 153, pp. 865–874). Elsevier BV. https://doi.org/10.1016/j.cherd.2019.10.049
14. Khalilzadeh Shirazi, E., Metzger, J. W., Fischer, K., & Hassani, A. H. (2020). Removal of textile dyes from single and binary component systems by Persian bentonite and a mixed adsorbent of bentonite/charred dolomite. In Colloids and Surfaces A: Physicochemical and Engineering Aspects (Vol. 598, p. 124807). Elsevier BV. https://doi.org/10.1016/j.colsurfa.2020.124807
15. Zheplinska, M., Mushtruk, M., & Salavor, O. (2021). Cavitational Impact on Electrical Conductivity in the Beet Processing Industry. In Lecture Notes in Mechanical Engineering (pp. 755–762). Springer International Publishing. https://doi.org/10.1007/978-3-030-68014-5_73
16. Chernenkova, A., Leonova, S., Chernykh, V., & Chernenkov, E. (2020). Influence of biologically active raw materials on rheological properties of flour confectionery products. In Acta Biologica Szegediensis (Vol. 63, Issue 2, pp. 195–205). University of Szeged. https://doi.org/10.14232/abs.2019.2.195-205

17. Muscalu, G., Voicu, G., Istudor, A., & Tudor, P. (2020). Bread Dough Rheological Behavior Under the Influence of the Geometry of the Kneading Arms. In Revista de Chimie (Vol. 71, Issue 9, pp. 295–307). Revista de Chimie SRL. https://doi.org/10.37358/rc.20.9.8340
18. Burova, Z., Vorobiov, L., Ivanov, S., & Dekusha, O. (2022). Measures and means to improve the energy efficiency of food production. In Animal Science and Food Technology (Vol. 13, Issue 2, pp. 7–15). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(2).2022.7-15
19. Xue, F., Zhang, W., Xue, F., Li, D., Xie, S., & Fleischer, J. (2021). A novel intelligent fault diagnosis method of rolling bearing based on a two-stream feature fusion convolutional neural network. In Measurement (Vol. 176, p. 109226). Elsevier BV. https://doi.org/10.1016/j.measurement.2021.109226
20. Zheplinska, M., Mushtruk, M., Vasyliv, V., Kuts, A., Slobodyanyuk, N., Bal-Prylypko, L., Nikolaenko, M., Kokhan, O., Reznichenko, Y., & Salavor, O. (2021). The micronutrient profile of medicinal plant extracts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 528–535). HACCP Consulting. https://doi.org/10.5219/1553
21. Mayer, M., Möller, S., Rubel, M., Widdowson, A., Charisopoulos, S., Ahlgren, T., Alves, E., Apostolopoulos, G., Barradas, N. P., Donnelly, S., Fazinić, S., Heinola, K., Kakuee, O., Khodja, H., Kimura, A., Lagoyannis, A., Li, M., Markelj, S., Mudrinic, M., Wang, Y. Q. (2019). Ion beam analysis of fusion plasma-facing materials and components: facilities and research challenges. In Nuclear Fusion (Vol. 60, Issue 2, p. 025001). IOP Publishing. https://doi.org/10.1088/1741-4326/ab5817
22. Vasyliv, V., Mushtruk, M., Zheplinska, M., Mukoid, R., & Tkachenko, S. (2021). Method of Electrohydraulic Activation of Water-Lime Suspension in Sugar Production. In Lecture Notes in Mechanical Engineering (pp. 664–673). Springer International Publishing. https://doi.org/10.1007/978-3-030-91327-4_6
23. Sharkova, N. O., Zhukotskyi, E. K., Turchyna, T. Y., Dekusha, H. V., & Makarenko, A. A. (2018). Research of effect of dpei-processing of shiitake fruit body on physical and mechanical properties of mushroom suspension. In Thermophysics and Thermal Power Engineering (Vol. 41, Issue 1, pp. 27–33). Institute of Engineering Thermophysics of NAS of Ukraine. https://doi.org/10.31472/ttpe.1.2019.4
24. Zheplinska, M., Mushtruk, M., Vasyliv, V., Slobodyanyuk, N., & Boyko, Y. (2021). The Main Parameters of the Physalis Convection Drying Process. In Lecture Notes in Mechanical Engineering (pp. 306–315). Springer International Publishing. https://doi.org/10.1007/978-3-030-77823-1_31
25. Mushtruk, M., Palamarchuk, I., Palamarchuk, V., Gudzenko, M., Slobodyanyuk, N., Zhuravel, D., Petrychenko, I., & Pylypchuk, O. (2023). Mathematical modelling of quality assessment of cooked sausages with the addition of vegetable additives. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 242–255). HACCP Consulting. https://doi.org/10.5219/1845
26. Orosz, T., Rassõlkin, A., Kallaste, A., Arsénio, P., Pánek, D., Kaska, J., & Karban, P. (2020). Robust Design Optimization and Emerging Technologies for Electrical Machines: Challenges and Open Problems. In Applied Sciences (Vol. 10, Issue 19, p. 6653). MDPI AG. https://doi.org/10.3390/app10196653
27. Zinchenko, R., & Slyva, Y. (2022). Analysis of the use of plant components in the production of meat products. In Animal Science and Food Technology (Vol. 13, Issue 4, pp. 19–29). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(4).2022.19-29
28. Ibrahim, M. G. (2022). Numerical simulation to the activation energy study on blood flow of seminal nanofluid with mixed convection effects. In Computer Methods in Biomechanics and Biomedical Engineering (Vol. 26, Issue 3, pp. 315–325). Informa UK Limited. https://doi.org/10.1080/10255842.2022.2063018
29. Savenkova, T. V., Soldatova, E. A., Misteneva, S. Yu., & Taleysnik, M. A. (2019). Technological properties of flour and their effect on quality indicators of sugar cookies. In Food Systems (Vol. 2, Issue 2, pp. 13–19). The Gorbatov's All-Russian Meat Research Institute. https://doi.org/10.21323/2618-9771-2019-2-2-13-19
30. Rödger, J.-M., Beier, J., Schönemann, M., Schulze, C., Thiede, S., Bey, N., Herrmann, C., & Hauschild, M. Z. (2020). Combining Life Cycle Assessment and Manufacturing System Simulation: Evaluating Dynamic Impacts from Renewable Energy Supply on Product-Specific Environmental Footprints. In International Journal of Precision Engineering and Manufacturing-Green Technology (Vol. 8, Issue 3, pp. 1007–1026). Springer Science and Business Media LLC. https://doi.org/10.1007/s40684-020-00229-z
31. Sá-Caputo, D., Paineiras-Domingos, L. L., Francisca-Santos, A., dos Anjos, E. M., Reis, A. S., Neves, M. F. T., Oigman, W., Oliveira, R., Brandão, A., Machado, C. B., Chiementin, X., Taiar, R., Sartório, A., & Bernardo-Filho, M. (2019). Whole-body vibration improves the functional parameters of individuals with metabolic syndrome: an exploratory study. In BMC Endocrine Disorders (Vol. 19, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12902-018-0329-0

32. Singla, M., & Sit, N. (2021). Application of ultrasound in combination with other technologies in food processing: A review. In Ultrasonics Sonochemistry (Vol. 73, p. 105506). Elsevier BV. https://doi.org/10.1016/j.ultsonch.2021.105506
33. Stadnyk, I., Mushtruk, M. M., Vasyliv, V., Zheplinska, M., Palamarchuk, I., Burova, Z., & Gudzenko, M. M. (2021). Dynamics of interfacial interaction between components during mixing. In Animal Science and Food Technology (Vol. 12, Issue 2, pp. 60–72). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.02.007
34. Palamarchuk, I. P., Mushtruk, M. M., Palamarchuk, V. I., Deviatko, O. S., Wójcik, W., Kalizhanova, A., & Kozbakova, A. (2021). Physical-mathematical modelling of the process of infrared drying of rape with vibration transport of products. In Mechatronic Systems 1 (pp. 243–253). Routledge. https://doi.org/10.1201/9781003224136-21
35. Nowak, D., & Jakubczyk, E. (2020). The Freeze-Drying of Foods—The Characteristic of the Process Course and the Effect of Its Parameters on the Physical Properties of Food Materials. In Foods (Vol. 9, Issue 10, p. 1488). MDPI AG. https://doi.org/10.3390/foods9101488
36. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/1711
37. Sun, W., Jo, S., & Seok, J. (2019). Development of the optimal bluff body for wind energy harvesting using the synergetic effect of coupled vortex-induced vibration and galloping phenomena. In International Journal of Mechanical Sciences (Vol. 156, pp. 435–445). Elsevier BV. https://doi.org/10.1016/j.ijmecsci.2019.04.019
38. Palamarchuk, I., Palamarchuk, V., Mushtruk, M., Shtefan, E., & Petrychenko, I. (2023). Using the Similarity Theory in designing vibroconveyor dryer for grain. In Advanced Manufacturing Processes V (pp. 550–560). Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-42778-7_51
39. Dima, C., Assadpour, E., Dima, S., & Jafari, S. M. (2020). Bioavailability of nutraceuticals: Role of the food matrix, processing conditions, the gastrointestinal tract, and nano delivery systems. In Comprehensive Reviews in Food Science and Food Safety (Vol. 19, Issue 3, pp. 954–994). Wiley. https://doi.org/10.1111/1541-4337.12547
40. Palamarchuk, I., Palamarchuk, V., Mushtruk, M., Shtefan, E., & Petrychenko, I. (2023). Using the Similarity Theory in Designing Vibroconveyor Dryer for Grain. In Advanced Manufacturing Processes V (pp. 550–560). Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-42778-7_51

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Anastasiia Blahopoluchna.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Volodymyr Piddubnyi, Kyiv National University of Trade and Economics, Faculty of Biotechnology and Food Sciences, Department of Technologies and Organization of Restaurant Business, Kyoto str. 19, 02156, Kyiv, Ukraine,
E-mail: a.poddubnaya@i.ua
ORCID: https://orcid.org/0000-0002-1497-7133

Anna Sabadosh, Uzhhorod Trade and Economic Institute DTEU, Department of Technology and Organization of the Restaurant Industry, str. Korotnunskogo 4, Uzhhorod, 88020, Ukraine,
E-mail: aasaa30@ukr.net
ORCID: https://orcid.org/ 0000-0002-4749-5608

***Mikhailo Mushtruk**, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Processes and Equipment for Processing of Agricultural Production, Heroes of Defense Str., 12 B, Kyiv, 03040, Ukraine,

E-mail: mixej.1984@ukr.net
ORCID: https://orcid.org/0000-0002-3646-1226

Andrii Chahaida, Zhytomyr Polytechnic State University, Department of tourism and hotel and restaurant business Chudnivska str., 103, 10005, Zhytomyr, Ukraine,
E-mail: andrey11081968@ukr.net
ORCID: https://orcid.org/0000-0003-1826-9545

Viktor Fedorov, Khmelnytskyi National University, Department of Industrial Mechanical Engineering and Agricultural Engineering, str. Instytutska, 11, Khmelnytskyi, 29016, Ukraine,
E-mail: fedoriv55@ukr.net
ORCID: https://orcid.org/0000-0002-4499-0910

Khrystyna Kravcheniuk, Ternopil Ivan Puluj National Technical University, Department of food biotechnology and chemistry, st. Ruska, 56, Ternopil, 46001, Ukraine,
E-mail: kravchenukx30@gmail.com
ORCID: https://orcid.org/0000-0002-7547-6834

Svitlana Krasnozhon, Kyiv National Economic University named after Vadym Hetman, Department of corporate finance and controlling faculty of finance, str. Degtyarivska, 49-g, Kyiv, 02156, Ukraine,
E-mail: svetle@ukr.net
ORCID: https://orcid.org/0000-0001-8068-2096

Iurii Radchenko, Azienda Darmi Fabarm, Travagliato Via averolda 31. Italy 25039,
E-mail: fauzzi@ukr.net
ORCID: https://orcid.org/0009-0001-7435-0738

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 14.12.2023
Revised: 25.1.2024
Accepted: 2.2.2024
Published: 5.3.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 268-280
https://doi.org/10.5219/1944
ISSN: 1337-0960 online
www.potravinarstvo.com

Comparison of essential indicators related to the personal income tax burden in the EU countries

Alexandra Ferenczi Vaňová, Renáta Krajčírová, Michal Munk, Andrea Ecková, Jozef Bojňanský

ABSTRACT

Agriculture performs productive functions and is an essential provider of job opportunities. The labour force is one of the important factors affecting agricultural and food production. In general, the business environment affects, among other things, employers' and employees' personal income tax burden. Increasing the efficiency of business activity is inevitably associated with achieving the lowest possible costs. It can be concluded that labor and other personal costs represent, on average, 30-60% of the costs of the agricultural product. In general, taxation of individuals engaged in agriculture with personal income tax does not differ from taxation of individual's income in other sectors of the national economy. The paper focuses on assessing indicators related to the personal income tax burden in the EU countries for the years 2008-2020 based on the data from the Eurostat Tax Classification presented by descriptive characteristics. Achieved results are compared within the EU Member States and for particular countries with an EU-27 average. The West EU countries implement tax and fiscal policies compared to the East EU countries, which are not subject to significant changes in the tax system and thus ensure relatively stable tax revenues to public budgets regarding total taxes and employment income, including unchanged personal income tax rates. The East EU countries declare a lower tax burden of personal income tax. Still, due to the competitiveness of the countries, this burden is also being gradually reduced in the reviewed period in the case of the West EU countries, thus bringing closer unification of the tax policies. Slovakia achieved below-average values of all assessed indicators and at the same time high variability.

Keywords: agriculture, employment income, personal income tax rate, tax and fiscal policy, tax burden

INTRODUCTION

Agriculture and food production in Slovakia are one of the main pillars of the national economy. The sustainability of these industries is crucial for further economic development, ensuring the country´s food security, and satisfying domestic demand **[1]**. Using the specific function and the importance of agriculture within the national economy this sector is the subject of government regulations **[2]**. The essential specific of entrepreneurship in agriculture is the active participation of the government and its agricultural policy in trying to sustain the food balance, utilize the domestic production potential, and fulfill outside production functions of agriculture **[3]**. Farmers and agriculture systems play an essential and increasingly important role in protecting the landscape, and rural environment, and contributing to the social and economic development of rural areas **[4]**. The structure of tax systems is one of the factors that significantly affect countries' economic growth. For this reason, it is important to look at individual taxes as a possible source of budget revenue and their impact on economic growth **[5]**. Major changes in the tax systems of the EU countries have resulted in the globalization and digitalization of the economy, which has substantially increased the geographical mobility of taxation. This has

created a competitive environment between the tax systems that raised concerns about the level and fairness of the tax policies from a global perspective **[6]**. The importance of taxation for the EU is visible through the four pillars of progress and development, namely, a true Economic Union, which will allow each Member State to have the structural characteristics for prosperity in the Monetary Union, Financial Union, which guarantees the integrity of the common currency and through which a fair risk distribution takes place; a Fiscal Union that would lead to fiscal stability and fiscal sustainability; a Political Union based on responsibility, legitimacy, and consolidation **[7]**. Taxation is one of the few fields of EU policy in which unanimity is required for new legislation to be implemented. EU Member States remain extremely reluctant to cede any of their sovereignty in tax matters to the EU **[8]**. Macroeconomic indicators are used to assess the state of a country´s economy and measure a country´s overall economic performance. These indicators are different quantified and focus on certain countries or sectors. The most important indicator of the performance of the economy of the given country as a whole, which is related to supply and demand on consumption, is GDP **[9]**, **[10]**. Given the relationship between collected tax and the gross domestic product, many economic components can affect the tax burden. The tax burden can be defined as the ratio of collected taxes in a particular period against the total product. Calculations of tax burden mean determining taxes' effects on national and international approaches **[13]**. One of the macroeconomic indicators represents tax quota, which is constructed as a ratio of total tax revenue and nominal GDP of the economy. The indicator itself includes direct and indirect taxes. It is necessary to distinguish between tax quota and so-called compound tax quota, which, besides direct and indirect taxes, also includes compulsory social security contributions, which predicate the tax burden precisely **[11]**. Calculating the tax burden becomes more important for the comparison of tax systems. Each country has its own tax system. It should be noted that the tax systems have gradually changed; they were adapted to the specificities and requirements of each country **[12]**. EU Member States have proposed a wide range of reforms across many types of taxes both in terms of direct and indirect taxation, from personal income tax to corporate income tax, from value-added tax to environmental taxation. Personal income tax-related reforms also took place due to the economic recovery after the COVID-19 pandemic as the temporary measures taken during the COVID-19 pandemic have slowly been adjusted back to those of normal times **[13]**. A different construction of taxes in the individual EU countries is the reason for the low information value of specific taxes in the international comparison **[14]**. The present tax policy of the EU prefers revenue from indirect taxes (e.g. value, added tax and excise taxes); the value of indirect taxes has continuously increased since 2001. The development of revenue from direct taxes (e.g. personal income tax and social security contributions) has fluctuated. On the other hand, the value of revenue from quasi-taxes, mainly from social contributions, has a steady development in the EU countries, and it only decreases very slowly **[15]**. The most important financial source of revenue for the state budget is taxes and is considered an instrument for the implementation of the state's fiscal policy with medium to long-term results **[16]**. Despite increasing globalization, income tax revenues have provided comparable inflows to state budgets over the last years **[17]**. Personal income tax is the most important fiscal instrument. Its share amounts to 20-25% of total fiscal revenues in many European countries and other developed economies because of its important role in enhancing social equity. On the other hand, a high personal income tax rate is considered a significant factor for inefficiencies in labor markets. Therefore, over the last decades, most industrialized countries initiated reforms aiming at reducing the overall tax burden, particularly personal income tax rates. The reforms in most of these countries focused on decreasing top marginal tax rates, but also on decreasing minimum marginal tax rates on low-income workers **[18]**. Decreasing personal income tax rates has a positive effect on the creation labor market and also a positive impact on domestic consumption. Labor tax burdens represent the relationship between personal income tax and social security contributions and are complicated since the relationship is simultaneously both close and distant **[19]**. The relationship between tax and social security contributions is complicated since the relationship is simultaneously both close and distant. The payments share some similar characteristics, but they are also very different in nature due to the divergent purpose of the payments **[20]**. Neither personal income tax nor social security is harmonized within the EU. In most EU countries, personal income tax and social security contributions are relatively distinct payments. Personal income tax and social security contributions are integral parts of the overall taxation system in modern economies **[21]**. Labour taxation (i. e. the taxation of employment income and social security contributions) contributes, on average, just over half of the tax revenues in the EU Labour taxation influences economic growth by affecting the incentives to work and hire. The European Commission and other international organizations such as the OECD and IMF have argued that shifting some of the tax burden from labor taxation towards consumption and property taxation could foster economic growth. This position is part of a large debate involving academic scholars and policymakers on the implications of the taxation structure **[13]**.

At the theoretical and practical level, several studies deal with the assessment of the tax burden through selected macroeconomic indicators in EU countries **[22]**, **[11]**, **[15]**, **[23]**, **[24]**, **[25]**, **[26]**, **[27]**, **[14]**, **[28]**, **[29]**, **[30]** and the others. In general, the tax quota is one of the most comprehensive indicators of the tax burden in

international comparisons of countries. It measures the share of taxes collected as a share of gross domestic product (further referred to as "GDP") in a given tax year. The composite or aggregate tax quota reflects the total tax and levy burden on entities. Our research builds on previous research that focused on assessing the selected countries' overall tax burden in 2002-2011.

Current research and hence the paper aim to assess the personal income tax burden through the selected indicators such as Total taxes, Employment income, and Top statutory personal income tax rate of 27 EU Member States as well as to compare each particular country with EU-27 average in the reviewed period of the years 2008-2020.

Scientific Hypothesis

The structure of taxation varies significantly in EU countries. It depends on several factors such as historical development, different conceptions of tax and social system, and tax and fiscal policy. The status of individuals and taxation of the labor force in agriculture is generally identical to other sectors of the national economy of individual countries. For this reason, it is necessary to first deal with the tax burden of personal income tax within the EU Member States. We assume that the average values of selected indicators such as Total taxes, Employment income, and Personal income tax rates in EU countries are significantly different for the reviewed period.

MATERIAL AND METHODOLOGY

Data sources that are tax revenue by type of tax are obtained from Eurostat and follow the tax classification defined by the ESA 2010 methodology. Eurostat supplements its database with the national tax lists supplied to Eurostat by the EU member states. Many revenue indicators are presented as a percentage of GDP. Considering the issue of personal income tax, our research focuses on the evaluation of the data obtained from Eurostat Tax Classification (data updated in June 2022 including tax revenue data up to 2020), in the years 2008-2020 in 27 particular Member States of the EU (without Iceland and Norway). These are specifically the following indicators:

- ✓ Total taxes, according to tax revenues by type of tax and level of government, present total taxes including social security contributions expressed as a percentage of GDP;
- ✓ Taxes on employed labor (further referred to as "Employment income"), according to tax revenues by the tax base, present tax revenues from employment income expressed as a percentage of GDP;
- ✓ In addition, we assess the other indicators such as the Top statutory personal income tax (further referred to as "PIT") rate expressed as a percentage.

Total taxes including social security contributions are defined as taxes on production and imports, current taxes on income and wealth, capital taxes, and social security contributions. Employment income comprises all taxes, directly linked to wages and mostly withheld at source, paid by employers and employees, including actual compulsory social contributions. This means the personal income tax is typically levied on different sources of income: labor income, including taxable social benefits. Apart from the aggregate data in national accounts, additional assessed data are the Top statutory PIT rates defined by national legislations and reported by each particular EU Member State. According to Eurostat, the Top statutory PIT rate does not differentiate by source of income, so surcharges and deductions specific to income source are not considered.

Statistical Analysis

The research is focused on the consideration of the selected indicators mentioned above (Total taxes, Employment income, and Top statutory PIT rate) and also their comparison with the EU-27 average presented by the descriptive characteristics **[31]**, **[32]**, **[33]** that are assessed in the period 2008-2020, i.e. for 13 years. Exploratory techniques were used to process the results obtained from public databases to compare monitored indicators between the EU-27 Member States and against the overall EU-27 average. The comparison was based on the calculation and visualization of point and interval estimates of location characteristics and variability (the point and interval mean estimate, the standard error of mean estimate, and the standard deviation) of the monitored indicators (Total taxes, Employment income, and Top statutory PIT rate) for the EU-27 and particular EU Member State.

RESULTS AND DISCUSSION

In general, it is currently difficult to find a comprehensive indicator of the tax burden suitable for international comparison, mainly because of the different tax systems constantly evolving and changing over time. The tax quota is one of the most widely used indicators for comparing the tax burden among countries. This macroeconomic indicator can take several forms and generally represents the share of taxes and social security contributions in relation to GDP.

For the indicator Total taxes (Table 1), based on the results of the mean, 95.00% confidence interval, and standard deviation, we found that compared to the EU-27 average values (Mean = 39.33164; 95.00% confidence interval of mean estimate between 38.83127 and 39.83201; Std. Dev. = 0.828018) recorded higher and therefore above-average values of the Total taxes indicator and at the same time lower variability of the selected indicator, i.e. homogeneous countries, which include Austria (Mean = 42. 02729; Std. Dev. = 0.679653), Italy (Mean = 42.11863; Std. Dev. = 0.771224), Sweden (Mean = 43.04061; Std. Dev. = 0.784335) and Belgium (Mean = 44.47251; Std. Dev. = 0.885526).

Table 1 Descriptive statistics for indicator Total taxes.

Country	N	Mean	Std.Dev.	Std.Err.	-95.00%	+95.00%
Denmark	13	45.88	1.19	0.33	45.16	46.60
France	13	44.70	1.53	0.42	43.78	45.62
Belgium	13	44.47	0.89	0.25	43.94	45.01
Sweden	13	43.04	0.78	0.22	42.57	43.51
Finland	13	42.34	1.07	0.30	41.70	42.99
Italy	13	42.12	0.77	0.21	41.65	42.58
Austria	13	42.03	0.68	0.19	41.62	42.44
Germany	13	38.78	0.88	0.24	38.25	39.31
Hungary	13	37.89	1.16	0.32	37.19	38.59
Slovenia	13	37.54	0.21	0.06	37.42	37.67
Netherlands	13	37.12	1.64	0.45	36.13	38.11
Luxembourg	13	36.68	1.47	0.41	35.79	37.56
Croatia	13	36.53	0.76	0.21	36.07	36.99
Greece	13	36.22	3.13	0.87	34.33	38.11
Czechia	13	34.54	1.16	0.32	33.84	35.24
Spain	13	33.22	1.84	0.51	32.10	34.33
Poland	13	33.15	1.55	0.43	32.22	34.09
Portugal	13	33.15	1.77	0.49	32.08	34.21
Cyprus	13	32.93	1.17	0.32	32.22	33.63
Estonia	13	32.82	1.11	0.31	32.15	33.49
Slovakia	13	31.44	2.53	0.70	29.91	32.97
Malta	13	30.82	0.85	0.24	30.31	31.33
Latvia	13	29.65	1.31	0.36	28.85	30.44
Lithuania	13	28.98	1.46	0.41	28.10	29.86
Bulgaria	13	28.41	1.91	0.53	27.25	29.56
Romania	13	26.67	1.09	0.30	26.01	27.33
Ireland	13	25.59	3.27	0.91	23.61	27.57
EU-27	13	39.33	0.83	0.23	38.83	39.83

Note: - 95.00% +95.00% - 95% confidence interval of the mean estimate.

On the contrary, the countries with below-average values of this indicator and high variability compared to the EU-27 values, i.e. heterogeneous countries, include Ireland (Mean = 25.59330; Std. Dev. = 3.273899), which recorded the lowest average value of this indicator among the assessed countries and the highest variability, as well as Greece (Mean = 36.22006; Std. Dev. = 3.133599), are among the most heterogeneous countries.

Slovakia (Mean = 31.44262; 95.00% confidence interval of mean estimate between 29.91385 and 32.97139; Std. Dev. = 2.529841) has the third highest variability within the EU countries, with the lowest average value of the Total taxes indicator and at the same time the highest variability compared to the surrounding selected countries such as Hungary (Mean = 37.88882; 95.00% confidence interval of mean estimate between 37.18663 and 38.59101; Std. Dev. = 1.162001), Czechia (Mean = 34.53868; Std. Dev. = 1.158392) and Poland (Mean = 33.15391; Std. Dev. = 1.548724), as well as in the comparison of Slovakia compared to EU-27 values (Mean = 39.33164; 95.00% confidence interval of mean estimate between 38.83127 and 39.83201; Std. Dev. = 0.82801). Countries such as Hungary, Czechia, and Poland have thus recorded higher average values of this indicator compared to Slovakia and approximately the same level of variability. The lowest variability was found for Slovenia (Mean = 37.54419; Std. Dev. = 0.211915). Besides Ireland, the lowest mean values of this indicator were recorded for countries such as Romania (Mean = 26.67193; Std. Dev. = 1.090078) and Bulgaria (Mean = 28.40535; Std. Dev. = 1.905741).

Also in the case of the Employment income indicator (Table 2), its above-average values compared to the EU-27 average (Mean = 18.23237; 95.00% confidence interval of mean estimate between 18.02898 and 18.43576; Std. Dev. = 0.336573) were achieved by the following countries: Sweden (Mean = 21.95575; Std. Dev. = 0.555988), France (Mean = 21.57219; Std. Dev. = 0.765308), Belgium (Mean = 21.36801; Std. Dev. = 0. 894024), Austria (Mean = 20.97773; Std. Dev. = 0.433196) and Italy (Mean = 18.35758; Std. Dev. = 0.440902), which also have the lowest variability of the indicator, i.e. they are homogeneous countries.

Table 2 Descriptive statistics for indicator Employment income.

Country	N	Mean	Std.Dev.	Std.Err.	-95.00%	+95.00%
Sweden	13	21.96	0.56	0.15	21.62	22.29
France	13	21.57	0.77	0.21	21.11	22.03
Belgium	13	21.37	0.89	0.25	20.83	21.91
Austria	13	20.98	0.43	0.12	20.72	21.24
Germany	13	19.18	0.75	0.21	18.73	19.64
Finland	13	19.04	0.69	0.19	18.62	19.46
Italy	13	18.36	0.44	0.12	18.09	18.62
Denmark	13	18.03	0.51	0.14	17.72	18.34
Slovenia	13	17.90	0.44	0.12	17.64	18.17
Hungary	13	17.46	0.83	0.23	16.95	17.96
Czechia	13	16.58	0.96	0.27	16.00	17.16
Estonia	13	15.82	0.55	0.15	15.49	16.16
Netherlands	13	15.70	0.28	0.08	15.53	15.87
Spain	13	15.48	0.81	0.22	14.99	15.96
Luxembourg	13	14.74	0.78	0.22	14.27	15.21
Croatia	13	14.27	0.60	0.17	13.91	14.64
Latvia	13	13.92	0.55	0.15	13.59	14.25
Slovakia	13	13.77	1.57	0.44	12.82	14.72
Greece	13	13.76	1.06	0.29	13.12	14.40
Lithuania	13	13.21	0.87	0.24	12.68	13.74
Portugal	13	12.61	0.87	0.24	12.08	13.14
Poland	13	12.30	0.87	0.24	11.77	12.83
Cyprus	13	11.60	1.17	0.32	10.89	12.31
Romania	13	11.03	0.82	0.23	10.54	11.53
Ireland	13	10.94	1.53	0.42	10.01	11.86
Bulgaria	13	9.71	1.03	0.28	9.09	10.33
Malta	13	9.50	0.55	0.15	9.17	9.84
EU-27	13	18.23	0.34	0.09	18.03	18.44

Note: - 95.00% +95.00% - 95% confidence interval of the mean estimate.

The countries that have below-average values of this indicator compared to EU-27 values and the highest variability within them, i.e. heterogeneous countries, can be classified as Slovakia (Mean = 13.77149; Std. Dev. = 1. 569211), which among all the assessed countries recorded the highest variability together with Ireland (Mean = 10.93575; Std. Dev. = 1.528891), Cyprus (Mean = 11.59966; Std. Dev. = 1.167690), Greece (Mean = 13.76254; Std. Dev. = 1.057570), Bulgaria (Mean = 9.70596; Std. Dev. = 1.025974). Countries such as Hungary (Mean = 17.45821; Std. Dev. = 0.834386) and Czechia (Mean = 16.57977; Std. Dev. = 0.957086) recorded higher mean values of this indicator and its lower variability value compared to Slovakia. Poland (Mean = 12.30156; Std. Dev. = 0.872068) achieved a lower mean value of this indicator than Slovakia and approximately the same variability as Hungary and Czechia.

Countries with below-average indicator values and the lowest variability, i.e. homogeneous countries, include the Netherlands (Mean = 15.70239; Std. Dev. = 0.280917). Similarly, as in the case of the indicator of Total taxes, in the case of Employment income, the lowest mean values of the indicator were recorded for countries such as Bulgaria (Mean = 9.70596; Std. Dev. = 1.025974), Ireland (Mean = 10.93575; Std. Dev. = 1.528891) and Romania (Mean = 11.03187; Std. Dev. = 0.820205).

In the case of the Top statutory PIT rate indicator (Table 3), above average values compared to the EU-27 average (Mean = 38.57962; 95.00% confidence interval of mean estimate between 38.33033 and 38.82891; Std. Dev. = 0.412527) were achieved by the following countries: Denmark (Mean = 56.67589; Std. Dev. = 2.46646),

Sweden (Mean = 56.46308; Std. Dev. = 1.28418), Belgium (Mean = 53.50006; Std. Dev. = 0.31294), which also recorded the lowest variability.

In contrast, countries such as Austria (Mean = 50.00000; Std. Dev. = 0.00000) and Germany (Mean = 47.47500; Std. Dev. = 0.00000) had zero variability in the indicator, Portugal (Mean = 51.54538; Std. Dev. = 5.39669) and Greece (Mean = 48.76923; Std. Dev. = 5.11784) had the highest variability in the indicator among the countries that scored above average values.

Countries with below-average values of this indicator compared to the EU-27 values and countries such as Austria (Mean = 50.00000; Std. Dev. = 0.00000) and Germany (Mean = 47.47500; Std. Dev. = 0.00000) achieved zero variability of this indicator, Portugal (Mean = 51. 54538; Std. Dev. = 5.39669) and Greece (Mean = 48.76923; Std. Dev. = 5.11784) the highest variability of the indicator among the countries that recorded above average values. with the highest variability include Hungary (Mean = 21.86154; Std. Dev. = 10.61638) and Lithuania (Mean = 17.92308; Std. Dev. = 5.79456). Countries with below-average values for this indicator, such as Malta (Mean = 35.00000), Czechia (Mean = 15.00000), and Bulgaria (Mean = 10.00000), recorded zero variability.

Table 3 Descriptive statistics for indicator Top statutory PIT rate.

Country	N	Mean	Std.Dev.	Std.Err.	-95.00%	+95.00%
Denmark	13	56.68	2.47	0.68	55.19	58.17
Sweden	13	56.46	1.28	0.36	55.69	57.24
Belgium	13	53.50	0.31	0.09	53.31	53.69
Netherlands	13	51.78	0.69	0.19	51.37	52.20
Portugal	13	51.55	5.40	1.50	48.28	54.81
Finland	13	50.53	1.09	0.30	49.87	51.18
Austria	13	50.00	0.00	0.00	50.00	50.00
France	13	49.14	2.44	0.68	47.66	50.61
Greece	13	48.77	5.12	1.42	45.68	51.86
Germany	13	47.48	0.00	0.00	47.48	47.48
Croatia	13	47.11	4.20	1.17	44.57	49.65
Italy	13	47.02	1.28	0.35	46.24	47.79
Ireland	13	46.62	2.79	0.77	44.93	48.30
Slovenia	13	46.54	4.56	1.26	43.78	49.29
Spain	13	45.69	3.67	1.02	43.47	47.91
Luxembourg	13	42.92	2.65	0.73	41.32	44.52
Malta	13	35.00	0.00	0.00	35.00	35.00
Cyprus	13	33.85	2.19	0.61	32.52	35.17
Poland	13	32.62	2.22	0.62	31.27	33.96
Latvia	13	25.78	3.34	0.93	23.77	27.80
Slovakia	13	22.69	3.04	0.84	20.86	24.53
Hungary	13	21.86	10.62	2.94	15.45	28.28
Estonia	13	20.54	0.52	0.14	20.22	20.85
Lithuania	13	17.92	5.79	1.61	14.42	21.42
Czechia	13	15.00	0.00	0.00	15.00	15.00
Romania	13	14.62	2.63	0.73	13.03	16.21
Bulgaria	13	10.00	0.00	0.00	10.00	10.00
EU-27	13	38.58	0.41	0.11	38.33	38.83

Note: - 95.00% +95.00% - 95% confidence interval of the mean estimate.

Slovakia (mean value of this indicator was at the level of Mean = 22.69231; 95.00% confidence interval of mean estimate between 20.85633 and 24.52828; Std. Dev. = 3.03822) achieved lower mean values of the above indicator compared to Poland (Mean = 32.61538; 95.00% confidence interval of mean estimate between 31.27458 and 33.95619; Std. Dev. = 2.21880). On the contrary, Hungary (Mean = 21.86154; 95.00% confidence interval of mean estimate between 15.44612 and 28.27695) and Czechia (Mean = 15.00000; 95.00% confidence interval of mean estimate between 15.00000 and 15.00000) recorded lower average indicator values than Slovakia. The lowest average values for this indicator are for countries such as Bulgaria (Mean = 10.00000; Std. Dev. = 0.00000) and Romania (Mean = 14.61538; 95.00% confidence interval of mean estimate between 13.02538 and 16.20539; Std. Dev. = 2.63117).

The calculation of descriptive characteristics and 95.00% confidence interval of mean estimate were chosen for the selected variables such as Total taxes, Employment income, and Top statutory PIT rate for Member States

of the EU as well as indicators comparison among the countries. These are the point and interval mean estimate, the standard error of the mean estimate, and the standard deviation for the individual countries. The average values were collected over the 13 years 2008-2020, which we visualize in Figure 1, where the EU countries are listed alphabetically. These countries are compared with the EU-27 average values through selected indicators.

The results show which countries have achieved above-average and below-average values compared to the EU-27 average for each selected indicator separately. For the period 2008-2020, Slovakia shows below-average values in all assessed indicators compared to Sweden, which, on the other hand, recorded above-average values for each indicator. For some countries, such as Croatia, the selected indicators Total taxes and Employment income reached below average values. In contrast, the indicator Top statutory PIT rate recorded an above average value, as is the case for Portugal.

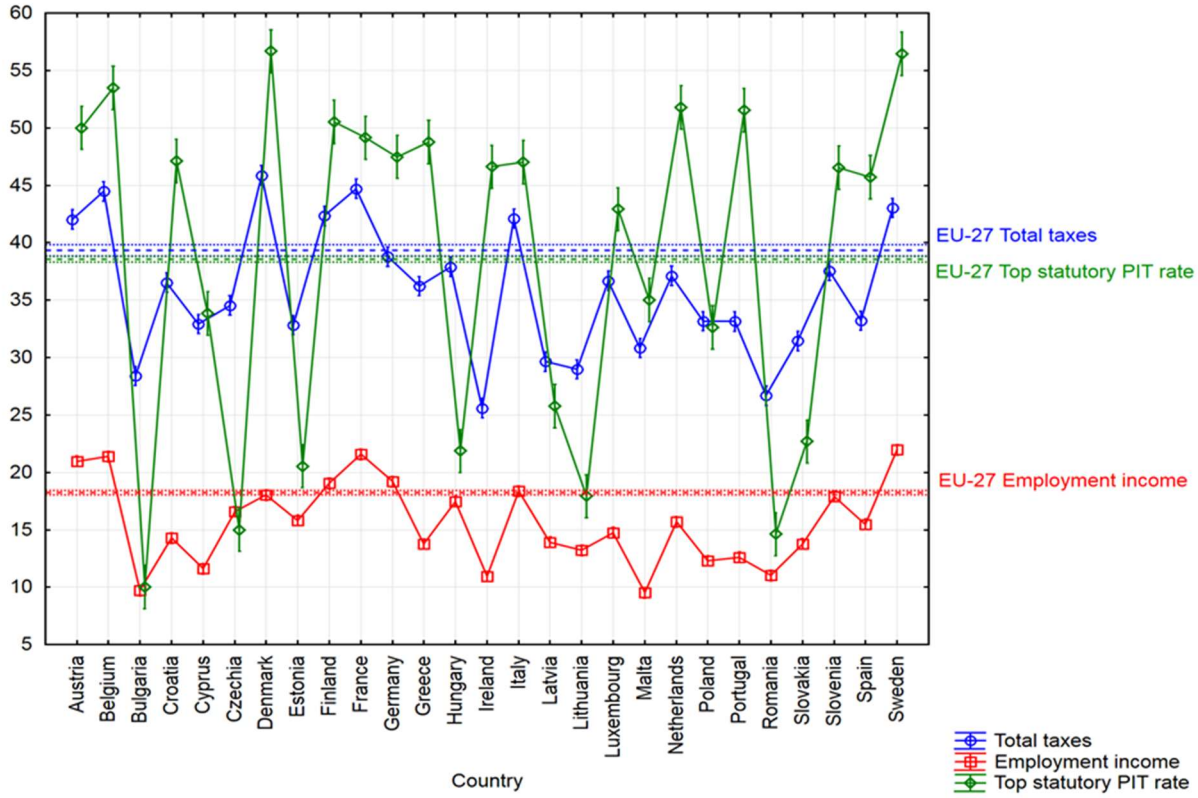


Figure 1 Diagram of averages and confidence intervals of the monitored indicators for EU-27 and individual EU Member States.

Based on the above-average values achieved for the selected indicators of Total Tax and Employment income, it is evident that the homogeneous countries, i.e. Denmark, Austria, Italy, Sweden, Belgium, and France, simultaneously showed a low variability of the above indicators. This means that in these countries total tax revenues from total taxes and tax revenues from employment income do not change over the years **[28]**. In our opinion, the above-mentioned countries perform a tax policy subject to insignificant changes in the tax system in the reviewed years, thus resulting in a stable collection of tax revenues for the public budgets of these countries **[34]**. The West EU countries present the highest values of tax quota in the long term including Denmark and Sweden **[35]**. These EU countries have a higher tax burden on individuals than the East EU countries but over reviewed years. A gradual reduction of this burden affects the competitiveness of particular EU countries **[26]**. We consider that this is a convergence of the particular tax systems of the West EU countries and the East EU countries. Relatively stable tax revenues to the public budgets of each country are ensured by a tax system with an unchanging administration and tax structure **[36]**.

Following the above, countries with below-average values of the selected indicators' Total taxes and Employment income, and at the same time with high variability of these indicators, i.e. heterogeneous countries, include the V4 countries, Ireland, Greece, Bulgaria, and Romania. This means that in these countries total tax revenues from total taxes and tax revenues from employment income vary over the years. It can be concluded that the EU countries mentioned above have been significantly affected by legislative tax and levy changes, the global financial crisis in 2009 and 2010 **[23]**, as well as the political situation affecting the fiscal and tax policies of these countries **[15]**. For example, the East EU countries have undertaken several tax reforms in the reviewed period

[11]. This fact is confirmed by several authors, e.g. **[22]** according to which OECD countries are experiencing an increase in inequality in income distribution due to reforms of their tax systems. In our view, the above countries are less stable regarding the tax revenues collected in their public budgets. At the same time, it should be stressed that these countries have a lower tax burden. Slovakia shows the lowest level of personal income taxation among OECD countries in the long term, followed by countries such as Czechia, Poland, and Hungary **[35]**. In this context, these countries' share of taxes to GDP is lower than the EU average. The Slovak Republic has the lowest overall tax burden, and the Czech Republic and Croatia are similar and below the EU average **[37]**.

The personal income tax belongs to direct taxes. Therefore, decision-makers should carefully prepare reforms in this area **[29]**. Above average values for the Top statutory PIT rate indicator with its lowest variability over the 2008-2020 period were achieved in countries such as Belgium, Netherlands, and Sweden as well as Austria and Germany with zero variability of this indicator. This means that in these homogeneous countries, there have been no changes in the PIT rate and no significant changes in the collection and payment of PIT. This is one of the reasons why a country can provide stable tax revenues to public budgets. On the contrary, below-average values of this indicator were recorded for Hungary and Lithuania, which also achieved the highest variability of the indicator. Malta, Czechia, and Bulgaria showed zero variability of this indicator in the years assessed.

The tax rate, which significantly influences the tax burden is important information for politicians and economists **[38]**. Progressivity is a typical feature of a PIT **[39]**. The very high degree of tax progressiveness affects the motivation to work, which is why, for example, in Slovakia or the Czech Republic **[14]**, the progressive tax rate was replaced by a nominal linear tax rate in the past **[30]**. In this context, the tax rates during the period of 2004-2012 were quite stable due to the flat tax rate and therefore it might be expected that the tax burden will be the same, but the tax quota is declining which suggests it was affected by the changes in GDP **[29]**. In our view, the tax reforms involve changes to the Top statutory PIT rates and items affecting the calculation of the PIT base and the settlement of the resulting PIT liability. Considering the Hungarian system personal income taxation is generally defined in terms of tax rates and tax brackets **[40]**. A progressive multifactorial system in terms of allowances was used in the Czech Republic in 2018, and this may be considered the simplest income tax system for the V4 countries **[41]**. The Slovak income tax system partially uses tools different from the Czech system. The super-grossing method determined the tax base in the Czech Republic. At the same time, in Slovakia the amount paid by the employee as a contribution to the social and health insurance system is not included in the tax base **[42]**.

Tax revenue as a share of GDP and how it changes can vary from country to country for several reasons **[24]**, **[25]**. The determination of the tax base and the level of PIT rates and the administration of the tax lead to differences in revenue collection for public budgets across countries. Each country has a different approach to the design and financing of the public sector, leading to differences in tax systems between countries. In times of economic change, the evolution of the tax revenue to GDP ratio needs to be analyzed from both perspectives: tax revenue and GDP growth **[27]**. In this context, it is important to note that in 2021 tax revenue grew faster than GDP in most EU countries, but this trend is not the same in all Member States **[13]**.

The aforementioned facts, i.e. the level of tax revenues and GDP within the EU countries, are influenced by trends and structural changes, which mainly include consumption decisions, production processes, political economy, governance-related matters, migration, labor force movement, demographic changes, globalization, technological advances, digitalization, and the others, which pose current challenges for the sustainability of each of national tax system **[43]**, **[44]**. In this context, it should be concluded that the labor costs in agriculture are approximately the same as in neighboring countries **[3]**. Differences in the area of PIT in individual EU member countries relate mainly to the adjustments of the income tax base, changes in PIT rates, settlement of the final tax liability as well as the collection method of this tax. In the taxation of the agricultural sector, it is necessary to observe differences specific to this economic sector, mainly because of the use of the various elements of the tax base **[1]**.

The need to keep Slovak agriculture requires the introduction of effective support instruments by the state that would ensure the competitiveness of employers **[2]**. We think that one such tool is the reduction or exemption from employers' and employees' tax and social contributions obligations. Low costs represent an important competitive advantage for entrepreneurs **[5]**. It is necessary to ensure comparable legislative conditions for Slovak employers doing agriculture business, as in neighboring EU countries.

The European Commission has long recommended the Slovak Republic move from labor taxation to environmental and property taxes, improve tax collection, combat tax evasion, and improve compliance with tax regulations. In our opinion, changes in the tax system should be aimed at the investing attractiveness and the motivation to work and employ people, especially in productive sectors which include also agriculture.

Current relief effective as of 1 August 2023 a part of social contributions (sickness insurance, retirement insurance, disability insurance, unemployment insurance, and insurance for the solidarity reserve fund) reduces

the employer's costs by approximately 24% of the assessment base of each employee per calendar month up to the minimum wage amounted to EUR 750 in the period until 30 June 2024. In selected subsectors of the agri-food sector of the Slovak Republic (in addition to food production this also includes viniculture, vegetable and fruit growing, and animal husbandry with plant production), the employer does not pay social insurance contributions for the employee. The objective of the relief in question is to increase the level of self-sufficiency and the competitiveness of this industry as well as to reduce the prices of final consumers. This relief is not currently adequate due to the limited period applied and in our opinion, it should concern the entire agri-food sector.

Further research in the area of PIT will focus on the assessment of selected indicators within the V4 countries, as they have historically similar tax systems, including the structure of tax systems, as well as a similar way of forming the tax base and calculating PIT, including the method of collecting this tax.

CONCLUSION

Assessment of tax burden becomes more important for comparing tax systems and is used to determine the effect of fiscal and tax policies. The optimal indicator for measuring the tax burden does not exist. The tax burden, in particular the tax quota in an international comparison, defines the share of a country's total revenue (taxes and levies) in GDP, i.e. it expresses what part of the economy's annual output a country collects and can subsequently redistribute. The overall impact on tax revenues and its relation to GDP is rather country-specific. It depends on the type of measures, the magnitude and length of the support, the economic structure of each country, the type of employment, and others. In 2020, tax revenue in the EU decreased less than GDP, which increased the tax-to-GDP ratio. Based on the results of the descriptive characteristic of individual indicators, it can be stated that for the observed period of 13 years, 2008-2020, the values of the selected indicators, Total taxes and Employment income, are approximately identical for homogeneous countries with above-average values of these two indicators, as well as for heterogeneous countries with below-average values of the indicators. Employment income is a component of Total taxes in all reviewed countries. The structure of taxation varies significantly across countries. Some countries have a higher share of Employment income in total tax revenues collected in public budgets. The East EU countries report the lowest tax burden. The West EU countries present the highest tax burden. Differences among them derive from the different conceptions of tax and social systems. The trend in tax revenue development within EU countries has not changed significantly, and we expect the values to remain unchanged for 2021-2023. The results of descriptive statistics for the indicator Top statutory PIT rate document that this indicator for the assessed period 2008-2020 varies within the EU countries. The homogeneous countries with an average Top statutory PIT rate compared to the EU-27 average include the West EU countries. Heterogeneous countries with below-average Top statutory PIT rate values compared to the EU-27 average include Hungary and Lithuania. In the case of countries with below-average values, such as Malta, Czechia, and Bulgaria, the Top statutory PIT rate did not change over the years under review. Progressivity of income taxation is still retained within the East EU countries. PIT is included in each national tax system, and the revenue from this tax forms a significant part of total taxes, the amount of which depends mainly on the structure of the overall tax system. Every fair tax system is subject to certain requirements that respect the interests of taxpayers on the one hand and the economic interests of the country on the other. One of the preferred requirements in designing a tax system is to ensure sufficient revenue for the public budgets. Another requirement concerns the adjustment of tax revenue to GDP development related to introducing floating rates in personal income tax. The European Commission also recommends a fairer, simpler, and more modern tax environment. The above research findings are consistent with similar studies that have assessed the PIT burden in selected countries in previous years. Economic conditions in agriculture are an important part of the business environment related to government participation. In most developed countries, the government actively intervenes in business, primarily to utilise domestic production resources and the sustainability of this sector. The government participation is determined in the EU's Common Agricultural Policy. The regional dimension also needs to be taken into consideration. From the long-term point of view, a concept involving predictable conditions and guarantees of state support for the domestic agri-food sector is important.

REFERENCES

1. Kozáková, J., & Urbánová, M. (2018). Is current system of direct payments suitable for farmers in Slovakia? In Potravinarstvo Slovak Journal of Food Sciences (Vol. 12, Issue 1, pp. 716–722). HACCP Consulting. https://doi.org/10.5219/941
2. Dvořáková, D. (2012). Specifika účetnictví a oceňování v zemědělství. (Specifics of accounting and assessing in the agriculture sector). Wolters Kluwer ČR. 156 p.
3. Grznár, M., Trsťanská, A., Szabo, Ľ., Šubertová, E., Foltínová, A., Vybíralová, J., & Hudáková, I. (2010). Podnikanie v agropotravinárstve (Doing business in the agri-food sector). 2nd ed. 218 p. Ekonóm.
4. Kozáková, J., & Hornáčková, E. (2021). Product line of selected agricultural entity in Slovakia and suggestion for its expansion based on analysis of consumers' interest in dairy and meat products. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 939–960). HACCP Consulting. https://doi.org/10.5219/1654
5. Andrejovská, A., & Konečná, V. (2019). Investors´ decision on the context of the effective taxation of agricultural companies. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 13, Issue 1, pp. 1040–1050). HACCP Consulting. https://doi.org/10.5219/1223
6. Andrejovská, A., Buleca, J., & Puliková, V. (2019). Capital taxation efficiency of agricultural businesses in the Slovak Republic. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 13, Issue 1, pp. 572–580). HACCP Consulting. https://doi.org/10.5219/1135
7. Juncker, J. C., Tusk, D., Dijsselbloem, J., Draghi, M. & Schulz, M. (2018). Completing Europe's Economic and Monetary Union (pp. 1–23). European Commission
8. Van der Hoek, P. (1999). European Countries. Handbook on Taxation, 1st Edition. 24 p. Routledge.
9. Cetlová, H., Velinov, E., Čížků, A., Linhartová, V., Skýpalová, R., & Novotná, B. (2023). The macroeconomic indicators influence the consumption of selected organic food under the conditions of global climate change – a case study from the Czech Republic. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 694–711). HACCP Consulting. https://doi.org/10.5219/1882
10. Mosteanu, R. N., & Alexandru, V. (2009). The flat-rate tax a measure against economic crisis in Romania. In Metalurgia International (Vol. 14, Issue 8, pp. 114–122). Editura Stiintifica F. M. R.
11. Kaštan, M. & Machová, Z. (2010). Tax burden in EU countries – a comparative study. In Bulletin of the Transylvania University of Brasov. Economic Sciences (Vol. 3, pp. 263–270). Transylvania University of Brasov.
12. Suhanyiová, A., Suhanyi, L. & Horváthová, J. (2015). Tax aspects of two neighboring Central European countries with an emphasis on the personal income tax. Proceedings paper of 13th International Scientific Conference on Hradec Economic Days. In Hradecké ekonomické dni 2015 (Vol. 5, Issue 3, pp. 145–154). University Hradec Králové.
13. European Commission. Directorate General for Taxation and Customs Union. (2023). Annual report on taxation 2023: review of taxation policies in the European Union. Publications Office. https://doi.org/10.2778/982557
14. Koštuříková, I. (2016). Tax burden in the Czech Republic and Slovakia. In Proceeding paper of International Scientific Conference on Region in the Development of the Society (pp. 470–479). Mendel University in Brno.
15. Szarowska, I. (2010). The effect of tax burden on economic growth in the European Union. In Proceedings paper of 28th International Conference on Mathematical Methods in Economics 2010 (pp. 596–601). Technical University of Ostrava.
16. Tofan, M., & Gavrilluta, A. F. (2019). Heterogeneity of taxation and fiscal challenges. In Proceedings paper of International Conference on European Financial Regulation (EUFIRE 2019) (pp. 771–779). Alexandru Ioan Cuza University of Iasi.
17. Karpowicz, A. (2018). Why revenues from payroll taxes do not follow trends in taxation of income of corporations? In Proceeding paper of the 36th International Scientific Conference on Economic and Social Development (ESD) - Building Resilient Society (ESD 2018) (pp. 190–202). Varazdin Development and Entrepreneurship Agency.
18. Popova, N. (2016). Personal Income Tax in Bulgaria. In Proceedings paper of 13th International Scientific Conference on Economic and Social Development (ESD) (pp. 152–160). Varazdin Development and Entrepreneurship Agency.
19. Mertens, K., & Ravn, M. O. (2013). The Dynamic Effects of Personal and Corporate Income Tax Changes in the United States. In American Economic Review (Vol. 103, Issue 4, pp. 1212–1247). American Economic Association. https://doi.org/10.1257/aer.103.4.1212

20. Kavelaars, P. (1992). Allocation Rules in European Social Security Law. In EC Tax Review (Vol. 1, Issue Issue 4, pp. 241–249). Kluwer Law International BV. https://doi.org/10.54648/ecta1992028
21. Tepperová, J. (2019). Personal Income Tax and Social Security Coordination in Cross-Border Employment – a Case Study of the Czech Republic and Denmark. In European Journal of Social Security (Vol. 21, Issue 1, pp. 23–41). SAGE Publications. https://doi.org/10.1177/1388262719833766
22. Costello, C., & Karp, L. (2004). Dynamic taxes and quotas with learning. In Journal of Economic Dynamics and Control (Vol. 28, Issue 8, pp. 1661–1680). Elsevier BV. https://doi.org/10.1016/s0165-1889(03)00082-4
23. Baranová, V. (2012). Methods of tax burden measurement: A practical case for European Union countries. In Proceedings Paper of 2nd International Scientific Conference Taxes in The World (pp. 7–26).
24. Kotlán, I., & Machová, Z. (2013). The impact of the tax burden on the living standard in OECD countries. In Ekonomický časopis (Vol. 61, Issue 9, pp. 951–962). Institute of Economic Research SAS.
25. Kotlán, I., & Machová, Z. (2013). The issue of using tax quota in the growth empiricism. In Proceedings of the 10th International Scientific Conference: Economic policy in the European Union Member States (pp. 145–160). Silesian University in Opava and School of Business Administration in Karviná.
26. Vybíralová, I. (2016). The Impact of Labour Taxation on the Economic Growth. In 34th International Conference Mathematical Methods (MME 2016) (pp. 893–903). Technical University of Liberec.
27. Zimčík, P. (2016). Tax quota vs. World tax index: panel data comparison. In Proceedings paper of the international conference: Quantitative methods in economics: multiple criteria decision making XVIII (pp. 411–415). University of Economics in Bratislava.
28. Powadová, T. (2018). Application of tax policy in selected countries of Scandinavia in comparison with the Czech Republic. In Proceedings from 19th International Scientific Conference on International Relations – Current Issues of World Economy and Politics (pp. 606–614). University of Economics in Bratislava.
29. Konôpková, Z. (2021). What is the Real Tax Burden in Slovakia? In Proceedings paper of 19th International Conference on Hradec Economic Days (Vol. 11, pp. 338–344). University of Hradec Králové.
30. Krajňák, M. (2022). Quo Vadis Tax and Levy Burden of Wages in the Czech Republic? Tax Reform in 2022. In Journal of Tax Reform (Vol. 8, Issue 1, pp. 25–39). Ural Federal University. https://doi.org/10.15826/jtr.2022.8.1.106
31. Munk, M., Pilková, A., Benko, Ľ., Blažeková, P., & Švec, P. (2021). Methodology of stakeholders' behaviour modelling based on time. In MethodsX (Vol. 8, p. 101570). Elsevier BV. https://doi.org/10.1016/j.mex.2021.101570
32. Munk, M., Pilková, A., Benko, Ľ., Blažeková, P., & Švec, P. (2021). Pillar 3–Pre-processed web server log file dataset of the banking institution. In Data in Brief (Vol. 39, p. 107672). Elsevier BV. https://doi.org/10.1016/j.dib.2021.107672
33. Munk, M., Pilková, A., Benko, Ľ., Blažeková, P., & Švec, P. (2021). Web usage analysis of Pillar 3 disclosed information by deposit customers in turbulent times. In Expert Systems with Applications (Vol. 185, p. 115503). Elsevier BV. https://doi.org/10.1016/j.eswa.2021.115503
34. Macek, R. (2015). The Impact of Taxation on Economic Growth: Case Study of OECD Countries. In Review of Economic Perspectives (Vol. 14, Issue 4, pp. 309–328). Walter de Gruyter GmbH. https://doi.org/10.1515/revecp-2015-0002
35. OECD. (2014). Revenue Statistics – Comparative tables. Retrieved from http://stats.oecd.org/Index.aspx?DataSetCode=REV. https://doi.org/10.1787/rev_stats-2014-en-fr.
36. Sokolovska, A., Zatonatska, T., Stavytskyy, A., Lyulyov, O., & Giedraitis, V. (2020). The Impact of Globalization and International Tax Competition on Tax Policies. In Research in World Economy (Vol. 11, Issue 4, p. 1). Sciedu Press. https://doi.org/10.5430/rwe.v11n4p1
37. Pavel, J., & Vítek, L. (2014). Tax Compliance Costs: Selected Post-transitional Countries and the Czech Republic. In Procedia Economics and Finance (Vol. 12, pp. 508–515). Elsevier BV. https://doi.org/10.1016/s2212-5671(14)00373-6
38. Bánociová, A., Gal, M., Mihóková, L. & Pavlíková, L. (2014). Zdaňovanie subjektov v slovenskej praxi. (Taxation of subjects in Slovak practice). 194 p. ELFA Press.
39. Krajňák, M. (2020). Is Personal Income Tax on Dependent Activity in the Czech Republic Progressive? In Politická ekonomie (Vol. 68, Issue 5, pp. 534–553). Prague University of Economics and Business. https://doi.org/10.18267/j.polek.1295
40. Szabóné Bonifert, É. (2021). Missed Opportunities of Simplification Regarding Personal Income Tax Systems in Hungary. In Pénzügyi Szemle = Public Finance Quarterly (Vol. 66, Issue 3, pp. 337–358). Corvinus University of Budapest. https://doi.org/10.35551/pfq_2021_3_2

41. Hájek, J., & Olexová, C. (2022). Comparing Personal Income Tax Gap in the Czech Republic and Slovakia. In Politická ekonomie (Vol. 70, Issue 1, pp. 27–50). Prague University of Economics and Business. https://doi.org/10.18267/j.polek.1341

42. Szabóné Bonifert, É. (2020). Opportunities for Simplification in the Personal Income Tax Systems of the Visegrad Countries. In Pénzügyi Szemle = Public Finance Quarterly (Vol. 65, Issue 4, pp. 531–553). Corvinus University of Budapest. https://doi.org/10.35551/pfq_2020_4_6

43. European Commission. Directorate General for Taxation and Customs Union. (2022). Taxation trends in the European Union: data for the EU Member States, Iceland, Norway: 2022 edition. Publications Office. https://doi.org/10.2778/417176

44. European Commission. (2020). Communication from the Commission to the European Parliament and the Council. An Action Plan for Fair and Simple Taxation Supporting the Recovery Strategy.

Funds:

The results of this paper are part of the VEGA project "Environment and food security: the role of agricultural and fiscal policies (1/0641/23)".

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

*__Alexandra Ferenczi Vaňová__, Slovak University of Agriculture in Nitra, Faculty of Economics and Management, Institute of Accounting and Informatics, Tr. Andreja Hlinku 2, 949 76 Nitra, Slovak Republic,
Tel.: +421376414157
E-mail: alexandra.vanova@uniag.sk
ORCID: https://orcid.org/0000-0003-1598-5271

__Renáta Krajčírová__, Slovak University of Agriculture in Nitra, Faculty of Economics and Management, Institute of Accounting and Informatics, Tr. Andreja Hlinku 2, 949 76 Nitra, Slovak Republic,
Tel.: +421376414151
E-mail: renata.krajcirova@uniag.sk
ORCID: https://orcid.org/0000-0001-8581-5994

__Michal Munk__, Constantine the Philosopher University in Nitra, Faculty of Natural Sciences and Informatics, Department of Informatics, Tr. Andreja Hlinku 1, 949 01 Nitra, Slovak Republic,
Tel.: +421376408673
E-mail: mmunk@ukf.sk
ORCID: https://orcid.org/0000-0002-9913-3596

__Andrea Ecková__, Prague University of Economics and Business, Faculty of Business, Department of Managerial Economics, Nám. W. Churchilla 1938/4, 130 67 Praha 3 – Žižkov, Czech Republic,
Tel.: +420224098342
E-mail: andrea.eckova@vse.cz
ORCID: https://orcid.org/0009-0001-6807-5098

__Jozef Bojňanský__, Slovak University of Agriculture in Nitra, Faculty of Economics and Management, Institute of Accounting and Informatics, Tr. Andreja Hlinku 2, 949 76 Nitra, Slovak Republic,
Tel.: +421376414199
E-mail: jozef.bojnansky@uniag.sk
ORCID: https://orcid.org/0009-0009-1609-1166

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 19.1.2024
Revised: 20.2.2024
Accepted: 28.2.2024
Published: 5.3.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 281-296
https://doi.org/10.5219/1956
ISSN: 1337-0960 online
www.potravinarstvo.com

Microbiological characteristics of hard cheese with flax seeds

Mykola Kukhtyn, David Arutiunian, Oleg Pokotylo, Khrystyna Kravcheniuk, Volodymyr Salata, Yulia Horiuk, Halyna Karpyk, Diana Dalievska

ABSTRACT

Highly nutritious dairy products such as hard cheeses are considered a good source of protein, fats, mineral substances, and vitamins and are consumed in significant quantities. At the same time, the disadvantages of cheeses include the presence of a large amount of saturated fatty acids in their composition, which are associated with the development of cardiovascular diseases. Therefore, modifying the composition of fatty acids in hard cheese by increasing the content of unsaturated fatty acids and reducing the amount of saturated fatty acids is extremely important for consumers' health. This research aimed to determine the dynamics of microbiological indicators in the ripening technology of hard rennet cheese with different contents of flax seeds as a source of omega-3 fatty acids. The technology of hard rennet cheese with 3-5% flax seed content was investigated. A 1.3 times higher content of lactic acid microflora was found in samples of hard cheese with 5% flax seeds during the first 10 days of ripening compared to the control sample of cheese. On the 60th day of ripening of cheese with a content of 5% flax seeds, the number of lactic acid bacteria was 9.4 ±0.3×10^9 CFU/g, and in the control cheese – 7.8 ±0.3×10^9 CFU/g. During the production and ripening of cheese with different content of flax seeds, no exceedance of normative values was found for the number of *Enterobacteriaceae* and *Staphylococcus aureus* bacteria. A method (washing the seeds in a sodium bicarbonate solution and drying at a temperature of 95 ±1 for 20 min) of processing flax seeds before adding them to the cheese mass was proposed, which reduced the number of mesophilic microorganisms by approximately 200 times to 3.9 ±0.1×10^1 CFU/ g, fungi by 160 times to 0.3 ±0.1×10^1 CFU/g and aerobic mesophilic bacilli by 78 times to 1.1 ±0.1×10^1 CFU/g. Therefore, the developed hard rennet cheese with flax seeds can be consumed as an additional source of omega-3 fatty acids and dietary fiber.

Keywords: rennet cheese, cheese technology, flax seeds, cheese ripening, lactic acid microflora

INTRODUCTION

Today, the industry is trying to produce food products intended not only to satisfy hunger and provide the necessary nutrients but also to prevent food-related diseases **[1]**, **[2]**. Milk and milk products are considered the primary sources of nutrients in the human ration, offering quality proteins, minerals, vitamins, and energy **[3]**. In addition, milk is an excellent matrix for the release of bioactive compounds **[4]**, and various dairy products are the basis for enrichment with other nutrients **[5]**, **[6]**. Currently, the development of new formulations of dairy products that consumers would like very much is one of the main forces in the dairy industry. Dairy products such as cheeses, ice creams, and yogurts are consumed worldwide, and improving the composition of these products with vitamins, antioxidants, fiber, and polyphenols can be achieved by integrating rich sources **[7]**, **[8]**, **[9]**, **[10]**. Additional components can be carrot paste **[11]**, **[12]**, **[13]**, broccoli **[14]**, grape extract and extracts **[15]**, **[16]**, **[17]**, sesame **[18]**, spinach powder **[19]**, tomato extracts **[20]**. In addition, consumers worldwide demand the development of cheeses with reduced content of synthetic additives such as flavors and colorants **[21]**, **[22]**.

Consequently, increasing knowledge of the relationship between nutrients and health has led to new product categories, such as functional foods and nutraceuticals. Among the various functional product groups, omega-3 fatty acids are popular **[23]**, **[24]**. Because lipids are considered one of the most essential nutrients for humans.

Among the fatty acids of food lipids, these are indispensable - α-linolenic (C18:3 omega-3), eicosapentaenoic (omega-3), docosahexaenoic (omega-3), and linoleic (C18: 2, omega-6), which are not synthesized by the human organism, so they must be got with food products **[25]**, **[26]**. Unsaturated fatty acids are used to replace saturated fatty acids in various products, as high consumption levels of the latter negatively influence people's health **[27]**.

It has been reported that modern dietary habits have significantly reduced the daily intake of omega-3 fatty acids to less than recommended. As a result, the need to fortify food with omega-3 fatty acids is increasing **[28]**. In most developed countries, health policy recommends reducing the intake of saturated fatty acids and increasing the intake of unsaturated fatty acids, especially α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n -3), which are beneficial for health **[29]**, **[30]**. Research has shown that the daily intake of long-chain polyunsaturated omega-3 fatty acids in the UK, USA, Canada, and Australia is below the recommended norm (less than 100-200 mg/day). According to the International Society for the Study of Fatty Acids and Lipids, their content in the ration should be about 650 mg/day of polyunsaturated fatty acids and 2.2 g/day of alpha-linolenic acid **[31]**. Omega-3 polyunsaturated fatty acids provide significant nutritional and health benefits, such as by preventing coronary heart disease, hypertension, type 2 diabetes, rheumatoid arthritis, and obstructive pulmonary disease **[32]**, **[33]**, **[34]**, **[35]**, **[36]**.

Including unsaturated fatty acids in dairy products interests food industry enterprises. These products can greatly strengthen health and prevent diseases **[25]**.

The main food sources of eicosapentaenoic and docosahexaenoic acids are tuna and salmon **[37]**; for alpha-linolenic acid, there are plant sources such as dark green leafy vegetables and flaxseed oil **[38]**. Flaxseed oil contains 52% alpha-linolenic acid, is an excellent source of omega-3, and is a good carrier of vitamin D_3 **[39]**. The disadvantage of introducing animal fats (fish oil) into dairy products as a source of omega-3 fatty acids is its fishy taste. At the same time, using vegetable oil as a source of omega-3 can avoid this and get enriched products that meet consumers' requirements in terms of taste properties **[39]**, **[40]**. Thus, sources of enrichment in unsaturated fatty acids can be vegetable raw materials such as nuts, chia seeds, flax, canola, and soybeans and oils such as olive, canola, soybean, chia, linseed, palm, and corn **[41]**, **[42]**. Significant concentrations of omega-3 alpha-linolenic acid, up to 9.5%, were found in wheat bran oil **[43]** and in rye bran oil (7.6%) **[44]**. However, flax is considered one of the best and most affordable additives for making cheeses with an increased content of essential omega-3 fatty acids **[45]**. Flaxseeds are grown worldwide for fiber, oil, medicinal purposes, and as a food product **[46]**. Flaxseed's nutritional, functional, probiotic, and phytoactive properties are attracting the attention of health food consumers and producers **[38]**. Flaxseed is considered a potential functional food ingredient as it provides a variety of health benefits along with nutritional value **[46]**. Flaxseed consists of 37-41% fat, 28-29% total dietary fiber, 20% of protein, 6.5-7.7% of moisture, and 2.4-3.4% of ash **[46]**. Worldwide, flaxseed is recognized as the well-known richest plant source of omega-3 fatty acids, containing alpha-linolenic acid (18:3), which accounts for 39.00 to 60.42% of total fatty acids (including polyunsaturated fatty acids 73%, monounsaturated fatty acids 18% and saturated fatty acids 9%), followed by oleic (18:1n-9) 13.44-19.39%, linoleic (18:2n-6) 12.25-17.44%, palmitic acid (16:0) 4.90-8.00% and stearic acid (18:0) 2.24-4.59% **[47]**.

The development of a yogurt recipe that was enriched with omega-3 fatty acids by adding flax and blackcurrant oils was reported to provide 10% of the recommended value for α-linolenic acid **[48]**. Researchers **[49]** added 10% linseed oil to the curd paste as a source of α-linolenic acid. To increase polyunsaturated fatty acids in cheese and various dairy products, researchers **[50]**, **[51]**, **[52]** added feed rich in these acids, flaxseed, and rapeseed to the ration of cows.

Thus, the demand for omega-3-rich foods is increasing worldwide and is expected to grow. This forces the food industry to constantly work on developing dairy products with good taste properties and significant demand. Therefore, the enrichment of hard cheeses with various sources of essential acids is promising, since cheeses have a significant demand and are consumed by different population categories in many countries. Therefore, developing flaxseed cheese will expand the range of dairy products rich in omega-3 fatty acids.

The work aimed to determine the dynamics of microbiological indicators in the ripening technology of hard rennet cheese with different contents of flax seeds as a source of omega-3 fatty acids.

Scientific Hypothesis

To develop a technology for producing hard rennet cheese with flax seeds and to set whether the added content of flax affects the ripening process of the cheese.

MATERIAL AND METHODOLOGY

Production researches were conducted at the Chortkiv cheese factory (Chortkiv, Ukraine), and laboratory research (physicochemical and microbiological) at the Department of Food Biotechnology and Chemistry of the Ivan Pulij Ternopil National Technical University.

Samples

20 samples of hard rennet cheese with different flaxseed content were investigated (5 samples with 1.5% flax, 5 samples with 3% flax, 5 samples with 5%, and 5 were controls without flax).

Chemicals

To produce hard cheese, milk of 3.5% fat was used; mesophilic sourdough CHN-19 (Chr. Hansen, Denmark), consisting of *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. lactis*, *Leuconostoc mesenteroides ssp. cremoris* and *Lactococcus lactis subsp. diacetylactis*; rennet enzyme (Natural Extra, Italy), which consists of 95% of chymosin and 5% of pepsin; calcium chloride (Novokhim, Ukraine) and "Debut" flax seeds.

Laboratory Methods

Microbiological testing was performed according to standard methods, which included preparing samples for the research, carrying out tenfold dilutions, and sowing them on selective and accumulating media. In particular, bacteria of the *Enterobacteriaceae* genus on the Endo medium (Pharmaktyv, Ukraine), lactic acid bacteria on the MRS Agar medium (HiMedia, India) according to the national standards of Ukraine DSTU 7357:2013 **[53]**, bacteria of the genus *Salmonella* and *Listeria* according to DSTU EN 12824:2004 **[54]** and DSTU ISO 11290-1:2003 **[55]** respectively. Staphylococci were isolated on BD Baird-Parker Agar medium (HiMedia, India). Bacteria of the genus Bacillus were determined by sowing cheese and its dilutions on meat peptone agar, followed by incubation at 30 0C for 72 hours. The samples were kept in a water bath at 85 °C for 15 minutes. Fungi on Saburo's medium (Pharmaktiv, Ukraine). The number of mesophilic bacteria on meat peptone agar medium with incubation of crops at a temperature of 30 ±1 °C for 72 hours.

Description of the Experiment

Sample preparation: The preparation of flax seeds: flax seeds (5-10 g) were washed in a 1% solution of baking soda (100 ml) at a temperature of 55 ±5 °C for 5 min, followed by washing in sterile tap water (100 ml), followed by drying in a drying cabinet in a Petri dish at a temperature of 95 ±1 °C for 20 min and stored in a sterile package.

Briefly, the cheese production technology with linseed was as follows. Pasteurized cow's milk was heated to a temperature of 34-35 °C, and dry leaven CHN-19 was applied to its surface, left for 3-4 min, and stirred for uniform distribution of the leaven and left alone for 30-35 min. Calcium chloride and rennet enzyme were added, previously dissolved in 50 ml of water. The mixture was evenly mixed and left for 40-45 minutes to form a clot. After that, the curd was checked for readiness (whey separation test), and the cheese clot was cut into cubes 1-1.5 cm in size. The cut cubes were mixed in a circular motion for 5 minutes and left to settle to the bottom. Then, 10% of the serum was removed, and the same volume of water was added at a temperature of 65 ±1 °C; the mass was kneaded for 10 minutes and left alone again for the grains to settle to the bottom. Then, a third of the whey was drained, and the same amount of water was added at a temperature of 42-43 °C; the cheese mass was stirred for 20 min, and the cheese grain was separated by draining the whey. We added sterilized flax seeds according to the method we developed and mixed the curd grain for uniform distribution of flax. The cheese grains were collected in molds and given 5-10 minutes for the whey to drain, then put under a press for 12 hours. After pressing, the cheese head was placed in a brine bath for 12 to 18 hours, depending on the duration of exposure. After half of the elapsed time, the cheese head was turned over. Then the heads of cheese were laid out on drainage mats to remove residual moisture and form a dry crust for 3-4 days, turning the head of cheese 2 times a day. After forming a dry crust, the head of cheese was placed for ripening at a temperature of 8-12 °C for 60 days.

The technology of preparation of the control sample of cheese was similar to that of cheese with flax seeds. Only flax was not added to the cheese grain.

Number of samples analyzed: we analyzed 20 samples.

Number of repeated analyses: All measurements of instrument readings were performed three times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was three times.

Design of the experiment: In the first stage, the microbiota of flaxseed was studied, and a disinfection method was developed. In the second stage, the technology of hard cheese with flax seeds was developed. On the third, the dynamics of microbiological changes in hard cheese with flax seeds during its ripening were studied.

Statistical Analysis

Statistical processing of the results was carried out using methods of variation statistics using Statistica 9.0 (StatSoft Inc., USA). Non-parametric methods of research were used (Wilcoxon-Mann-Whitney test). The arithmetic mean (x) and the mean (SE) standard error were determined. The difference between the comparable values was considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

In the first stage of the work, the microbiota of flax seeds was researched before washing and after our proposed processing in a 1% solution of baking soda with subsequent drying at a temperature of 95 ±1 °C for 20 minutes. The results are given in Table 1 and Figure 1.

Table 1 Characteristics of flaxseed microbiota during preparation for use in the production technology of hard cheese with an increased content of omega-3 fatty acids, x ±SE; n =5.

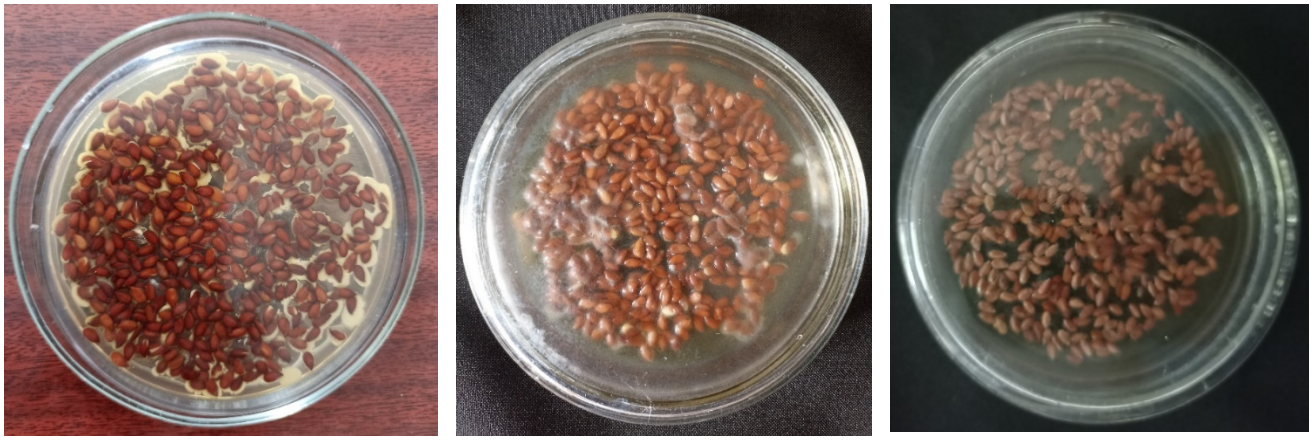
Indicator	Technological operations of flax seed preparation		
	before processing	after soaking in a 1% soda solution	after drying in a drying cabinet
Number of mesophilic bacteria, CFU/g	$7.8 \pm 0.3 \times 10^4$	$5.9 \pm 0.2 \times 10^{3*}$	$3.9 \pm 0.1 \times 10^{1**}$
Number of *Bacillus* spp., CFU/g	$8.6 \pm 0.3 \times 10^2$	$8.5 \pm 0.3 \times 10^{1*}$	$1.1 \pm 0.1 \times 10^{1**}$
Number of fungi, CFU/g	$4.8 \pm 0.1 \times 10^2$	$5.1 \pm 0.2 \times 10^{1*}$	$0.3 \pm 0.1 \times 10^{1**}$

Note: * - $p < 0.05$, ** $p < 0.001$ – compared to the amount before processing.

It was found that the surface of ordinary linseed is quite significantly contaminated with bacterial and fungal microflora. Usually, such seeds can only be used with disinfecting processing in dairy product production technology. In particular, insemination with mesophilic bacteria before processing was $7.8 \pm 0.3 \times 10^4$ CFU/g, which is a significant amount. Reducing microbial insemination by soaking in a 1% sodium bicarbonate solution for 10 min followed by washing in running drinking water provided an average of 13.2 times ($p < 0.05$) decrease in mesophilic microbiota on the seed surface. The next processing, which included drying the flax seed in an oven at a temperature of 95 ±1 °C for 20 min, ensured its almost complete disinfection since a small amount of mesophilic microorganisms – $3.9 \pm 0.1 \times 10^1$ CFU/g – was isolated from the surface. That is, the number of mesophilic microorganisms was 150 times ($p < 0.001$) less than after washing.

The processing proposed by us also had a significant influence on the reduction of spore-forming microflora since their content after soaking in sodium bicarbonate solution was 10.1 times lower ($p < 0.05$) compared to the amount on the surface of flax seeds before processing – $8.6 \pm 0.3 \times 10^2$ CFU/g. Excessive content of spore-forming microflora in the raw material can affect the cheese ripening process because this microbiota may contain anaerobic representatives that cause defects (swelling of the cheese head) in the later stages of its ripening. Temperature processing of seeds in a drying cabinet did not have such a significant influence on reducing the number of spore-forming microbiota as on the mesophilic one. Since the content of Bacillus spp. after such processing was decreased by 7.3 times ($p < 0.05$), and their number was $1.1 \pm 0.1 \times 10^1$ CFU/g. This content of *Bacillus* spp. is non-essential and cannot influence cheese production technology when adding flax seeds, as they belong to aerobic microflora.

The influence of the proposed processing on the fungal microflora was also investigated because these microorganisms are ubiquitous and can show their activity in a wide range of temperatures and pH of the environment. Fungal microbiota was also well washed off during soaking in the soda solution, as their number was decreased on average by 9.4 times ($p < 0.05$) to $5.1 \pm 0.2 \times 10^1$ CFU/g. This content of fungal microorganisms on the surface of flax seeds is still quite significant, such seeds cannot be introduced into the production technology of rennet cheeses, where the temperature and humidity conditions will be favorable for their growth. Using the drying mode in the cabinet under the selected mode made it possible to reduce the contamination of fungal microbiota on the surface of flax to $0.3 \pm 0.1 \times 10^1$ CFU/g, i.e., 17 times ($p < 0.001$). The results of microbiological research are shown in Table 1 and are partially illustrated in Figure 1. It can be seen that before processing, flax seeds are covered with continuous colonies of microorganisms that permeate the thickness and surface of the nutrient medium (Figure 1a). After soaking in a sodium bicarbonate solution and washing in water, the number of microorganisms on the surface decreased significantly, as indicated by the growth of colonies (Figure 1c). Sowing flax seeds after the drying process did not reveal the growth of microflora on the surface of the nutrient medium (Figure 1c).



a b c

Figure 1 Growth of flaxseed microbiota in Petri dishes after the proposed processing. Note: a) before processing; b) after washing; c) after drying at 95 ±1 °C for 15 min.

In general, our proposed processing of flax seeds to reduce the number of microorganisms on its surface is quite effective. It allows us to introduce raw materials into the recipe for producing hard rennet cheese enriched with omega-3 fatty acids.

Adding flax seeds to the rennet cheese production technology can affect microbiota development and maturation. Therefore, research was conducted to substantiate the parameters of the microbiological process for the production technology of hard rennet cheese with different amounts of flax seeds. Mathematical modelling of the cheese recipe according to the content of omega-3 fatty acids showed that the optimal amount of flax seeds as a source of essential acids would be from 1.5 to 5%. In the given range of flaxseed concentrations, three samples of rennet cheese with a flaxseed content of 1.5%, 3.0, and 5.0% were produced (Figure 2).

Figure 2 Hard cheese with 3% flax seeds after ripening.

Research of changes in lactic microbiota in cheese samples with different flaxseed content (Figure 3) revealed a more intense microbiological process with the participation of lactic microflora in cheese samples with flaxseed during the first 10 days of ripening, compared to the control sample of hard rennet cheese.

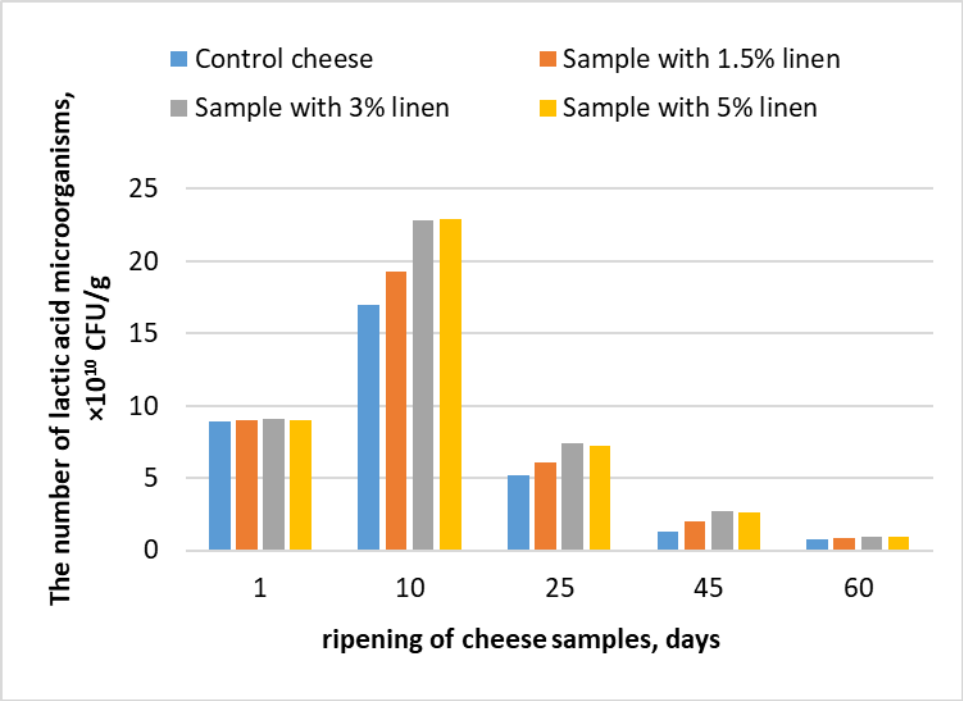


Figure 3 Development of lactic acid microorganisms in cheese samples with flax seeds.

In particular, with an almost identical amount of lactic microflora on the first day – 9.0 ±0.1×10^{10} CFU/g, after ten days of maturation, their number in the sample of rennet cheese with the highest content of flax seeds (5%) increased by 2.5 times (p <0.05), which is on average 1.2 times more lactic acid bacteria than in the cheese sample with the lowest flaxseed content (1.5%). In the control cheese sample, the content of lactic acid bacteria was 1.3 times (p <0.05) less than in the cheese sample with 5% linseed oil. The ripening of cheese from 10 to 25 days revealed a rapid decrease in lactic acid microflora, both in experimental samples of rennet cheese with flax and in the control sample. At the same time, a statistically significant difference between the dynamics of the reduction of lactic acid bacteria in the experimental cheese samples and the control was not set since the content of lactobacilli in the tested cheese samples during this period was decreased by an average of 3.2 times (p <0.05). This indicates that during this period, the lactic acid microflora has insufficient carbohydrates for nutrition and intensive development, so it gradually dies.

From the 25th to the 45th day of ripening of the experimental cheese samples, the process of dying of lactic acid bacteria continued. At the same time, in experimental samples of cheese with flax content, the number of lactobacilli during this period was decreased by 3.0 and 2.7 times (p <0.05), against 4.0 times (p <0.05) in the control sample of rennet cheese.

Research of the microbiota of experimental samples of hard rennet cheese with flax on the 60th day from the beginning of ripening revealed a decrease in the rate of death of lactic acid microbiota compared with the previous periods of the research. That is, on the 60th day of ripening of experimental samples of hard rennet cheese with flax, the number of lactobacilli in the cheese was 0.85-0.94×10^{10} CFU/g, which depended on the concentration of added flaxseed in the control cheese their number was 0.78 ±0. 03×10^{10} CFU/g.

Technically harmful fungal microflora, which accidentally enters the product during production in the process of vital activity, produces a much more diverse number of enzymes than lactic acid. Therefore, due to enzymatic processes, a complex of chemical substances is formed, which negatively influences the quality of rennet cheese. We determined the dynamics of fungal microbiota development in hard rennet cheese with different amounts of flax seeds during its two-month ripening process (Figure 4).

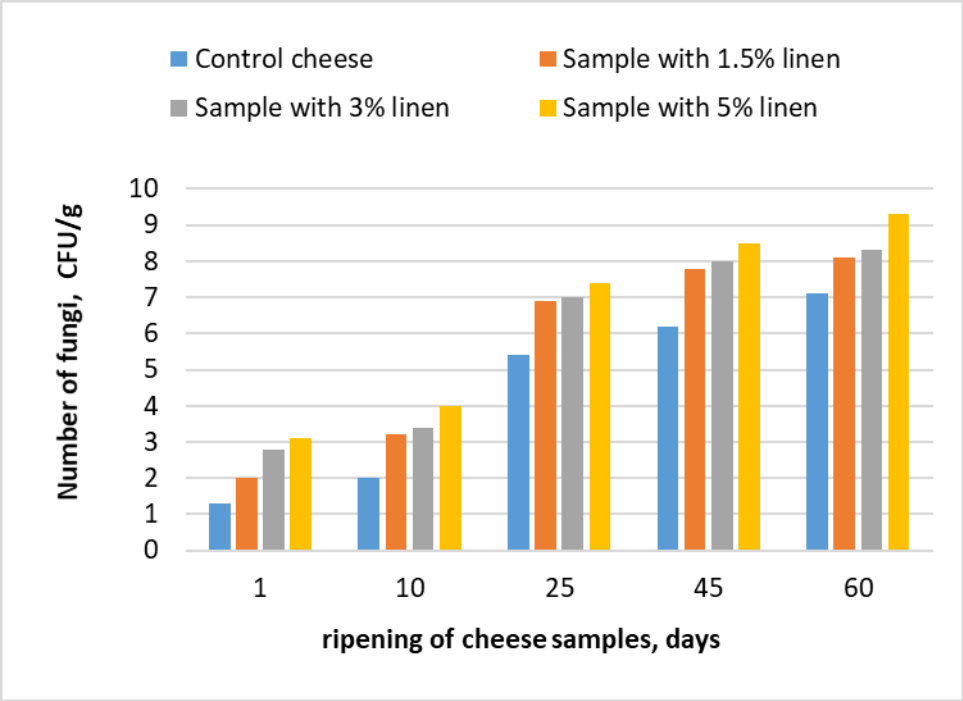


Figure 4 Development of fungal microbiota in samples of cheese with flax seeds.

After the first day of the technological process, the amount of fungal microflora in experimental cheese samples with flax seeds was 2 to 3.1 CFU/g. At the same time, the highest contamination was noted in the sample with the highest content of flax, and in the control sample of cheese, the number of fungi was, on average, 1 cell per g. During the 60-day ripening process of the experimental cheese samples, the number of fungal cells gradually increased, and in the finished product, it was within the range of 8-10 cells per 1 g. This content of fungal microflora in rennet cheese poses no threat to the deterioration of its microbiological and organoleptic evaluation, as in fresh cheese, as well as during storage. As such, several fungi cells cannot show significant enzymatic processes.

Because flax seeds are contaminated with spore-forming bacilli that belong to the epiphytic microbiota, and the frugal method of reducing the microflora from the seeds proposed by us does not entirely inactivate them, we determined the possibility of their development in cheese during the ripening process (Figure 5).

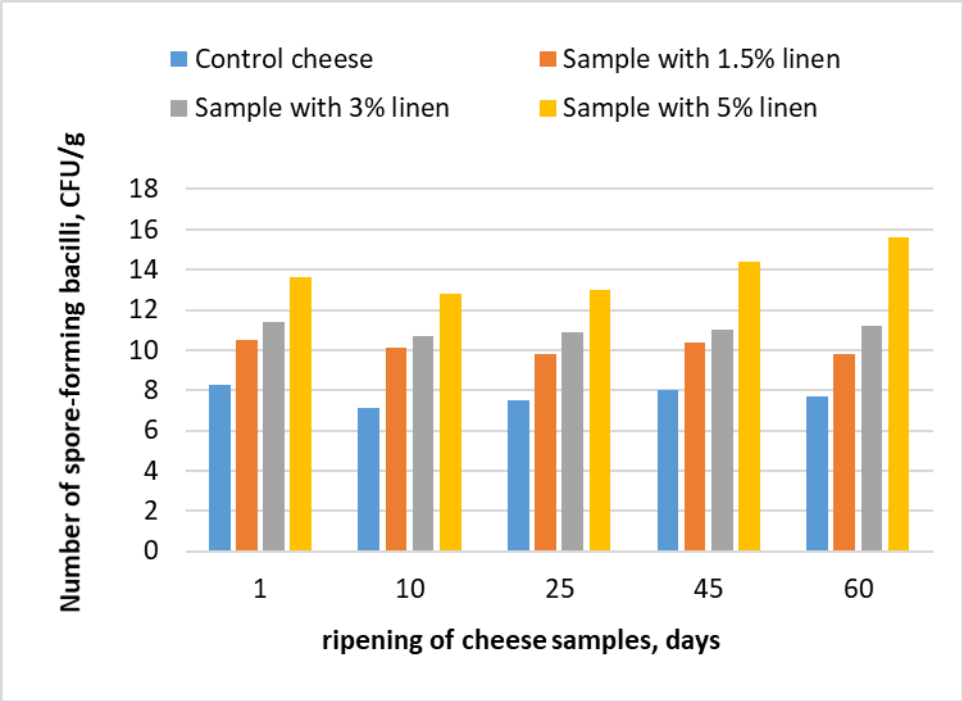


Figure 5 Development of spore-forming bacilli in samples of cheese with flax seeds.

We observe the dependence between the content of spore-forming bacilli on the first day of cheese production and the amount of flaxseed added to the cheese mass. In particular, with an increase in the concentration of linseed in cheese samples, the content of Bacillus bacteria increased. On the first day of the research, the number of spore-forming bacteria in the sample with 5% flaxseed content was 1.6 times greater (p <0.05) than in the control sample of cheese and 1.3 times (p <0.05) greater compared with the sample with 1.5% flaxseed content. It becomes evident that these bacilli are sources of flax seeds, which maintain their vitality during the first day of the production process. During this, there are favorable conditions for their development since, at this stage, the technological process takes place with good aeration of the environment, which is essential for the vital activity of aerobic bacilli. However, even this amount of Bacillus bacteria at this stage of cheese production is insignificant and does not influence the beneficial microbiota of sourdough and biochemical changes in the product.

During the quantitative evaluation of bacilli on the 10^{th}-60^{th} day of ripening of samples of hard rennet cheese with different flaxseed content, their development was stopped, as the content was almost at the same level as the value of the first day, within 10-15 CFU/g.

The influence of hard rennet cheese production technology with added flax seeds on the quantitative changes of sanitary-indicative and pathogenic microorganisms was determined (Table 2).

Table 2 Research of changes in sanitary-indicative microorganisms and pathogenic ones according to the production technology of hard rennet cheese with flax seeds, x ±SE, n = 5.

Indicators	The term maturation, day	Norms (DSTU 6003: 2008)	Control cheese	Experimental samples of hard rennet cheese with flax seeds		
				1.5 %	3.0 %	5.0 %
Enterobacteriaceae	1	in 0.01 g of the product is not allowed	>1	>1	>1	>1
	10		>1	>1	>1	>1
	25		>1	>1	>1	>1
	45		>1	>1	>1	>1
	60		>1	>1	>1	>1
Staphylococcus aureus	1 10 25 45 60	no more than 500 CFU/g	Not found	Not found	Not found	Not found
L. monocytogenes, *Salmonella* spp.	1 10 25 45 60	in 25 g of the product is not allowed	Not found	Not found	Not found	Not found

It was found during hard rennet cheese production and ripening technology with flax seeds. These microorganisms indicate compliance with the entire complex of sanitary requirements - the *Enterobacteriaceae* genus was not detected in 1 g of the product.

In general, from the results of a series of experiments on the influence of the added different amounts of flax seeds on the microbiological processes of ripening hard rennet cheese, we found that the main representatives of the autochthonous microbiota – lactic acid microflora develop somewhat more intensively in samples with 5% flax, compared to 1.5% content and in the control sample. However, the general dynamics of the growth of lactic acid microorganisms in the experimental samples corresponds to the dynamics in the control. At the same time, developing technically harmful microorganisms (spore-forming and fungal microbiota) in cheese samples with flax and control does not lead to the deterioration of their microbiological indicators. This makes it possible to use flax seeds in the production technology of hard rennet cheeses at 5%.

Highly nutritious dairy products such as hard cheeses are considered a good source of protein, fats, minerals, and vitamins **[56]**, **[57]** and are consumed in significant quantities in the traditional European diet **[50]**. At the same time, the disadvantages of cheese include the presence in their composition of a large amount of saturated fatty acids **[58]**, the consumption of which causes the development of cardiovascular diseases **[30]**, **[32]**, **[33]**, **[34]**, **[35]**, **[36]**. Therefore, modifying the composition of fatty acids in hard cheese by increasing the content of unsaturated fatty acids and reducing the amount of saturated fatty acids is extremely important for consumers' health. Enriching cheese with sources rich in unsaturated fatty acids increases the quality of the product by improving its fatty acid profile **[38]**, **[39]**. Alternative sources of unsaturated fatty acids include flaxseed and oil,

echium, walnut, algal oil, etc. **[41]**, **[42]**, **[44]**. This research shows that the problem of consumption of foods rich in polyunsaturated fatty acids can be partially solved by developing a hard rennet cheese with flax seeds.

It has been set that flax seeds are contaminated with saprophytic microflora of plants and the environment. Therefore, it can only produce hard cheese with prior disinfection processing. In particular, insemination with mesophilic bacteria before processing was 7.8 $\pm 0.3 \times 10^4$ CFU/g, which is a significant amount. The normative indicator of the content of mesophilic microflora for non-traditional plant raw materials (which includes flax seeds) should not exceed the amount of 5 $\times 10^4$ CFU/g **[59]**. The method proposed by us (washing the seeds in a solution of sodium bicarbonate and drying at a temperature of 95 ±1 for 20 min) of processing flax seeds before adding them to the cheese mass reduced the number of mesophilic microorganisms to 3.9 $\pm 0.1 \times 10^1$ CFU/g. This microbial number of mesophilic bacteria is not significant for breaking the production technology of rennet cheese **[60]**. Also, with this processing, the amount of fungal microbiota on the surface of the seeds was decreased approximately 160 times to 0.3 $\pm 0.1 \times 10^1$ CFU/g. Such content of fungal microflora on the surface of flax seeds is relatively safe for its introduction as an additive rich in omega-3 fatty acids in hard rennet cheese production technology.

Of interest was research on the influence of different amounts of added flaxseed on the microbiological processes in cheese during its ripening. It revealed a 1.3 times higher content of lactic acid microflora in samples of hard cheese with 5% flax seeds during the first 10 days of ripening compared to a control sample of cheese. Probably, the more intensive development of lactic acid bacteria in samples of cheese containing flax seeds is associated with additional enrichment of the cheese with minerals and B vitamins, which flax is rich in **[28]**, **[46]**, **[47]**, and which are necessary for the development of lactic acid microbiota **[11]**, **[12]**, **[13]**, **[61]**. In addition, the growth of a more significant number of lactic acid microflora in the experimental samples will contribute to faster ripening of the rennet cheese.

Compared to control cheese, an average of 1.4 times slower dying off of lactic acid microbiota was also revealed in the period from 25 to 45 days of ripening of experimental cheese samples. In our opinion, this decrease in the intensity of the death of lactic microflora in rennet cheese with flax during ripening is related to its enrichment with biologically active substances of the seeds, which serve as an additional source of nutrition for lactic acid bacteria.

So, it follows from the research that the ripening process of hard rennet cheese with the addition of 1.5 to 5% of flax seeds to the cheese grain had a beneficial influence on the development of lactic acid microflora, as their number was greater than in the control sample of cheese, and the dying process was slower. This indicates that adding 1.5 to 5% of flax seeds in the production technology of hard rennet cheese does not disrupt the general dynamics of the development of lactic acid microflora inherent to this type of cheese.

Adding various phytosupplements to the technology of hard rennet cheese can contribute to additional contamination with fungal microbiota since the spores of these microorganisms are usually present on raw plant materials **[62]**, **[63]**. In our research, when adding flax seeds to the hard cheese production technology, the fungal microbiota increases to 10 cells per g in experimental samples during 60 days of ripening, against 7 CFU/g in control. At the same time, the largest amount was in the sample of cheese, which contained 5% of flax seeds. This content of fungal microbiota did not exceed the permissible content of 50 CFU/g for most dairy products provided by the national standard **[60]**. Therefore, we believe that the use of flax seeds according to our proposed method of disinfection, which involves washing in a soda solution, drying, processing at pasteurization temperatures, and adding to the production technology of hard rennet cheeses, does not lead to a statistically probable growth of fungal microflora in the test samples during the entire ripening process.

At the beginning of cheese ripening (the first day), a 1.6 times higher content of spore-forming aerobic bacilli was found in the sample with the highest flax seeds compared to the content in the control cheese. At the same time, during 60 days of ripening of the cheese, no process of their reproduction was detected since their content practically corresponded to the initial amount of 10-15 CFU/g in the experimental samples and about 8 in the control. This indicates that anaerobic conditions are created in the middle of the cheese head during the technological process, unfavorable for developing this type of bacteria. Such data are consistent with research **[2]** that aerobic spore bacilli cannot reproduce in hard cheese technology. At the same time, their number should be at most 10 cells in raw materials **[59]**. Therefore, a small (up to 10 CFU/g of product) amount of aerobic bacilli introduced with flax seeds does not harm the production technology of hard rennet cheese with flax content.

In standard 6003:2008, hard rennet cheese is evaluated according to sanitary indicator microorganisms – *Enterobacteriaceae* and pathogens: *Staphylococcus aureus*, *Listeria monocytogenes,* and *Salmonella*. Bacteria of the *Enterobacteriaceae* genus should not be detected in 0.01 g of cheese at the time of sale, and the species Staphylococcus aureus in 1 g of rennet cheese should not exceed 500 CFU/g. At the same time, the pathogenic bacteria *Listeria monocytogenes* and *Salmonella* spp., traditionally used in dairy products, should not be in 25 g. Evaluation of cheese samples according to microbiological indicators of safety, respectively **[60]** during the entire

ripening process with different content of flax seeds did not reveal an excess in the number of bacteria of the genus *Enterobacteriaceae*, *Staphylococcus aureus*, and such pathogenic bacteria as *L. monocytogenes* and *Salmonella* spp. were not detected in 25 g of the product. This indicates that all cheese samples have a significant reserve of storage stability. It can also be stated that according to indicators of microbiological safety, all test samples were not inferior to the control cheese, and no pathogenic microorganisms were isolated from them.

In general, more and more data have recently appeared, and research was conducted on the enrichment of various types of cheese [48], [49] with vegetable oils to improve their lipid composition **[64]**, **[65]**. The addition of oils, particularly linseed, is a perspective in developing recipes for new types of products. In contrast, in hard cheese technology, adding vegetable oils will change the product category from cheese to cheesy, which consumers do not like very much. Therefore, in our research, flax seeds were added as a source of omega-3 fatty acids during the production of hard rennet cheese, increasing the product's biological value and enriching it with dietary fibers. The data received is consistent with investigations **[66]**, which studied the addition of whole flax seeds and their flour to the technological parameters of soft cheese. It was found that enriching the cheese with flax seeds is more expedient since the proportion of polyunsaturated fatty acids decreases during grinding. At the same time, adding flax seeds and flax flour slightly enhanced the development of the cheese's bifidobacteria and lactic acid microflora. Enrichment of such dairy products as yogurt and curd paste with linseed oil did not influence microbiological processes during production and storage **[48]**, **[49]**. At the same time, researchers **[67]** showed that adding linseed mucilage to cream cheese increased protein, ash, and total solids content, while moisture content and pH values decreased. In addition, it was found that the combination of flaxseed mucilage and probiotic bacteria enhanced the antibacterial action against pathogenic bacteria such as *Pseudomonas aeruginosa* and *Yersinia enterocolitica* **[67]**. In general, we can conclude that flax seeds, flax oil, or flax products positively influenced the microbiota of dairy products. Although we support the opinion of scientists **[50]**, **[51]**, **[52]**, **[68]** that a promising direction for the production of dairy products rich in polyunsaturated fatty acids is the correction of the ration of animals to get milk of improved lipid profile. At the same time, this direction is just emerging. It needs scientific justification and the possibility of industrial production of a sufficient amount of such milk.

Therefore, the disinfection of ice seeds according to our proposed method significantly reduces its contamination with epiphytic microorganisms, which allows it to be added to the technological process of production of hard rennet cheese in an amount of up to 5%. With such a quantity of flax seeds in the hard cheese recipe, no significant changes in the microbiological process occur during two months of ripening.

CONCLUSION

The proposed method (washing the seeds in a solution of sodium bicarbonate and drying at a temperature of 95 ±1 for 20 min) of processing flax seeds before adding them to the cheese mass reduced the number of mesophilic microorganisms to 3.9 $\pm 0.1 \times 10^1$ CFU/g, fungi in 160 times up to 0.3 $\pm 0.1 \times 10^1$ CFU/g and aerobic mesophilic bacilli 78 times up to 1.1 $\pm 0.1 \times 10^1$ CFU/g. The technology of hard rennet cheese with a flax seed content of 3-5% has been developed. A 1.3 times higher content of lactic acid microflora was found in samples of hard cheese with 5% flax seeds during the first 10 days of ripening compared to the control sample of cheese. On the 60th day of ripening of cheese with a content of 5% flax seeds, the number of lactic acid bacteria was 9.4 $\pm 0.3 \times 10^9$ CFU/g, and in the control cheese – 7.8 $\pm 0.3 \times 10^9$ CFU/g. During the technology of production and ripening of cheese with different content of flax seeds, no excess of the normative values was found for the number of bacteria of the genus *Enterobacteriaceae*, *Staphylococcus aureus*, and such pathogenic bacteria as *L. monocytogenes* and *Salmonella* spp. were not detected in 25 g of the product. Therefore, the developed hard rennet cheese with flax seeds can be consumed as an additional omega-3 fatty acids and dietary fiber source.

REFERENCES

1. Kukhtyn, M., Vichko, O., Kravets, O., Karpyk, H., Shved, O., & Novikov, V. (2019). Biochemical and microbiological changes during fermentation and storage of a fermented milk product prepared with Tibetan Kefir Starter. In Archivos Latinoamericanos de Nutrición (Vol. 68, Issue 4, pp. 336–343). Sociedad Latinoamericana de Nutricion. https://doi.org/10.37527/2018.68.4.007
2. Skril, Y. (2023). Analytical review of biotechnological problem of Ukrainian hard cheeses. In Biotechnologia Acta (Vol. 16, Issue 3, pp. 5–23). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/biotech16.03.005
3. Kukhtyn, M., Salata, V., Horiuk, Y., Kovalenko, V., Ulko, L., Prosyanyi, S., Shuplyk, V., & Kornienko, L. (2021). The influence of the denitrifying strain of Staphylococcus carnosus No. 5304 on the content of nitrates in the technology of yogurt production. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 66–73). HACCP Consulting. https://doi.org/10.5219/1492

4. Yukalo, V. G., & Storozh, L. A. (2018). Isolation of κ-CN-1P and B-CN-5P fractions from native casein micelles. In The Ukrainian Biochemical Journal (Vol. 90, Issue 4, pp. 74–79). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka). https://doi.org/10.15407/ubj90.04.074
5. Gumus, C. E., & Gharibzahedi, S. M. T. (2021). Yogurts supplemented with lipid emulsions rich in omega-3 fatty acids: New insights into the fortification, microencapsulation, quality properties, and health-promoting effects. In Trends in Food Science & Technology (Vol. 110, pp. 267–279). Elsevier BV. https://doi.org/10.1016/j.tifs.2021.02.016
6. Nadirova, S., & Sinyavskiy, Yu. (2023). Biotechnological approaches to the creation of new fermented dairy products. In Eurasian Journal of Applied Biotechnology (Issue 4, pp. 128–136). National Center for Biotechnology. https://doi.org/10.11134/btp.4.2022.16
7. Arioui, F., Ait Saada, D., & Cheriguene, A. (2016). Physicochemical and sensory quality of yogurt incorporated with pectin from peel of Citrus sinensis. In Food Science & Nutrition (Vol. 5, Issue 2, pp. 358–364). Wiley. https://doi.org/10.1002/fsn3.400
8. Dabija, A., Codină, G. G., Ropciuc, S., Gâtlan, A.-M., & Rusu, L. (2018). Assessment of the Antioxidant Activity and Quality Attributes of Yogurt Enhanced with Wild Herbs Extracts. In Journal of Food Quality (Vol. 2018, pp. 1–12). Hindawi Limited. https://doi.org/10.1155/2018/5329386
9. Yousefi, M., & Jafari, S. M. (2019). Recent advances in application of different hydrocolloids in dairy products to improve their techno-functional properties. In Trends in Food Science & Technology (Vol. 88, pp. 468–483). Elsevier BV. https://doi.org/10.1016/j.tifs.2019.04.015
10. Meybodi, N. M., Mortazavian, A. M., Arab, M., & Nematollahi, A. (2020). Probiotic viability in yoghurt: A review of influential factors. In International Dairy Journal (Vol. 109, p. 104793). Elsevier BV. https://doi.org/10.1016/j.idairyj.2020.104793
11. Farahat, E. S. A., Mohamed, A. G., El-Loly, M. M., & Gafour, W. A. M. S. (2021). Innovative vegetables-processed cheese: I. Physicochemical, rheological and sensory characteristics. In Food Bioscience (Vol. 42, p. 101128). Elsevier BV. https://doi.org/10.1016/j.fbio.2021.101128
12. Salehi, F. (2021). Quality, physicochemical, and textural properties of dairy products containing fruits and vegetables: A review. In Food Science & Nutrition (Vol. 9, Issue 8, pp. 4666–4686). Wiley. https://doi.org/10.1002/fsn3.2430
13. Kamath, R., Basak, S., & Gokhale, J. (2022). Recent trends in the development of healthy and functional cheese analogues-a review. In LWT (Vol. 155, p. 112991). Elsevier BV. https://doi.org/10.1016/j.lwt.2021.112991
14. Sharma, K. D., Stähler, K., Smith, B., & Melton, L. (2011). Antioxidant capacity, polyphenolics and pigments of broccoli-cheese powder blends. In Journal of Food Science and Technology (Vol. 48, Issue 4, pp. 510–514). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-010-0211-1
15. Lavelli, V., Sri Harsha, P. S. C., Torri, L., & Zeppa, G. (2014). Use of winemaking by-products as an ingredient for tomato puree: The effect of particle size on product quality. In Food Chemistry (Vol. 152, pp. 162–168). Elsevier BV. https://doi.org/10.1016/j.foodchem.2013.11.103
16. Marchiani, R., Bertolino, M., Ghirardello, D., McSweeney, P. L. H., & Zeppa, G. (2015). Physicochemical and nutritional qualities of grape pomace powder-fortified semi-hard cheeses. In Journal of Food Science and Technology (Vol. 53, Issue 3, pp. 1585–1596). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-015-2105-8
17. Marchiani, R., Bertolino, M., Belviso, S., Giordano, M., Ghirardello, D., Torri, L., Piochi, M., & Zeppa, G. (2015). Yogurt Enrichment with Grape Pomace: Effect of Grape Cultivar on Physicochemical, Microbiological and Sensory Properties. In Journal of Food Quality (Vol. 39, Issue 2, pp. 77–89). Wiley. https://doi.org/10.1111/jfq.12181
18. Slozhenkina, M. I., Gorlov, I. F., Kryuchkova, V. V., Serkova, A. E., Ryaskova, A. D., & Belik, S. N. (2019). Vegetable ingredient in cheese product. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 13, Issue 1, pp. 1018–1025). HACCP Consulting. https://doi.org/10.5219/1207
19. El-Sayed, S. M. (2020). Use of spinach powder as functional ingredient in the manufacture of UF-Soft cheese. In Heliyon (Vol. 6, Issue 1, p. e03278). Elsevier BV. https://doi.org/10.1016/j.heliyon.2020.e03278
20. Alenisan, M. A., Alqattan, H. H., Tolbah, L. S., & Shori, A. B. (2017). Antioxidant properties of dairy products fortified with natural additives: A review. In Journal of the Association of Arab Universities for Basic and Applied Sciences (Vol. 24, Issue 1, pp. 101–106). Informa UK Limited. https://doi.org/10.1016/j.jaubas.2017.05.001
21. Grażyna, C., Hanna, C., Adam, A., & Magdalena, B. M. (2017). Natural antioxidants in milk and dairy products. In International Journal of Dairy Technology (Vol. 70, Issue 2, pp. 165–178). Wiley. https://doi.org/10.1111/1471-0307.12359

22. Adetuyi, B. O., Olajide, P. A., Adetunji, C. O., & Adetunji, J. B. (2024). Beneficial uses of essential oils in diary products. Applications of Essential Oils in the Food Industry. https://doi.org/10.1016/B978-0-323-98340-2.00021-3
23. Baba, W. N., Jan, K., Punoo, H. A., Wani, T. A., Dar, M. M., & Masoodi, F. A. (2018). Techno-functional properties of yoghurts fortified with walnut and flaxseed oil emulsions in guar gum. In LWT (Vol. 92, pp. 242–249). Elsevier BV. https://doi.org/10.1016/j.lwt.2018.02.007
24. Rao, S., Radhakrishnan, P., Valiathan, S., & M, S. (2023). Rosehip oil nanoemulsion as a stable delivery system for omega-3 fatty acids to enhance the nutritional value of yogurt. In Food Chemistry Advances (Vol. 3, p. 100545). Elsevier BV. https://doi.org/10.1016/j.focha.2023.100545
25. Ganesan, B., Brothersen, C., & McMahon, D. J. (2013). Fortification of Foods with Omega-3 Polyunsaturated Fatty Acids. In Critical Reviews in Food Science and Nutrition (Vol. 54, Issue 1, pp. 98–114). Informa UK Limited. https://doi.org/10.1080/10408398.2011.578221
26. Sąsiadek, W., Michalski, J., & Ulatowski, P. (2018). Charakterystyka nienasyconych kwasów tłuszczowych zawartych w rybach. In Prace Naukowe Uniwersytetu Ekonomicznego we Wrocławiu (Issue 542, pp. 161–176). Wroclaw University of Economics and Business. https://doi.org/10.15611/pn.2018.542.12
27. Briggs, M., Petersen, K., & Kris-Etherton, P. (2017). Saturated Fatty Acids and Cardiovascular Disease: Replacements for Saturated Fat to Reduce Cardiovascular Risk. In Healthcare (Vol. 5, Issue 2, p. 29). MDPI AG. https://doi.org/10.3390/healthcare5020029
28. Feizollahi, E., Hadian, Z., & Honarvar, Z. (2018). Food Fortification with Omega-3 Fatty Acids; Microencapsulation as an Addition Method. In Current Nutrition & Food Science (Vol. 14, Issue 2, pp. 90–103). Bentham Science Publishers Ltd. https://doi.org/10.2174/1573401313666170728151350
29. Lock, A. L., & Bauman, D. E. (2004). Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. In Lipids (Vol. 39, Issue 12, pp. 1197–1206). Wiley. https://doi.org/10.1007/s11745-004-1348-6
30. Russo, G. L. (2009). Dietary n−6 and n−3 polyunsaturated fatty acids: From biochemistry to clinical implications in cardiovascular prevention. In Biochemical Pharmacology (Vol. 77, Issue 6, pp. 937–946). Elsevier BV. https://doi.org/10.1016/j.bcp.2008.10.020
31. Iafelice, G., Caboni, M. F., Cubadda, R., Di Criscio, T., Trivisonno, M. C., & Marconi, E. (2008). Development of Functional Spaghetti Enriched with Long Chain Omega-3 Fatty Acids. In Cereal Chemistry (Vol. 85, Issue 2, pp. 146–151). Wiley. https://doi.org/10.1094/cchem-85-2-0146
32. Singh, K. K., Mridula, D., Rehal, J., & Barnwal, P. (2011). Flaxseed: A Potential Source of Food, Feed and Fiber. In Critical Reviews in Food Science and Nutrition (Vol. 51, Issue 3, pp. 210–222). Informa UK Limited. https://doi.org/10.1080/10408390903537241
33. Xie, R., & Zhang, Y. (2023). Association between 19 dietary fatty acids intake and rheumatoid arthritis: Results of a nationwide survey. In Prostaglandins, Leukotrienes and Essential Fatty Acids (Vol. 188, p. 102530). Elsevier BV. https://doi.org/10.1016/j.plefa.2022.102530
34. Bhat, S., Sarkar, S., Zaffar, D., Dandona, P., & Kalyani, R. R. (2023). Omega-3 Fatty Acids in Cardiovascular Disease and Diabetes: a Review of Recent Evidence. In Current Cardiology Reports (Vol. 25, Issue 2, pp. 51–65). Springer Science and Business Media LLC. https://doi.org/10.1007/s11886-022-01831-0
35. Kaur, G., Mason, R. P., Steg, P. G., & Bhatt, D. L. (2024). Omega-3 Fatty Acids for Cardiovascular Event Lowering. In European Journal of Preventive Cardiology. Oxford University Press (OUP). https://doi.org/10.1093/eurjpc/zwae003
36. De Marchi, F., Vignaroli, F., Mazzini, L., Comi, C., & Tondo, G. (2024). New Insights into the Relationship between Nutrition and Neuroinflammation in Alzheimer's Disease: Preventive and Therapeutic Perspectives. In CNS & Neurological Disorders - Drug Targets (Vol. 23, Issue 5, pp. 614–627). Bentham Science Publishers Ltd. https://doi.org/10.2174/1871527322666230608110201
37. Tur, J. A., Bibiloni, M. M., Sureda, A., & Pons, A. (2012). Dietary sources of omega 3 fatty acids: public health risks and benefits. In British Journal of Nutrition (Vol. 107, Issue S2, pp. S23–S52). Cambridge University Press (CUP). https://doi.org/10.1017/s0007114512001456
38. Oliver, L., Dietrich, T., Marañón, I., Villarán, M. C., & Barrio, R. J. (2020). Producing Omega-3 Polyunsaturated Fatty Acids: A Review of Sustainable Sources and Future Trends for the EPA and DHA Market. In Resources (Vol. 9, Issue 12, p. 148). MDPI AG. https://doi.org/10.3390/resources9120148
39. Lee, S. H., Kim, Y. B., Kim, D.-H., Lee, D.-W., Lee, H.-G., Jha, R., & Lee, K.-W. (2021). Dietary soluble flaxseed oils as a source of omega-3 polyunsaturated fatty acids for laying hens. In Poultry Science (Vol. 100, Issue 8, p. 101276). Elsevier BV. https://doi.org/10.1016/j.psj.2021.101276

40. Muñoz-Tébar, N., De la Vara, J. A., Ortiz de Elguea-Culebras, G., Cano, E. L., Molina, A., Carmona, M., & Berruga, M. I. (2019). Enrichment of sheep cheese with chia (Salvia hispanica L.) oil as a source of omega-3. In LWT (Vol. 108, pp. 407–415). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.03.092
41. Dams, S., Holasek, S., Tsiountsioura, M., Edelsbrunner, M., Dietz, P., Koefeler, H., Malliga, D.-E., Gürbüz, A., Meier-Allard, N., Poncza, B., Lackner, S., Schwarzenberger, E., Jansenberger, Y., & Lamprecht, M. (2020). Effects of a plant-based fatty acid supplement and a powdered fruit, vegetable and berry juice concentrate on omega-3-indices and serum micronutrient concentrations in healthy subjects. In International Journal of Food Sciences and Nutrition (Vol. 71, Issue 6, pp. 769–780). Informa UK Limited. https://doi.org/10.1080/09637486.2020.1725960
42. Otero, P., Carpena, M., Fraga-Corral, M., Garcia-Oliveira, P., Soria-Lopez, A., Barba, F. J., Xiao, J., Simal-Gandara, J., & Prieto, M. A. (2021). Aquaculture and agriculture-by products as sustainable sources of omega-3 fatty acids in the food industry. In eFood (Vol. 2, Issue 5, pp. 209–233). Wiley. https://doi.org/10.53365/efood.k/144603
43. Kumar, G. S., & Krishna, A. G. G. (2013). Studies on the nutraceuticals composition of wheat derived oils wheat bran oil and wheat germ oil. In Journal of Food Science and Technology (Vol. 52, Issue 2, pp. 1145–1151). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-013-1119-3
44. Górnaś, P., Rudzińska, M., Raczyk, M., & Soliven, A. (2015). Lipophilic bioactive compounds in the oils recovered from cereal by-products. In Journal of the Science of Food and Agriculture (Vol. 96, Issue 9, pp. 3256–3265). Wiley. https://doi.org/10.1002/jsfa.7511
45. Kaur, P., Waghmare, R., Kumar, V., Rasane, P., Kaur, S., & Gat, Y. (2018). Recent advances in utilization of flaxseed as potential source for value addition. In OCL (Vol. 25, Issue 3, p. A304). EDP Sciences. https://doi.org/10.1051/ocl/2018018
46. Kajla, P., Sharma, A., & Sood, D. R. (2014). Flaxseed—a potential functional food source. In Journal of Food Science and Technology (Vol. 52, Issue 4, pp. 1857–1871). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-014-1293-y
47. Yawale, P., Upadhyay, N., Ganguly, S., & Kumar, S., Ashish. (2022). A comprehensive review on recent novel food and industrial applications of flaxseed: 2014 onwards. In Food and Feed Research (Issue 00, pp. 16–16). Centre for Evaluation in Education and Science (CEON/CEES). https://doi.org/10.5937/ffr0-35420
48. Dal Bello, B., Torri, L., Piochi, M., & Zeppa, G. (2015). Healthy yogurt fortified with n-3 fatty acids from vegetable sources. In Journal of Dairy Science (Vol. 98, Issue 12, pp. 8375–8385). American Dairy Science Association. https://doi.org/10.3168/jds.2015-9688
49. Lialyk, A., Pokotylo, O., Kukhtyn, M., Beyko, L., Horiuk, Y., Dobrovolska, S., & Mazur, O. (2020). Fatty acid composition of curd spread with different flax oil content. In Nova Biotechnologica et Chimica (Vol. 19, Issue 2, pp. 216–222). University of Saints Cyril and Methodius. https://doi.org/10.36547/nbc.v19i2.776
50. Gebreyowhans, S., Lu, J., Zhang, S., Pang, X., & Lv, J. (2019). Dietary enrichment of milk and dairy products with n-3 fatty acids: A review. In International Dairy Journal (Vol. 97, pp. 158–166). Elsevier BV. https://doi.org/10.1016/j.idairyj.2019.05.011
51. Drouin, G., Catheline, D., Sinquin, A., Baudry, C., Le Ruyet, P., Rioux, V., & Legrand, P. (2018). Incorporation of Dairy Lipids in the Diet Increased Long-Chain Omega-3 Fatty Acids Status in Post-weaning Rats. In Frontiers in Nutrition (Vol. 5). Frontiers Media SA. https://doi.org/10.3389/fnut.2018.00042
52. Nguyen, Q. V., Malau-Aduli, B., Cavalieri, J., Malau-Aduli, A. E. O., & Nichols, P. (2019). Enhancing Omega-3 Long-Chain Polyunsaturated Fatty Acid Content of Dairy-Derived Foods for Human Consumption. In Nutrients (Vol. 11, Issue 4, p. 743). MDPI AG. https://doi.org/10.3390/nu11040743
53. DSTU 7357:2013. Milk and dairy products. Methods of microbiological control. National Standard of Ukraine.
54. DSTU EN 12824:2004. Microbiology of food products and animal feed. Horizontal method of detection of Salmonella. National Standard of Ukraine.
55. DSTU ISO 11290-1:2003. Microbiology of food products and animal feed. Horizontal method of detection and counting of Listeria monocytogenes. Part 1. National Standard of Ukraine.
56. Guinee, T. P., & Fox, P. F. (2017). Salt in Cheese: Physical, Chemical and Biological Aspects. In Cheese (pp. 317–375). Elsevier. https://doi.org/10.1016/b978-0-12-417012-4.00013-2
57. Yukalo, V., Datsyshyn, K., & Storozh, L. (2019). Comparison of products of whey proteins concentrate proteolysis, obtained by different proteolytic preparations. In Eastern-European Journal of Enterprise Technologies (Vol. 5, Issue 11 (101), pp. 40–47). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2019.177314
58. Manuelian, C. L., Currò, S., Penasa, M., Cassandro, M., & De Marchi, M. (2017). Characterization of major and trace minerals, fatty acid composition, and cholesterol content of Protected Designation of Origin

cheeses. In Journal of Dairy Science (Vol. 100, Issue 5, pp. 3384–3395). American Dairy Science Association. https://doi.org/10.3168/jds.2016-12059

59. Kovalova, O., Vasylieva, N., Stankevych, S., Zabrodina, I., Haliasnyi, I., Gontar, T., Kotliar, O., Gavrish, T., Gill, M., & Karatieieva, O. (2023). Determining the effect of plasmochemically activated aqueous solutions on the bioactivation process of sea buckthorn seeds. In Eastern-European Journal of Enterprise Technologies (Vol. 2, Issue 11 (122), pp. 99–111). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2023.275548
60. DSTU 6003:2008. Cheese hard. General specification. National Standard of Ukraine
61. Kukhtyn, M., Vichko, O., Berhilevych, O., Horyuk, Y., & Horyuk, V. (2016). Main microbiological and biological properties of microbial associations of" Lactomyces tibeticus". In Research Journal of Pharmaceutical, Biological and Chemical Sciences (Vol. 7, Issue 6, pp. 1266–1272). GVGS.
62. Garnier, L., Valence, F., & Mounier, J. (2017). Diversity and Control of Spoilage Fungi in Dairy Products: An Update. In Microorganisms (Vol. 5, Issue 3, p. 42). MDPI AG. https://doi.org/10.3390/microorganisms5030042
63. Kure, C. F., & Skaar, I. (2019). The fungal problem in cheese industry. In Current Opinion in Food Science (Vol. 29, pp. 14–19). Elsevier BV. https://doi.org/10.1016/j.cofs.2019.07.003
64. Achachlouei, B. F., Hesari, J., Damirchi, S. A., Peighambardoust, S., Esmaiili, M., & Alijani, S. (2013). Production and characterization of a functional Iranian white brined cheese by replacement of dairy fat with vegetable oils. In Food Science and Technology International (Vol. 19, Issue 5, pp. 389–398). SAGE Publications. https://doi.org/10.1177/1082013212455341
65. Garbowska, M., Berthold-Pluta, A., Stasiak-Różańska, L., Kalisz, S., & Pluta, A. (2023). The Impact of White Mulberry, Green Barley, Chia Seeds, and Spirulina on Physicochemical Characteristics, Texture, and Sensory Quality of Processed Cheeses. In Foods (Vol. 12, Issue 15, p. 2862). MDPI AG. https://doi.org/10.3390/foods12152862
66. Mironova, I. V., Nigmatyanov, A. A., Varivoda, A. A., Temerbaeva, M. V., Topuria, G. M., & Ginzburg, N. A. (2020). Soft cheese with flax seeds. In IOP Conference Series: Earth and Environmental Science (Vol. 613, Issue 1, p. 012084). IOP Publishing. https://doi.org/10.1088/1755-1315/613/1/012084
67. Akl, E. M., Abdelhamid, S. M., Wagdy, S. M., & Salama, H. H. (2020). Manufacture of Functional Fat-free Cream Cheese Fortified with Probiotic Bacteria and Flaxseed Mucilage as a Fat Replacing Agent. In Current Nutrition & Food Science (Vol. 16, Issue 9, pp. 1393–1403). Bentham Science Publishers Ltd. https://doi.org/10.2174/1573401316666200227112157
68. Garnier, L., Valence, F., Pawtowski, A., Auhustsinava-Galerne, L., Frotté, N., Baroncelli, R., Deniel, F., Coton, E., & Mounier, J. (2017). Diversity of spoilage fungi associated with various French dairy products. In International Journal of Food Microbiology (Vol. 241, pp. 191–197). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2016.10.026

Funds:

This research received no external funding.

Acknowledgments:

The researchers also thanked the Chortkiv cheese factory, which facilitated this research.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Mykola Kukhtyn, Ternopil Ivan Pului National Technical University, Faculty of Engineering of Machines, Structures and Technologies, Department of Food Biotechnology and Chemistry, Ruska, 56, 46001, Ternopil, Ukraine,
Tel.: +380972392057
E-mail: kuchtynnic@gmail.com
ORCID: http://orcid.org/0000-0002-0195-0767

David Arutiunian, Ternopil Ivan Pului National Technical University, Faculty of Engineering of Machines, Structures and Technologies, Department of Food Biotechnology and Chemistry, Ruska, 56, 46001, Ternopil, Ukraine,
Tel.: +380984639564
E-mail: david46395642020@gmail.com
ORCID: https://orcid.org/0009-0005-9272-0703

Oleg Pokotylo, Ternopil Ivan Pului National Technical University, Faculty of Engineering of Machines, Structures and Technologies, Department of Food Biotechnology and Chemistry, Ruska, 56, 46001, Ternopil, Ukraine,
Tel.: +380972079605
E-mail: pokotylo_oleg@ukr.net
ORCID: http://orcid.org/0000-0001-8693-8240

Khrystyna Kravcheniuk, Ternopil Ivan Pului National Technical University, Faculty of Engineering of Machines, Structures and Technologies, Department of Food Biotechnology and Chemistry, Ruska, 56, 46001, Ternopil, Ukraine,
Tel.: +380978615477
E-mail: kravchenukx30@gmail.com
ORCID: https://orcid.org/0000-0002-7547-6834

Volodymyr Salata, Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj, Faculty of Veterinary Hygiene, Ecology and Law, Department of Veterinary-Sanitary Inspection, Pekarska, 50, 79010, Lviv, Ukraine,
Tel.: +380677288933
E-mail: salatavolod@ukr.net
ORCID: http://orcid.org/0000-0002-7175-493X

***Yulia Horiuk**, Podillia State University, Faculty of Veterinary Medicine and Technologies in Livestock, Department of Veterinary Obstetrics, Internal Pathology and Surgery, Schevchenko, 12, 32301, Kamianets-Podilskyi, Ukraine,
Tel.: +380976617964
E-mail: goruky@ukr.net
ORCID: https://orcid.org/0000-0002-7162-8992

Halyna Karpyk, Ternopil Ivan Pului National Technical University, Faculty of Engineering of Machines, Structures and Technologies, Department of Food Biotechnology and Chemistry, Ruska, 56, 46001, Ternopil, Ukraine,
Tel.: +380685541587
E-mail: galya_karpik@ukr.net
ORCID: https://orcid.org/0000-0002-5374-8368

Diana Dalievska, Ternopil Ivan Pului National Technical University, Faculty of Engineering of Machines, Structures and Technologies, Department of Food Biotechnology and Chemistry, Ruska, 56, 46001, Ternopil, Ukraine,
Tel.: +380972110257
E-mail: dalievska.d@gmail.com
ORCID: http://orcid.org/0000-0003-0578-9971

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 28.1.2024
Revised: 17.2.2024
Accepted: 29.2.2024
Published: 5.3.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 297-312
https://doi.org/10.5219/1959
ISSN: 1337-0960 online
www.potravinarstvo.com

Modelling the centrifugal mixing process of minced meat to optimise the production of chopped meat semi-finished products

Igor Palamarchuk, Mikhailo Mushtruk, Volodymyr Vasyliv, Eugeniy Stefan, Olesia Priss, Iryna Babych, Inna Karpovych, Nataliia Pushanko

ABSTRACT

One of the most important problems in ensuring the quality of mincemeat preparation in the production of sausages is the effective structuring of components and mixing of their ingredients. To solve this problem, researchers added a multifunctional admixture based on whey protein in the process of centrifugal mixing of the components, which determined the composition of the factor space of the investigated process. Based on the results of the research, the effective content of whey protein, sodium alginate, and soy fiber in the developed recipe was proven, which showed high characteristics in terms of fat-retaining and moisture-retaining ability, digestibility, pH level – activity, and other parameters. The developed formulation made it possible to improve the general indicator of the balance of amino acids in the product and increase the functional-technological and quality parameters of the developed products. The physical and mechanical characteristics of the obtained meat product were evaluated based on the results of physical and mathematical modelling. Modelling was carried out using Federman-on-Buckingham's second similarity theory and the "dimension theory" method, which allows the processing of the obtained experimental data in the form of a criterion equation, which was compiled using Froude, Euler, and Sherwood criteria. The purpose of this study was to obtain dependencies between such process factors as product density, the coefficient of dynamic viscosity of the technological medium, the ultimate shear stress, the change in the concentration of the main impurities of lactic acid in the raw material, the value of the diffusion coefficient and the coefficient of mass transfer in the loading mass, the weight of one load of products, the angular frequency of rotation of the screws of the minced meat mixer, the radius of the rotating working bodies, the characteristic size of the products after grinding. Using the complex criterion equation and the developed program, we find a recommended set of operating mode parameters for preparing minced meat under the conditions of centrifugal influence on the mixing process and the action of the specified factors.

Keywords: dimension theory, similarity numbers, whey proteins, centrifugal driving forces, lactic acid admixture, chopped meat products, complex food additives

INTRODUCTION

To improve the nutrition structure, the tendency to create an assortment of products enriched with vitamins, minerals, dietary fibers, and other biologically active substances is becoming increasingly widespread. At the same time, preservation of the necessary taste qualities is achieved due to functional indicators, physical and chemical composition, and structural combination of the main components. The development of the meat industry involves the production of combined meat products due to the mutual enrichment of their structures, a combination of functional and technological properties, an increase in biological value, and an improvement in organoleptic

properties, technical indicators of the finished product, a decrease in its value **[1]**. The generally accepted daily physiological norm of protein for an adult is 80 g on average, including about 50 g of animal protein **[2]**. The shortage of animal proteins contributes to the intensive development of food technologies with the optimal combination of meat and other raw materials to obtain biologically complete food products, which led to the development of the scientific direction of the development and production of meat products of combined composition. Serum proteins albumin and globulin have valuable biological properties, which allow us to assert the optimal set of vital amino acids from the point of view of nutrition physiology **[3]**. Such structures approach the amino acid scale of an "ideal" protein, a protein in which the ratio of amino acids meets the body's needs. Therefore, developing new food products using animal protein, particularly whey protein, allows providing meat products with complete animal protein, which is an urgent task.

The expansion and improvement of the range of chopped meat semi-finished products is traced in the works of Mushtruk et al. **[4]**; the development of meat-containing products by introducing multi-component additives into their composition for public catering establishments takes place in the works of Bal-Prylypko et al. **[5]**. The effective combination of proteins of animal and plant origin was proven by the works of Klymenko and Vinnikov, in particular, when replacing meat raw materials with complex additives to improve indicators of nutritional and biological value with enhanced organoleptic characteristics and a decrease in their cost **[6]**, **[7]**. Pasichny et al., Riabovol and Bal-Prylypko proved that the ratio of vegetable protein, animal, and food additives in the ratio of 45-50: 40-45: 5-15 in the composition of cooked sausage products provides the product with high functional and organoleptic properties **[8]**, **[9]**. Filin et al. and Kutlu et al. based on the study of the partial replacement of meat raw materials with wheat germ and fucus algae proved the enrichment of the food product with biologically active iodine, alginic acids, vitamins, and dietary fibers **[10]**, **[11]**. The positive effect of c on the formation of organoleptic and functional properties of sausage products was confirmed by the works of Sonko et al. **[12]**; in particular, the practical addition of boiled groats is salted in the works of Israelian et al. **[13]**, mashed beans – in the works of Shrana et al. **[14]**.

Research by Pylypchuk et al. revealed a nitrite-reducing bacterial preparation based on denitrifying microorganisms *Staphylococcus carnosus*, *S.cfrnosusssp*. utilize and cooled catholyte with a final pH of 8.86 and a redox potential (ORP) as part of the brine – 215-300 mV contributes to the formation of high organoleptic properties in sausage products and increases the nutritional value **[15]**.

The analysis of the presented and other literary sources revealed the need for systematized data and methods of effectively modelling the quality of chopped semi-finished products with a combined composition of raw materials and insufficient justification of recommendations regarding the use of complex multi-component additives of a given composition.

Thus, evaluating the effectiveness of expanding the range of semi-finished meat products with a multifunctional additive based on raw animal and vegetable materials using physical and mathematical modelling is an urgent task. This study aims to obtain the dependence between the main parameters of the process of forming the structure of chopped meat semi-finished products with a multifunctional admixture based on milk whey protein by applying the necessary experimental basis, which was developed using the similarity theory.

Scientific Hypothesis

The main hypotheses of this scientific work can be considered as follows: when forming the mass of semi-finished meat, the Sherwood criterion was chosen as the primary evaluation criterion because the provision of lactic acid diffusion is paramount; the use of the Froude criterion will allow to assess the increase in the driving force of the process due to the centrifugal forces used; the need to overcome the resistance forces of the technological environment justified the introduction of the Euler number. It is expected that the comprehensive assessment of the presented similarity numbers will allow the creation of a mathematical algorithm for describing the researched process, taking into account the main factors affecting it.

MATERIAL AND METHODOLOGY

Samples

The following were used for conducting experimental research:

• beef bone, in which the muscle tissue with a mass fraction of connective and fatty tissue does not exceed 10%, realtor Agrofirma "Polyssya LTD", Kyiv region, Ukraine to DSTU 6030:2008 **[16]**;
• pork without cysts and semi-fat, in which muscle tissue with a mass fraction of fat tissue from 45% to 80%, realtor Agrofirma "Polyssya LTD", Kyiv region, Ukraine to DSTU 7158:2010 **[17]**;
• multi-component stuffing, which includes lean pork and beef;
• melange according to DSTU 8719:2017 **[18]**;
• onion, realtor Agrofirma "Polyssya LTD", Kyiv region, Ukraine;

• specialty, realtor ATB Market.

Chemicals

Sodium hydroxide, NaOH (grade A, analytical grade, (Khimlaborreakt) Limited Liability Company, Ukraine).

Methyl red, $C_{15}H_{15}N_3O_2$ (grade A, analytical grade, (Khimlaborreakt) Limited Liability Company, Ukraine).

Sulfuric acid, H_2SO_4 (grade A, chemically pure, (Khimlaborreakt) Limited Liability Company, Ukraine).

Petroleum ether, $H_3C\text{-}O\text{-}CH_3$ (excise, analytical grade, (Khimlaborreakt) Limited Liability Company, Ukraine).

Animals, Plants and Biological Materials

The meat of bulls obtained after the slaughter of animals up to 12 months of age and the meat of pigs up to 9 months of age, which came from the agricultural company "Polyssya LTD" in Kyiv region, Ukraine, were used for the research.

The system of proteinases, which consists of pepsin and trypsin, acts on protein substances (LLC "Alex", Kyiv, Ukraine)

Instruments

A mince mixer L5-FM2-M-340 was used to implement the mincemeat preparation process.

Drying cabinet (SNOL, producer (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Muffle furnace (SNOL, producer (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Fat analyzer (SOX 406, producer (Khimlaborreaktyv) Limited Liability Company, China).

Mineralizer (Velp Scientifica, producer (Khimlaborreaktyv) Limited Liability Company, Italy).

Distiller for steam distillation (Velp Scientifica UDK 129 producer (Khimlaborreaktyv) Limited Liability Company, Italy).

Automatic penetrometer (K95500, producer (Khimlaborreaktyv) Limited Liability Company, USA).

pH meter (HI8314 HANNA, producer (Spectro lab) Limited Liability Company, Ukraine).

Thermometer (digital laboratory thermometer TH310 Milwaukee, producer (Spectro lab) Limited Liability Company, Ukraine).

Laboratory scales (AXIS BDM 3, (Spectro lab) Limited Liability Company, Ukraine).

Laboratory Methods

The assessment of the chemical composition of sausage products was conducted following established protocols:

• Moisture content was determined using the drying method specified in DSTU ISO 1442:2005 **[19]**.

• Fat content was analyzed using the Soxhlet method by DSTU 8380:2015 **[20]**.

• Protein proportion was determined through the Kjeldahl method **[21]**.

• Ash content was measured using the Velp Scientifica DK6 device for ash mass fraction determination, employing the weight method as per DSTU ISO 936:2008 **[22]**.

• Active acidity was studied by assessing pH levels according to DSTU ISO 2917:2001 **[23]**.

• Protein mass fraction was determined utilizing the Lowry method with Folin's reagent, resulting in a blue coloration of the protein solution **[24]**.

• Plasticity of minced meat was evaluated through the pressing method based on the area of the meat stain on filter paper.

• Content of the mycotoxin patulin was assessed via liquid chromatography with spectrophotometric detection **[25]**.

• Heavy metal content was determined using the atomic absorption method **[26]**.

• The content of radionuclides was measured using the gamma spectrometric method.

• The temperature of the samples was recorded using a TH310 Milwaukee thermometer.

• Sample weighing was carried out using AXIS BDM 3 scales.

Description of the Experiment

Sample preparation: The preparation of samples for the above studies was carried out by the DSTU 7963:2015 standard **[27]**. Sample selection was carried out by the requirements specified in DSTU 7992:2015 **[28]** and DSTU 8051:2015 **[29]**.

Number of samples analyzed: 8 samples were examined: 4 samples using pork and 4 from beef.

Number of repeated analyses: The study was repeated 5 times, with the experimental data processed using mathematical statistics.

Number of experiment replications: Each study was carried out five times, and the number of samples was three, resulting in fifteen repeated analyses.

Design of the experiment: In the first stage, raw materials were prepared. The meat raw material, after curing, was ground into a sieve with a grid diameter of 2-3 mm, i.e., the characteristic size of the product after grinding is $\ell = 2$ mm. During experimental research, mélange, a food additive, onion, spelled flour, and spices

were used as additional components in the production of chopped semi-finished products. The L5-FM2-M-340 minced meat mixer is used to implement the process of preparing minced meat, in the working capacity of which screws with screw-type blades are mounted, which rotate towards each other.

In the second stage, the resulting mass with admixtures of mélange, onion, breadcrumbs, and spices, with the addition of water, was processed in a minced meat mixer.

The third stage was the laboratory analysis of the obtained substance. The main characteristics of the obtained semi-finished meat were determined, namely, moisture content, protein content, fat content, table salt content, moisture-binding capacity, plasticity of minced meat, digestibility of proteins, and amino acid composition of proteins. The critical stage was the statistical analysis of the obtained experimental data.

Statistical Analysis

The results were evaluated using statistical software Statgraphics Centurion XVII (StatPoint, USA) – multifactor analysis of variance (MANOVA), LSD test. Statistical processing was performed in Microsoft Excel 2016 in combination with XLSTAT. Values were estimated using mean and standard deviations. The reliability of the research results was assessed according to the Student's test at a significance level of $p \leq 0.05$

RESULTS AND DISCUSSION

The main factors of this process are the product density ρ, the coefficient of dynamic viscosity of the technological medium μ, the ultimate shear stress τ, the change in the concentration of the main impurities in the raw material ΔC, the value of the diffusion coefficient D and the mass transfer coefficient in the loading mass β, the weight of one loading of products Pz, angular frequency ω of rotation of the screws of the minced meat mixer, the radius of rotating working bodies r, the characteristic size of products after grinding ℓ.

Modelling was carried out using Federman-Buckingham's second similarity theory and the "theory of dimensions" method, which allows the processing of the obtained experimental data in the form of a criterion equation.

The authors of the scientific manuscripts **[30]**, **[31]** investigated the centrifugal and vibrational technological effects on the hydrolysis of plant raw materials for producing pectin. The researchers found that with pulsed amplification of such a process, it is possible to increase its efficiency and design compact equipment, reduce electricity consumption, and improve the final product quality **[32]**. The authors confirmed the scientific hypothesis according to which vibrocentrifugal intensification of hydrolysis increases the driving force of the process, not only by activating the material flows of raw materials and reagents, due to the reduction of stability in the technological environment, therefore, a similar series of experiments should be repeated in our research **[33]**, **[34]**.

Taking into account the presented factors of the researched process and the peculiarities of its course, it is advisable to use the following criteria of similarity in the calculation: the Froude number Fr, as a measure of the ratio of inertial forces and weight **[35]**; Euler's number Eu, as a measure of the ratio of pressure forces and pressure velocity **[36]**; Sherwood's number Sh, as a measure of the ratio of the intensity of diffusion flows at the boundary of separation of interacting phases **[37]**, **[38]**. At the first stage of the calculation, the parameters of the studied process presented above were decomposed according to the dimensions in Table 1.

Table 1 Initial data for determining the strength characteristics of the researched process of minced meat preparation.

No	τ, Pa	μ, Pa·s	ΔC	t, s	Δh_i, mm	Δτ, Pa	$\frac{\Delta\tau}{\tau}$	$\varrho \frac{kg}{m^3}$
1	300	850	0,04	420	0,5	80	0,26	1100
2	400	1020	0,06	420	0,7	120	0,3	1150
3	500	1100	0,08	420	0,9	200	0,4	1180

The Froude criterion can be represented by the ratio of the acceleration of the force field induced in the process of mixing by centrifugal forces to the acceleration of free fall g:

$$Fr = \frac{a_B}{g} = \frac{r \cdot \omega^2}{g} \quad (1)$$

Where:

the acceleration of the force field $a_B = r \cdot \omega^2$; g is the acceleration of gravity.

The formula can determine Euler's criterion:

$$Eu = \frac{P}{\rho \cdot S \cdot \upsilon^2} = \frac{\tau}{\rho \cdot r^2 \cdot \omega^2} \quad (2)$$

Where:

P is the resistance of the medium, H; S – an area of force contact action, m^2; $\tau = \frac{P}{S}$ – ultimate shear stress, Pa; υ is the speed of movement of the executive bodies of the stuffing mixer: $v = r \cdot \omega$.

The Sherwood criterion *Sh* is classically calculated as:

$$Sh = \beta \cdot \ell / D = \beta \cdot A / D \quad (3)$$

Where:

ℓ – the characteristic size of the product after grinding, which we accept ℓ = 2 mm under the conditions of the studied mass transfer; *D* – the diffusion coefficient, which for minced masses can be taken $D = 0.5 \cdot 10^{-9}$ m^2/s.

The following ratio can determine the mass transfer coefficient for the process under study:

$$\beta = \frac{m}{(t \cdot \Delta C \cdot S)} = \frac{\tau}{(t \cdot \Delta C \cdot g)} \quad (4)$$

Where:

t is the processing time and one product load.

In further studies, we use experimental data for control samples, in particular, ultimate shear stress; minced meat density ϱ$_0$ = 1100-1180 kg/m^3; coefficient of dynamic viscosity; the depth of penetration of the cone into the mass of products according to studies of the penetration process **[39]**, **[40]**.

When the concentration of the main impurities in the raw material ΔC changes, the ultimate shear stress increases significantly, which leads to a significant increase in the density of minced meat ρ. For the specified power characteristics, we assume that Δτ/τ = Δϱ/ϱ = Δμ/μ, where Δτ, Δϱ, Δμ are the growth of the parameters of the mincemeat preparation process, respectively, when the ingredients under study are added to the mincemeat, i.e., for changes in its concentration in the form of ΔC, which is illustrated in Table 1.

Then by the method of linear extrapolation:

$$\rho_i = \rho_0 \left(1 + \frac{\Delta\rho}{\rho_i}\right) \quad (5)$$

Where:

g values Δh_i is the reduction of the immersion depth of the cone according to studies of the penetration process in three product samples.

Given a fairly large number of factors determining the process, let's replace the relationship between them with dependencies between the presented criteria of similarity. For this, we use the matrix of dimensions, which we compile with the help of Table 2.

Table 2 Matrix of dimensions of the studied process of mixing the ingredients of minced sausages.

Options	***ρ***	***D***	***ℓ***	***ω***	***τ***	***g***	***r***	*β*
M, Kg	1				1			1
L, m	-3	2	1		-1	1	1	
T, s		-1		-1	-2	-2		-1
Power coefficients	*n*	*m*	*c*	*h*	*t*	*f*	*j*	

In general, the relationship between the presented parameters can be written in the form of a function: $\beta = f(\rho, D, r, \omega, \tau, \ell, g)$.

Based on the matrix of dimensions compiled in Table 2, we rewrite the presented function in the form of a power series:

$$\beta = K \cdot \rho^{n} \cdot D^{m} \cdot r^{j} \cdot \omega^{h} \cdot \tau^{t} \cdot g^{f} \cdot \ell^{c} \quad (6)$$

Where:
K is a constant coefficient.

For the presented factor space, the number of variables is 6 and the number of dimensionless components is 6 – 3 = 3 according to the π – theorem, that is, it corresponds to the number of selected similarity criteria, in particular, Sherwood, Euler, and Froude numbers. The matrix of dimensions compiled in Table 3 is reproduced in the system of equations for the power coefficients of the mass transfer equations (6):

$$\begin{gathered} n + t = 1 \\ -3n + 2m + c + t + f + j = 0 \\ -m + h - 2t - 2f = -1 \end{gathered} \quad (7)$$

From equations (7) and (8) we obtain the following dependencies:

$$-2n + 2m + c + f + j = 1 \quad (8)$$

From equation (7):

$$t = 1 - n \quad (9)$$

From equation (9):

$$h = 1 - m - 2t - 2f \quad (10)$$

From equation (8):

$$j = 1 - 2n - 2m - c - f \quad (11)$$

From equation (7):

$$2n = 2 - 2t \quad (12)$$

Using the obtained equations (7) – (12), we transform the mass transfer equation (6) of the studied minced meat preparation process sequentially in the following forms.

$$\beta \cdot \ell / D = Sh = (\omega^2 \cdot r/g)^{-2m} \cdot \ell^{(c+1)} \cdot \omega^{(1+3m-2t-2f)} \cdot r^{(1+2n-c-f)} \cdot g^{(3n-c+t-j)} \cdot \rho^{(1-t)} \cdot D^{(m-1)} \cdot \tau^{t} \quad (13)$$

$$Sh = Fr^{-2m} \cdot [\tau/(\rho \cdot \omega^2 \cdot r^2)]^{t} \cdot \omega^{(1+3m-2f)} \cdot \rho \cdot r^{(3-c-f)} \cdot g^{(2n-c-j)} \cdot D^{(m-1)} \cdot \ell^{(c+1)} \quad (14)$$

$$Sh = Eu^{t} \cdot Fr^{(-2m-2f)} \cdot \omega^{(1+3m)} \cdot r^{(3-c)} \cdot g^{(2n-c-j-f)} \cdot D^{(m-1)} \cdot \ell^{(c+1)} \quad (15)$$

Taking into account equations (13-18), we obtain the general expression of the mass transfer equation of the process under study:

$$Sh = K \cdot Eu^{t} \cdot Fr^{(-2m-2f)} \quad (16)$$

$$K = \omega^{(1+3m)} \cdot r^{(3-c)} \cdot g^{(2n-c-j-f)} \cdot D^{(m-1)} \cdot \ell^{(c+1)} \quad (17)$$

To obtain initial data when performing a graph-analytical analysis of the researched process, the values of the similarity criteria presented above, parameters when using experimental data, and the results of the calculations were determined (Table 2). Using the data in Table 4 and the graph-analytical method of studying power functions, a graph of the function Sh = f (Eu) was constructed. This function is linear, the graph of which makes an angle α with the abscissa axis (Figure 1).

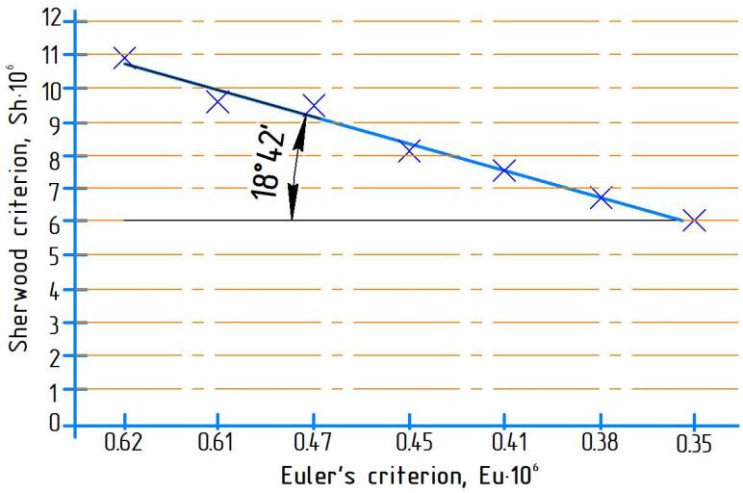


Figure 1 Graph of a linear function *Sh = f (Eu)*.

Then the value of the first power coefficient is:

$$t = tg\ \alpha = 0{,}333 \quad (18)$$

Using the previous method of calculation, we built a function graph using experimental data, found the angle γ (Figure 2) of its inclination to the abscissa axis, and determined the value of the second power coefficient.

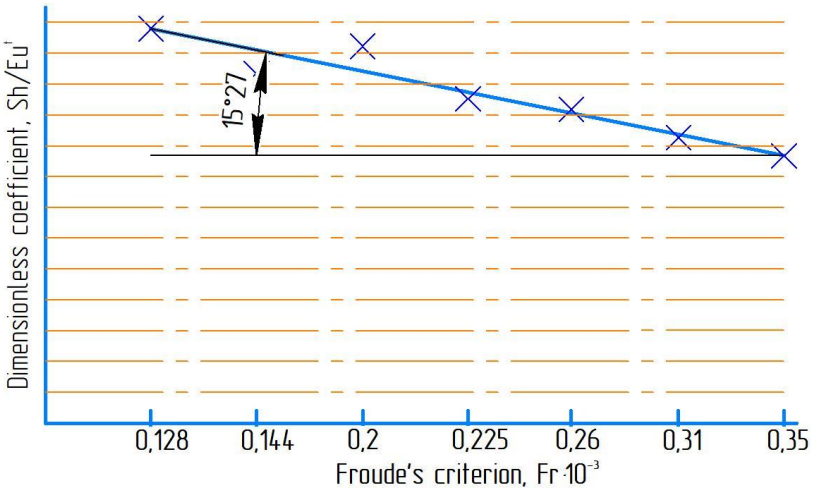


Figure 2 Graphic functions $Sh/Eu^{t} = f(Fr)$.

$$-2m = tg\gamma = 0.237 \text{ та } m = -0.1365 \quad (19)$$

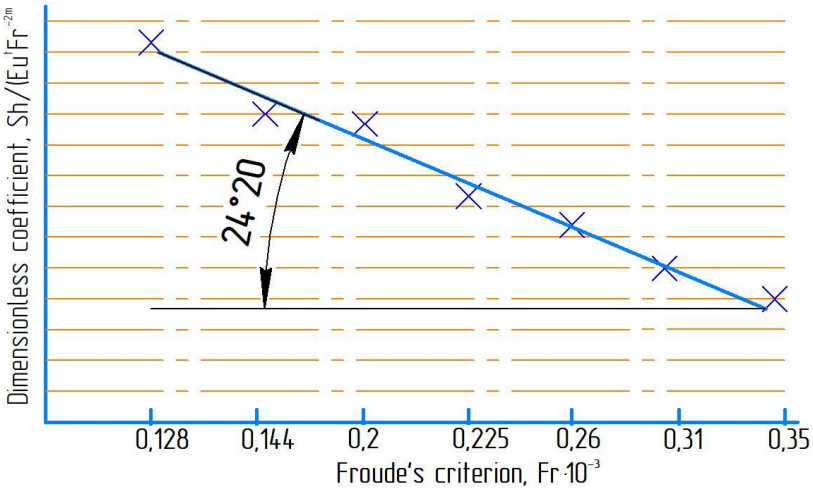


Figure 3 Graphic functions Graphic functions $Sh/(Eu^t \cdot Fr^{-2m}) = f(Fr)$.

Next, we built a graph of the function $Sh/(Eu^t \cdot Fr^{-2m}) = f(Fr)$ (рис. 3), from which the angle of the φ was determined:

$$-2f = tg\varphi = 0.449 \quad (20)$$

Then f = - 0.2245
From the equation (7):
n = 1- t = 0.667
From the equation (9):
h = 1 – m -2t – 2f = 1+ 0.1365 – 0.667 + 0.449 = 0.9195
From the equation (8):
– c - j = -3n + 2m – t + f = - 3·0.667 – 0.273 – 0.333 – 0.2245 = - 2.8315
Next, let's find the necessary dependencies:
1 + 3m = 1 - 3·0.1365 = 0.591
m -1 = - 1.1365
2n – c –j – f = 2·0.667 – 2.883 + 0.2245 = - 1.273
$g^{(2n-c-j-f)} = g^{-1.273} = 1/ 9.81^{1.273} = 0.055$
-2m -2f = 0.273 + 0.449 = 0.772

Taking into account the dependencies (16) and (17), the criterion equation of the studied process of cooking minced meat can be represented as:

$$Sh = 0.055 \cdot Eu^{0,333} \cdot Fr^{0,722} \cdot D^{-1,1365} \cdot \omega^{0,591} \cdot r^{(3-c)} \cdot \ell^{(c+1)} \quad (21)$$

Dependencies $r^{(3-c)} \cdot \ell^{(c+1)}$ can be neglected due to their insignificant impact on the minced meat preparation process under study. Thus, the definitively sought criterion equation of the process of cooking minced meat with given components is:

$$Sh = 0.055 \cdot Eu^{0.333} \cdot Fr^{0.722} \cdot D^{-1.1365} \cdot \omega^{0.591} \quad (22)$$

To improve the quality and functional-technological characteristics of semi-finished meat, in the developed recipe, a combination of such elements as milk whey protein, sodium alginate, and soy fiber was provided, encouraging the synergistic effect of the resulting composition (Table 1).

Table 1 The main components of the food additive to the meat products under investigation.

Product	Content, %			
	Protein	Moisture	Fat	Ash
Soy fiber	25.0	8.0	0.8	6.5
Whey protein	69.5	8.0	6.0	7.2
Sodium Alginate	-	9.0	-	18.0

Based on research, increasing the amount of whey protein and soy fiber in experimental samples, the viscosity of the mixture decreases under the conditions of increasing the speed of rotation of the working capacity. The best characteristics were shown by product samples containing 7.5 g of sodium alginate, 2 g of whey protein, and 3 g of soy fiber per 100 g of product.

According to such evaluation criteria, the fat-retaining and moisture-retaining ability was observed in the test sample, which contained a food additive of 60% sodium alginate, 16% whey protein, and 24% soy fiber. At the same time, the indicators of fat-holding capacity were 1.5 ml of fat/g of product and moisture-holding capacity – 1.32 g of water/g of product.

With a change in the content of food additives from 8 to 16% in the mass of chopped semi-finished meat, the best digestibility indicators were observed, respectively within (72-74) %, which can be explained by the fact that in the presence of pepsin and trypsin, whey proteins quickly and are almost completely digested, while raw meat is digested by trypsin more slowly and incompletely.

The influence of the content of the food additive on changes in the values of fat and water-holding capacity is presented in Table 2. All experimental samples showed sufficiently high indicators of these evaluation criteria, samples 4 and 5 were the best.

Table 2 Indicators of functional and technological properties of a complex food additive.

No	Ingredients of food additives, g (per 100 g of product)			Fat-retaining capacity, ml/g (1 ml of fat per 1 g of product)	Water-holding capacity, g/g (1 g of water per 1 g of product)
	Sodium alginate, g	Whey protein, g	Soy fiber, g		
1	7.5	1	4	1.25 ±0.021	1.11 ±0.016
2	7.5	1.5	3	1.15 ±0.041	1.12 ±0.016
3	7.5	2	2	1.25 ±0.041	1.23 ±0.024
4	7.5	2	3	1.5 ±0.163	1.32 ±0.016
5	7.5	2	4	1.7 ±0.163	1.44 ±0.033

According to the comparative analysis of the values of the active acidity of the mixture as a whole and its components (Table 3), it can be noted that in general the complex admixture is characterized by less than that of the components, except for sodium alginate; and a sufficiently low pH indicator, which justifies the feasibility of using the developed admixture in meat products.

Table 3 Indicators of active acidity for the structural components of the investigated food additive based on animal and vegetable raw materials.

No	Title	Active acidity (pH)
1	Whey protein	6.501 ±0.002
2	Sodium Alginate	6.375 ±0.079
3	Soy fiber	6.93 ±0.065
4	Food additives based on animal and vegetable raw materials	6.462 ±0.066

For conducting experimental studies, the following rate of products (net) was used per 1 kg of meat pulp, g: wheat bread – 250 (25%), milk – 300 (30%), salt – 20 (2%), ground pepper – 1 (0.1%). Cutlets and balls were made from the prepared mass. The cutlet mass was portioned (1-2 pieces per portion), rolled in breadcrumbs, and given an oval-flattened shape up to 2.0 cm thick, 10-12 cm long, and 5 cm wide and fried (Figure 4).

a)

b)

Figure 5 Samples of chopped semi-finished products: a – experimental sample, b – control sample.

The organoleptic valuation of taste and physicochemical properties of tested products showed that all tested parameters of quality of the experimental product surpassed the ones of the control one (Figure 5).

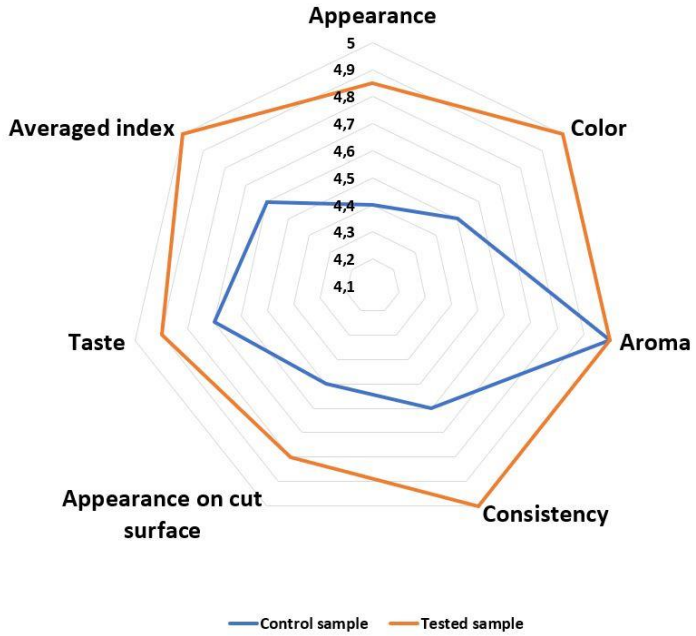


Figure 5 Results of organoleptic valuation of control quality and experimental mincemeat samples.

Several scientific papers discuss modelling processes related to sausage product production. These papers introduce various models, employing up to 10 main parameters in some cases **[41]**. However, some models neglect reference indicators or incorporate only one reference indicator **[42]**. This omission makes it challenging to verify

the reliability of the results obtained. In contrast, the authors of the paper **[43]** conducted a modelling process using only 2 parameters, potentially leading to imprecise results.

Similar studies are outlined in the scientific works of diverse research groups, focusing on the design of technological equipment **[44]**, **[45]**, sausage product production **[46]**, **[47]**, and the development of technological schemes for various food industry products **[48]**. However, these studies often need to pay more attention to time parameters, which can significantly impact the quality indicators of the final products.

In scientific works **[49]**, **[50]**, research was conducted on mixing minced meat, such as traditional mixing, vacuum mixing, and mechanical mixing. The authors claim that compared to the traditional method, which uses mechanical mixing, the centrifugal method is characterized by greater mixing efficiency with less processing time.

The authors of the scientific works **[51]**, **[52]** claim that the process of vacuum mixing in combination with the centrifugal method can be faster and more efficient because it uses the force of rotation to mix the components homogeneously and allows to reduce the effect of oxidation and preserve more natural taste and improve indicators quality of the finished product. However, organoleptic studies of the finished product were not conducted in the above-mentioned robots.

From an economic point of view, considering the costs of energy, time, and materials, the authors **[53]**, **[54]** confirmed the benefits of using the centrifugal method. Reducing processing time leads to lower energy costs, and efficient mixing can reduce raw material waste, contributing to savings.

The authors **[55]** studied the possibility of adapting the centrifugal method for different types of meat, such as chicken, pork, or beef. This can significantly impact the universality of the method and its applicability in various branches of the meat processing industry.

Therefore, the proposed method can be a promising solution for optimising the production process of semi-finished meat products, ensuring improved quality and economic efficiency.

The results obtained from the research can help improve production efficiency and final product quality. Below are the possible directions of research in this area:

Simulation of the process of centrifugal mixing:

- Development of mathematical models that describe the physical processes of centrifugal mixing of minced meat.
- It considers various parameters, such as the speed of the centrifugal device, the size and shape of the centrifugal drum, the composition of the minced meat, and other factors that affect the mixing quality.

Process optimisation:

- Development of optimisation algorithms that allow finding optimal parameters of the centrifugal mixing process.
- Consideration of budget, production volume, and product quality constraints.

Study of the effect of minced meat properties:

- Analysis of the influence of different types of meat, texture, moisture, and other characteristics of minced meat on the mixing process and the quality of the final product.
- Development of recommendations on the optimal selection of the composition of minced meat for a certain type of chopped semi-finished products.

Improvement of equipment:

- Development of new technologies and improvement of centrifugal devices to increase mixing efficiency.
- We are studying the possibility of using automated control systems to control process parameters.

Quality control:

- Development of product quality control methods at each stage of the process, including control of the homogeneity and structure of minced meat.
- I use modern analysis methods like data mining and machine learning to identify anomalies and improve product quality.

Study of the influence of environmental factors:

- Study the influence of temperature, humidity, pressure, and other environmental factors on the mixing process and product quality.
- Development of methods to compensate for the influence of variable conditions on the process. These studies can be helpful for meat cut manufacturers, helping to increase production efficiency, reduce costs, and improve product quality.

CONCLUSION

The presented similarity numbers show both the depth of penetration of the oscillatory effect of the force field inside the product and the impact of the change in the concentration of the projected components in minced meat ΔC on the value of the diffusion coefficient D and the mass transfer coefficient in the loading mass. It should be noted that the ω parameter, which characterizes the action of centrifugal forces during minced meat preparation, significantly impacts the studied process. Using the Composite criterion equation and the developed program, we find the recommended range of operating parameters for the process of cooking minced meat under conditions of centrifugal influence on the mixing process and the action of these factors in the implementation of the developed technology using an additive based on whey protein. When making mincemeat from chopped semi-finished products based on cutlet beef and lean pork, it turned out to be effective to use the researched complex food additive based on animal and vegetable raw materials in the amount of 0.5 to 1.5% in dry form; an increase in the mass fraction of whey protein in the range of (1.32-1.44)% was observed; which improves the general indicator of the balance of amino acids in the product, increases the functional-technological and quality parameters of the developed products. Taking into account the above-mentioned aspects will allow you to get a more complete picture of the effectiveness and advantages of the centrifugal method compared with other techniques for producing semi-finished meat products.

REFERENCES

1. He, J., Evans, N. M., Liu, H., & Shao, S. (2020). A review of research on plant-based meat alternatives: Driving forces, history, manufacturing, and consumer attitudes. In Comprehensive Reviews in Food Science and Food Safety (Vol. 19, Issue 5, pp. 2639–2656). Wiley. https://doi.org/10.1111/1541-4337.12610
2. Finger, A., Dibner, C., & Kramer, A. (2020). Coupled network of the circadian clocks: a driving force of rhythmic physiology. In FEBS Letters (Vol. 594, Issue 17, pp. 2734–2769). Wiley. https://doi.org/10.1002/1873-3468.13898
3. Alagawany, M., Elnesr, S. S., Farag, M. R., Tiwari, R., Yatoo, Mohd. I., Karthik, K., Michalak, I., & Dhama, K. (2020). Nutritional significance of amino acids, vitamins and minerals as nutraceuticals in poultry production and health – a comprehensive review. In Veterinary Quarterly (Vol. 41, Issue 1, pp. 1–29). Informa UK Limited. https://doi.org/10.1080/01652176.2020.1857887
4. Mushtruk, M., Palamarchuk, I., Palamarchuk, V., Gudzenko, M., Slobodyanyuk, N., Zhuravel, D., Petrychenko, I., & Pylypchuk, O. (2023). Mathematical modelling of quality assessment of cooked sausages with the addition of vegetable additives. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 242–255). HACCP Consulting. https://doi.org/10.5219/1845
5. Bal-Prylypko, L., Yancheva, M., Paska, M., Ryabovol, M., Nikolaenko, M., Israelian, V., Pylypchuk, O., Tverezovska, N., Kushnir, Y., & Nazarenko, M. (2022). The study of the intensification of technological parameters of the sausage production process. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 27–41). HACCP Consulting. https://doi.org/10.5219/1712
6. Samoichuk, K., Kiurchev, S., Oleksiienko, V., Palyanichka, N., & Verholantseva, V. (2016). Research into milk homogenization in the pulsation machine with a vibrating rotor. In Eastern-European Journal of Enterprise Technologies (Vol. 6, Issue 11 (84), pp. 16–21). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2016.86974
7. Yong, W., Amin, L., & Dongpo, C. (2019). Status and prospects of nutritional cooking. In Food Quality and Safety (Vol. 3, Issue 3, pp. 137–143). Oxford University Press (OUP). https://doi.org/10.1093/fqsafe/fyz019
8. Pasichnyi, V., Shubina, Y., Svyatnenko, R., & Moroz, O. (2021). The effect of freezing on the characteristics of semifinished products in a dough covering using non-conventional protein-containing raw materials. In Animal Science and Food Technology (Vol. 13, Issue 1, pp. 47–56). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(1).2022.47-56
9. Riabovol, M. V., & Bal-Prylypko, L. (2021). Research and development of a technology for the production of healthy sausages. In Animal Science and Food Technology (Vol. 12, Issue 1, pp. 39–47). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.01.039
10. Filin, S., Bal-Prylypko, L., Nikolaenko, M., Holembovska, N., & Kushnir, Y. (2023). Development of technology for plant-based minced semi-finished products. In Animal Science and Food Technology (Vol. 14, Issue 2, pp. 100-112). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.2.2023.100
11. Kutlu, I., Gulmezoglu, N., & Smoleń, S. (2022). Comparison of Biologically Active Iodine and Potassium Iodide Treatments in Increasing Grain Iodine Content and Quality of Oats. In Journal of Plant Growth Regulation (Vol. 42, Issue 5, pp. 2776–2786). Springer Science and Business Media LLC. https://doi.org/10.1007/s00344-022-10744-9

12. Sonko, N., Sukhenko, V., & Shtonda, O. (2021). Determination of the biological value of minced semi-finished products with a complex food additive by the enzymatic method. In Animal Science and Food Technology (Vol. 12, Issue 1, pp. 48–55). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.01.048
13. Israelian, V., Holembovska, N., & Slobodianiuk, N. (2021). Application of the papain enzyme in meat products technology. In Animal Science and Food Technology (Vol. 12, Issue 3, pp. 60–67). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.03.005
14. Sharan, S., Zanghelini, G., Zotzel, J., Bonerz, D., Aschoff, J., Saint-Eve, A., & Maillard, M. (2020). Fava bean (Vicia faba L.) for food applications: From seed to ingredient processing and its effect on functional properties, anti-nutritional factors, flavor, and color. In Comprehensive Reviews in Food Science and Food Safety (Vol. 20, Issue 1, pp. 401–428). Wiley. https://doi.org/10.1111/1541-4337.12687
15. Pylypchuk, O., Tyshchenko, L., Israelian, V., & Mushtruk, N. (2022). Influence of parameters of marinating meat semi-finished products on the quality of the finished product. In Animal Science and Food Technology (Vol. 13, Issue 2, pp. 44–52). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(2).2022.44-52
16. DSTU 6030:2008. Meat. Beef and veal in carcasses, half carcasses, and quarters. Quality management systems – Requirements.
17. DSTU 7158:2010 Meat. Pork in carcasses and half-carcasses. Quality management systems – Requirements.
18. DSTU 8719:2017 Egg products. Quality management systems – Requirements
19. DSTU ISO 1442:2005 Meat and meat products. Method for determining moisture content (control method). Quality management systems – Requirements.
20. DSTU 8380:2015 Meat and meat products. The method of measuring the mass fraction of fat. Quality management systems – Requirements.
21. DSTU 8063:2015 Dry dairy products. Determination of total protein by the Kjeldahl method. Quality management systems – Requirements.
22. DSTU ISO 936:2008 Meat and meat products. The method of determining the mass fraction of total ash. Quality management systems – Requirements.
23. DSTU ISO 2917:2001. Meat and meat products. Determination of pH (control method). Quality management systems – Requirements.
24. Bal'-Prylypko, L. V., Derevyanko, L. P., Slobodyanyuk, N. M., Starkova, E. R., & Androshchiuk, O. S. (2018). Using the Ampullaria glauca snails' caviar for correction of the effects of the ionizing radiation exposure in a small dose. In Nuclear Physics and Atomic Energy (Vol. 19, Issue 2, pp. 159–165). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/jnpae2018.02.159
25. DSTU 7992:2015 Meat and meat raw materials. Methods of sampling and organoleptic assessment of freshness. Quality management systems – Requirements.
26. Cherednichenko, O., & Bal-Prylypko, L. (2019). Modern condition and development of the specialized enterprises – rape producers. In IOP Conference Series: Earth and Environmental Science (Vol. 315, Issue 2, p. 022018). IOP Publishing. https://doi.org/10.1088/1755-1315/315/2/022018
27. DSTU 7963:2015 Food products. Preparation of samples for microbiological analyses. Quality management systems – Requirements.
28. DSTU 7992:2015 Meat and meat raw materials. Methods of sampling and organoleptic assessment of freshness. Quality management systems – Requirements.
29. DSTU 8051:2015 Food products. Sampling methods for microbiological analyses. Quality management systems – Requirements.
30. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/1711
31. Gavahian, M., Mathad, G. N., Pandiselvam, R., Lin, J., & Sun, D.-W. (2021). Emerging technologies to obtain pectin from food processing by-products: A strategy for enhancing resource efficiency. In Trends in Food Science & Technology (Vol. 115, pp. 42–54). Elsevier BV. https://doi.org/10.1016/j.tifs.2021.06.018
32. Shanina, O., Minchenko, S., Gavrysh, T., Sukhenko, Y., Sukhenko, V., Vasyliv, V., Miedviedieva, N., Mushtruk, M., Stechyshyn, M., & Rozbytska, T. (2020). Substantiation of basic stages of gluten-free steamed bread production and its influence on quality of finished product. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 189–201). HACCP Consulting. https://doi.org/10.5219/1200

33. Palamarchuk, I., Tsurkan, O., Sevin, S., Palamarchuk, V., & Vasyliv, V. (2023). Analysis of energy characteristics of vibration mixing of multicomponent mixtures of agricultural raw materials. In Animal Science and Food Technology (Vol. 14, Issue 1, pp. 69–75). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.1.2023.65

34. Zheplinska, M., Mushtruk, M., Vasyliv, V., Slobodyanyuk, N., & Boyko, Y. (2021). The Main Parameters of the Physalis Convection Drying Process. In Lecture Notes in Mechanical Engineering (pp. 306–315). Springer International Publishing. https://doi.org/10.1007/978-3-030-77823-1_31

35. Avezmurodovich, Q. R. (2021). Psychological aspects of left-handedness: Concept, causes, and peculiarities. In Academicia: An International Multidisciplinary Research Journal (Vol. 11, Issue 1, pp. 623–631). Diva Enterprises Private Limited. https://doi.org/10.5958/2249-7137.2021.00094.x

36. Liu, Y., Zhang, J., Wei, J., & Liu, X. (2020). The optimum structure of a laval nozzle for an abrasive air jet is based on nozzle pressure ratio. In Powder Technology (Vol. 364, pp. 343–362). Elsevier BV. https://doi.org/10.1016/j.powtec.2020.01.086

37. Palamarchuk, I., Palamarchuk, V., Mushtruk, M., Shtefan, E., & Petrychenko, I. (2023). Using the Similarity Theory in Designing Vibroconveyor Dryer for Grain. In Advanced Manufacturing Processes V (pp. 550–560). Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-42778-7_51

38. Filin, S., Bal-Prylypko, L., Nikolaenko, M., Holembovska, N., & Kushnir, Y. (2023). Development of technology for plant-based minced semi-finished products. In Animal Science and Food Technology (Vol. 14, Issue 2, pp. 100–112). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.2.2023.100

39. Shtonda, O., Kulyk, V., & Semeniuk, K. (2023). Application of beekeeping products in the technology of raw smoked sausages. In Animal Science and Food Technology (Vol. 14, Issue 3, pp. 86–98). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.3.2023.86

40. Zheplinska, M., Mushtruk, M., Vasyliv, V., Kuts, A., Slobodyanyuk, N., Bal-Prylypko, L., Nikolaenko, M., Kokhan, O., Reznichenko, Y., & Salavor, O. (2021). The micronutrient profile of medicinal plant extracts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 528–535). HACCP Consulting. https://doi.org/10.5219/1553

41. Riabovol, M. (2022). Mathematical modelling of the process of vibration mixing of minced sausage. In Animal Science and Food Technology (Vol. 13, Issue 2). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(2).2022.53-62

42. Austrich-Comas, A., Jofré, A., Gou, P., & Bover-Cid, S. (2023). Assessing the Impact of Different Technological Strategies on the Fate of Salmonella in Chicken Dry-Fermented Sausages Using Challenge Testing and Predictive Models. In Microorganisms (Vol. 11, Issue 2, p. 432). MDPI AG. https://doi.org/10.3390/microorganisms11020432

43. Bonilauri, P., Merialdi, G., Ramini, M., Bardasi, L., Taddei, R., Grisenti, M. S., Daminelli, P., Cosciani-Cunico, E., Dalzini, E., Frustoli, M. A., Giacometti, F., Tomasello, F., Piva, S., & Serraino, A. (2021). Modelling the behavior of Listeria innocua in Italian salami during the production and high-pressure validation of processes for exportation to the U.S. In Meat Science (Vol. 172, p. 108315). Elsevier BV. https://doi.org/10.1016/j.meatsci.2020.108315

44. Bal-Prylypko, L., Nikolaenko, M., Stepasyuk, L., Cherednichenko, O., & Lialyk, A. (2022). Forecasting the selling price of pork in agricultural enterprises. In Agricultural and Resource Economics: International Scientific E-Journal (Vol. 8, Issue 4, pp. 170–187). Institute of Eastern European Research and Consulting. https://doi.org/10.51599/are.2022.08.04.08

45. Palamarchuk, I., Kiurchev, S., Kiurcheva, L., & Verkholantseva, V. (2019). Analysis of Main Process Characteristics of Infrared Drying in the Moving Layer of Grain Produce. In Modern Development Paths of Agricultural Production (pp. 317–322). Springer International Publishing. https://doi.org/10.1007/978-3-030-14918-5_33

46. Delgado, J., Rondán, J. J., Núñez, F., & Rodríguez, A. (2021). Influence of an industrial dry-fermented sausage processing on ochratoxin A production by Penicillium nordicum. In International Journal of Food Microbiology (Vol. 339, p. 109016). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2020.109016

47. Schopfer, B., Mitrenga, S., Boulaaba, A., Roolfs, K., Plötz, M., & Becker, A. (2022). Red beet and Swiss chard juice extract as natural nitrate sources for the production of alternatively-cured emulsion-type sausages. In Meat Science (Vol. 188, p. 108780). Elsevier BV. https://doi.org/10.1016/j.meatsci.2022.108780

48. Mushtruk, M., Bal-Prylypko, L., Slobodyanyuk, N., Boyko, Y., & Nikolaienko, M. (2022). Design of Reactors with Mechanical Mixers in Biodiesel Production. In Lecture Notes in Mechanical Engineering (pp. 197–207). Springer International Publishing. https://doi.org/10.1007/978-3-031-06044-1_19

49. Witte, F., Sawas, E., Berger, L., Gibis, M., Weiss, J., Röser, A., Upmann, M., Joeres, E., Juadjur, A., Bindrich, U., Heinz, V., & Terjung, N. (2022). Influence of Finely Chopped Meat Addition on Quality Parameters of Minced Meat. In Applied Sciences (Vol. 12, Issue 20, p. 10590). MDPI AG. https://doi.org/10.3390/app12201059

50. Palamarchuk, I., Palamarchuk, V., Paziuk, V., Hulevych, R., Kalizhanova, A., & Sarsembayev, M. (2023). Analysis of power and energy parameters of the conveyor infrared dryer of oil-containing raw materials. In Informatyka, Automatyka, Pomiary w Gospodarce i Ochronie Środowiska (Vol. 13, Issue 2, pp. 10–14). Politechnika Lubelska. https://doi.org/10.35784/iapgos.3487

51. Vasyliv, V., Mushtruk, M., Zheplinska, M., Mukoid, R., & Tkachenko, S. (2021). Method of Electrohydraulic Activation of Water-Lime Suspension in Sugar Production. In Lecture Notes in Mechanical Engineering (pp. 664–673). Springer International Publishing. https://doi.org/10.1007/978-3-030-91327-4_64

52. Liao, C., & Yu, Y. (2020). An innovative method of immersion vacuum cooling for cooked meat products: immersion vacuum cooling with ultrasonic assistance. In International Journal of Food Science & Technology (Vol. 55, Issue 7, pp. 2771–2782). Wiley. https://doi.org/10.1111/ijfs.14530

53. Naumenko, O., Hetman, I., Chyzh, V., Gunko, S., Bal-Prylypko, L., Bilko, M., Tsentylo, L., Lialyk, A., Ivanytska, A., & Liashenko, S. (2023). Improving the quality of wheat bread by enriching teff flour. In Eastern-European Journal of Enterprise Technologies (Vol. 3, Issue 11 (123), pp. 33–41). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2023.279286

54. Fang, K., Tang, H., Li, C., Su, X., An, P., & Sun, S. (2023). Centrifuge modelling of landslides and landslide hazard mitigation: A review. In Geoscience Frontiers (Vol. 14, Issue 1, p. 101493). Elsevier BV. https://doi.org/10.1016/j.gsf.2022.101493

55. Kostelac, D., Gerić, M., Gajski, G., & Frece, J. (2022). Probiotic bacteria isolated from fermented meat display high antioxidant and anti-inflammatory potential. In Mutagenesis (Vol. 38, Issue 1, pp. 58–63). Oxford University Press (OUP). https://doi.org/10.1093/mutage/geac023

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Dr. for I. Palamdrchuk

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Igor Palamarchuk, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Processes and Equipment for Processing of Agricultural Production, Heroes of Defense Str., 12 B, 03040, Kyiv, Ukraine,
E-mail: vibroprocessing@gmail.com
ORCID: https://orcid.org/0000-0002-0441-6586

***Mikhailo Mushtruk**, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Processes and Equipment for Processing of Agricultural Production, Heroes of Defense Str., 12 B, 03040, Kyiv, Ukraine,
E-mail: mixej.1984@ukr.net
ORCID: https://orcid.org/0000-0002-3646-1226

Volodymyr Vasyliv, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Processes and Equipment for Processing of Agricultural Production, Heroes of Defense Str., 12 B, 03040, Kyiv, Ukraine,
E-mail: vasiliv-vp@ukr.net
ORCID: https://orcid.org/ 0000-0002-2109-0522

Eugeniy Stefan, National Technical University of Ukraine, Department of Reprography, Peremohy Ave., 37, 03056, Kyiv, Ukraine,
E-mail: eshtefan@ukr.net

ORCID: https://orcid.org/0000-0002-0697-7651

Olesia Priss, Tavria State Agrotechnological University, Faculty of agricultural technology and ecology, Department of Food Technology and Hotel and Restaurant Business, B. Khmelnitsky ave., 18, 72312, Melitopol, Ukraine,
E-mail: olesia.priss@tsatu.edu.ua
ORCID: https://orcid.org/0000-0002-6395-4202

Iryna Babych, National University of Food Technologies, Faculty name, Educational and Scientific Institute of Food Technology, Department of Biotechnology of fermentative products and wine-making, Volodymyrska Str., 68, 01601, Kyiv, Ukraine,
E-mail: 5613694@ukr.net
ORCID: https://orcid.org/0000-0002-3058-3062

Inna Karpovych, National University of Food Technologies, Department of Sugar Technology and Water Preparation, Volodymyrska Str., 68, 01601, Kyiv, Ukraine,
E-mail: inkarp@ukr.net
ORCID: https://orcid.org/0000-0002-5912-7466

Nataliia Pushanko, National University of Food Technologies, Department of Sugar Technology and Water Preparation, Volodymyrska Str., 68, 01601, Kyiv, Ukraine,
E-mail: oks-n@ukr.net
ORCID: https://orcid.org/0000-0001-7369-2827

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 28.1.2024
Revised: 21.2.2024
Accepted: 1.3.2024
Published: 5.3.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 313-330
https://doi.org/10.5219/1960
ISSN: 1337-0960 online
www.potravinarstvo.com

Influence of starter cultures on microbiological and physical-chemical parameters of dry-cured products

Larysa Bal-Prylypko, Svitlana Danylenko, Olena Mykhailova, Liana Nedorizanyuk, Alla Bovkun, Nataliia Slobodyanyuk, Alina Omelian, Anastasiia Ivaniuta

ABSTRACT

Using the antagonistic competitive interaction of the microbiological cultures has become one of the potential and modern ways to improve the quality of dry-cured meat products. These studies aim to substantiate the use of the starter cultures for producing fermented pork meat products. The studies' physicochemical, microbiological, organoleptic, and statistical methods were used for their implementation. Two starter cultures were used on the basis of *Lactobacillus plantarum*, *L. rhamnosus* and *Kocuria rosea* (SC 1); *Staphylococcus carnosus*, *L. plantarum*, *L. Rhamnosus* and *L. paracasei* (SC 2). The dynamics of microbiota development, the dynamics of acidity, the content of sodium nitrite, parameters of water activity, the dynamics of moisture changes, the content of nitrogen-containing substances, the accumulation of free cyclic and acyclic amino acids, as well as the organoleptic characteristics were studied. It had been established that the undesirable microflora is suppressed due to the active development of the starter cultures in fermented meat products. The study results confirm that using the starter cultures while producing the dry-cured meat contributes to their dehydration and reduction of the parameters of water activity. Based on the tasting results, the samples with the addition of the compositions of the starter cultures received a total score higher than the control sample, in particular, they had a beautiful appearance, a cut of red color, an elastic consistency, a delectable flavor with a characteristic sour after-taste. The use of the starter culture based on the combination of *Staphylococcus carnosus, L. plantarum, L. rhamnosus,* and *L. paracasei* (SC 2) for the dry-cured meat products to be produced activates the microbiological processes, which improves the structural-mechanical properties of the meat product, increases the water-binding power and, as a whole, positively influences on the quality of the finished product. It has been shown that the use of the drugs SC 2 and SC 1, when the dry-cured pork balyks are produced, decreases the duration of the technological cycle by 3-4 days, as well as ensures a high degree of sanitary and epidemic safety of the finished product: the absence of pathogenic and opportunistic microorganisms, the low residual content of sodium nitrite of up to 0.003%.

Keywords: fermented meat products, starter cultures, lactic bacteria, staphylococci, product quality

INTRODUCTION

Fermentation is a technology, which is used to increase the safety and nutritional value of food products, reduce production time, extend their shelf life, as well as create unique food tastes. Fermented meat is a typical and popular traditional meat product **[1]**. One of the modern ways for solving the technology development problem of meat products is related to the biotechnological modification principle of the meat raw materials and is aimed at regulating the biotechnological, physical-chemical, and microbiological processes, as a result of which the

structure, color and taste-aromatic characteristics of the finished product are formed **[2]**. The primary function of the starter cultures is the acidulation process by converting sugars, which were added to the meat mixture, into an acid. The acid, which is produced during this fermentation (primarily lactic acid), contributes to the spiciness (sour taste) of the product and contributes to the release of water as the pH value is decreased to the isoelectric point of the meat proteins for desired final water activity (a_w) to be obtained, protects against food-borne pathogens or enterotoxin production, contributes to the final texture by modifying the meat proteins, as well as has a part in fixing the red color of the meat. The acid-producing starter cultures, which are commonly used, belong to the genera *Pediococcus* and *Lactobacillus* **[3]**. Only those species that are relatively stable to salt while fermenting the meat are used **[4]**. The role of lactic acid bacteria in the formation of nitrosamine and their indirect or direct influence on the reduction of nitrosamines due to inhibiting development of bacteria, which are produced by precursors such as biogenic amines, has been discussed in the paper of Sallan et al. **[5]**.

The behaviour of combined starter cultures based on *Penicillium chrysogenum, Penicillium digitatum, Penicillium Sangiovese, Debaryomyces hansenii, Lactobacillus plantarum, Lactobacillus acidophilus, Pediococcus pentosaceus, Micrococcus varians* and *L. plantarum, L. acidophilus, P. rentosaceus, M. varians* and their influence on the microbiological and physicochemical characteristics of the cured ham had been evaluated by Toledano, A.M. et al. It has been proven that using the selected starter cultures did not influence the cured ham's main characteristics and increased the non-protein nitrogen content **[6]**.

Staphylococcus xylosus, Staphylococcus equorum, Staphylococcus saprophyticus, and *Staphylococcus carnosus* are the most commonly used among catalase-negative staphylococci **[7]**. Usually, *Micrococcaceae* and staphylococci fulfill the denitrifying and aroma-producing function, as well as have a slight influence on the pH value. For these reasons, it is advisable to use them together with acid-producing microorganisms – lactic acid bacteria [8].

Having studied the functioning of compositions made of *L. sakei, S. equorum, and S. success* in the production process, Montel, M.-C. et al. concluded that the use of autochthonous "starter" cultures contributes not only to an increase in hygienic safety but also guarantees to obtain products with traditional aroma and taste **[9]**. This was recorded during the production of "Napoli" salami with the use of cultures *S.* xylosus and *L. curvatus* **[10]**, Turkish dry-cured sausage "Sujuk" – with strains of *L. plantarum* GM 77 and *S. xylosu*s GM 92 **[11]**, as well as during spontaneous fermentation of Chinese Sichuan sausages **[12]**.

While ripening, the protein components of the meat raw materials undergo great changes: the activity of the tissue enzymes of the meat and microorganisms is activated, destroying the cellular structure of the muscle tissue and proteolysis, alongside the content of free amino acids can double **[13]**. Irish researchers observed a higher proteolysis degree in salami made with the composition of cultures *L. sakei* LAD and *S. carnosus* MC compared to the control sample. Thus, the total content of free amino acids in the finished product was 1292 mg per 100 g of dry matter, which is 1.7 times more than in the control sample; accordingly, more intensive hydrolysis of the myofibrillar proteins, especially myosin and actin, was occurred **[14]**. Japanese scientists, having investigated the functioning of commercial bacterial compositions in fermented sausages, had noticed a higher proteolysis degree in the variants made with cultures *L. sakei S. carnosus P. pentosaceus,* and *S. xylosus*. All samples were similar in the content of free amino acids at the beginning of the fermentation. Still, the finished products differed significantly due to the starter strains' specific properties **[15]**.

Staphylococci are critical for product lipid oxidation to be prevented due to the production of superoxide dismutase **[16]**, a lesser activity was found in *L. sake* **[17]**.

Peptidase activity is the most developed in micrococci, especially in strains *M. ristinae* and *M. varians*, however, strains *S. xylosus* and *S. carnosus* are the pronounced producers of aroma precursors, in particular, 3-methyl butanol, and from representatives of lactic-acid bacteria – *L. casei* and *L. plantarum* **[18]**.

The species and qualitative composition of commercial bacterial drugs are quite diversified and depend on the technological direction **[19]**. Well-matched cultures have a multifunctional action and can provide the accumulation of the required amount of lactic acid in a short period, intensify the formation process of carbonyl compounds and volatile fatty acids, reduce the proportion of residual sodium nitrite, and, most importantly, prevent the development of foreign microflora, in particular, pathogenic and sanitary-indicative ones **[20]**.

Sausage flavor is made due to salt, spices, and acid, formed by fermentation, various secondary fermentation metabolites of carbohydrates, and by-products of proteolytic and lipolytic activity of the starter culture and additional flora. Lactic-acid bacteria, used as starter cultures, have relatively weak proteolytic and lipolytic/osteolytic activity, while micrococci and staphylococci have more pronounced proteolytic and lipolytic enzymes. Peptides and amino acids, obtained due to the metabolism of lactic acid bacteria, can be further converted into aromatic carbonyls, alcohols, and esters by micrococci and staphylococci. This pool of aroma-contributing components is complemented by the proteolytic and lipolytic activity of micrococci and/or staphylococci **[21]**.

Nitrite-reducing microorganisms *Micrococcus*, which are used in the composition of the bacterial drug "Lakmik," provide a bright coloring of the product and guarantee a low residual content of sodium nitrite up to 0.003%. It has been proven that the sausage, which is produced with the use of this bacterial drug based on *L. casei*, *L. rhamnosus*, *L. plantarum*, *and M. variant,* have a high content of free amino acids and flavour-aromatic compounds **[22]**.

It has been proven by Burtseva G.V. et al. that the microbiota of the bacterial drug "MKS" based on *L. rhamnosus*, *L. plantarum*, and *S. simulans*, which are adapted to be developed in various types of meat raw materials, contribute to the reduction of the ripening period of the dry-cured whole-muscle products by 3-4 days, provide the formation of the traditional organoleptic characteristics, as well as guarantees a low content of residual nitrites. The strains of the above-mentioned compositions perform several metabolites, which can suppress the development of the undesirable microbiota (lactic acid, ammonia, volatile acids, ethers, alcohol, acetone, diacetyl) and specific antibacterial substances – bacteriocins **[23]**.

It has been proven **[24]**, **[25]** that the typical taste of the cured product is due to the enzymatic activity of the microbiota of the starter cultures. Product safety is also preserved due to the suppression of spoilage microorganisms, and pathogenic and opportunistic pathogen microorganisms, improving sensory qualities and stability during storage.

All these prerequisites arouse interest in testing the starter cultures based on LAB and CNC. Therefore, this scientific work aims to check the behavior and dynamics of the selected combined starter cultures and their influence on the characteristic parameters of fermented meat during its production.

Scientific Hypothesis

Microsoft Excel editor processed experimental data using mathematical statistics methods. The accuracy of the obtained experimental data was determined using the Student'st-test with confidence coefficient $p \leq 0.05$ with many parallel definitions of at least 5 (confidence probability $p = 0.95$). Linear programming problems were solved using the MS Excel table processor's'Search for a solution setting (Excel Solver).

MATERIAL AND METHODOLOGY

Samples

The study was conducted with three samples:
- control sample obtained by classical technology;
- experimental sample with starter culture SC 1;
- experimental sample with starter culture SC 2.

Chemicals

Sodium chloride, NaCl (TOV Khimlaborreaktiv, Ukraine).
Distilled water, H_2O (TOV Novokhim, Ukraine).
Selective medium (Oxoid, Basingstoke, UK).
Hydrochloric acid, hcl (TOV Khimlaborreaktiv, Ukraine).
Agar de Man-Rogosa-Sharpa, (Conda, Ukraine).
Mannitol-salt agar (himedia Laboratories Pvt. Ltd.).
Egg yolk emulsion (himedia Laboratories Pvt. Ltd.).
Agar Saburo ("Pharmaktiv" LLC, Ukraine).
Chloramphenicol ("Farmaktiv" LLC, Ukraine).
The Slanets-Bartli environment (Conda, Ukraine).
Bile-glucose agar (himedia Laboratories Pvt. Ltd.).
Pseudomonas Agar Base with the addition of C-F-C (himedia Laboratories Pvt. Ltd.).
Tryptone soy agar (Merck, Germany).
Sulfadiazine agar (Merck, Germany).
N-(1-naphthyl)-ethylenediamine-dihydrochloride (TOV Khimlaborreaktiv, Ukraine).
Concentrated hydrochloric acid H_2SO_4 (Shostka Chemical Reagents Plant, Ukraine).
Trichloroacetic acid ccl_3cooh (TOV Khimlaborreaktiv, Ukraine).
Chemically pure reagents were used.

Animals, Plants and Biological Materials

Bacterial preparation 1: (Institute of Food Resources NAAS of Ukraine, Ukraine) containing *Lactobacillus rhamnosus, L. plantarum, and Kocuria rosea.* In 1 g, the number of viable lactic acid bacteria and micrococci was 3.5×10^{10} CFU/g and 2.9×10^{8} CFU/g, respectively Bacterial preparation.

Bacterial preparation 2: (Institute of Food Resources NAAS of Ukraine, Ukraine) containing *Staphylococcus carnosus, L. plantarum, L. rhamnosus, L. paracasei* in 1 g, the number of viable lactic acid bacteria and

staphylococci was 5.1×10^{10} CFU/g and 3.3×10^{8} CFU/g back muscle *Longissimus dorsi* of the Great White pig (SE "DG Stepne", Institute of Pig Breeding and Agro-Industrial Production of the National Academy of Sciences, Poltava Region)

Bacterial preparations are manufactured under industrial conditions of the Institute of Food Resources of the National Academy of Sciences of Ukraine in accordance with TU U 15.5-00419880-101-2010. Bacterial preparations for the production of fermented meat products. Specifications.

Instruments

Lab Blender Stomacher (Seward Medical, London, United Kingdom)
Ph meter MP 512 ("Ulab").
Petri dishes (LLC Ukragrotest).
Thermostat TSO-80 (LLC Ukragrotest).
Gas Chromatograph (GE Lifesciences BPG 100/500, Germany).
Drying Scarf SNOL 60/300 LSN 11(Lithuania).
Unico S 2100 spectrophotometer (LLC Ukragrotest).
Analytical balances (Thermoengineering LLC, Ukraine).
Kjeldahl apparatus UDK 149 Velp Scientifica™.
AquaLab 3TE Series (США).
LC-2000 Biotronic (Germany).
"SANS" of the SMT 2000 series, model 2503 (Shenzhen SANS Testing Co. Ltd.) with Warner-Blatzler nozzle.

Laboratory Methods

The following microbiological parameters were determined:

- the amount of *Staphylococcus carnosus* CFU in 1 g of the product – on salt broth with mannitol at 30 ±1 °C for 72 ±2 hours **[26]**;
- the number of CFU lactic acid bacteria in 1 g of product on de Man, Rogoza, and Sharp (MRS) agar (Oxoid CM359) at 30 °C for 72 ±2 hours;
- the total number of microorganisms, CFU in 1 g of product – on tryptone-soy agar during incubation at a temperature of 30 °C for 72 hours;
- the number of lactic acid bacteria – on MRS-agar during incubation at a temperature of 30 °C for 5 days **[27]**;
- the amount of mold and yeast, CFU in 1 g of product – on Sabouraud's medium with 200 mg/l chloramphenicol during incubation at 24 °C for 5 days **[28]**;
- the total number of *Enterobacteriaceae* was checked on violet-red bile-glucose agar and incubated at 35-37 °C for 24 hours. Results were expressed as log CFU/g;
- to detect *Salmonella and L. monocytogenes*, 25 g of each sample was aseptically taken. DSTU ISO 6579-1:2017 method **[29]** was used to determine *Salmonella*. In the case of L. monocytogenes, the DSTU EN ISO 11290-2:2017 method was used **[30]**;
- *Clostridium spp.* was counted on sulfite polymyxin-sulfadiazine agar at 45 °C for 48 hours under anaerobic conditions. Results were expressed as log CFU/g;
- fifty grams of each sample was taken and crushed to obtain a homogeneous sample and placed in clean and dry containers at 4 °C until analysis. Non-protein nitrogen (NPN) was analyzed using the procedure described by Bandeira et al. **[31]**;
- the mass fraction of moisture was determined gravimetrically by drying a weight of the product in a drying cabinet at a temperature of 105 °C to a constant mass **[32]**;
- the content of sodium nitrite was determined by the reaction with N-1-naphthyl ethylenediamine dihydrochloride in an acidic environment with the formation of diazo compounds, the color intensity of which was measured photometrically. the mass fraction of protein – by the content of total nitrogen by the Kjeldahl method with the subsequent distillation of ammonia **[33]**;
- the water activity (aw) of the meat samples was measured using an Aqua Lab Series 3 TE METER Group Inc. USA according to ISO 21807:2004 **[34]**;
- the pH value was measured with an MP 512 pH meter ("Ulab", China) after mixing 10 g of the sample with 90 ml of distilled water ISO 2917:1999 **[35]**;
- the amino acid composition of proteins was studied after hydrolysis of product samples with a mixture of 6 n. hydrochloric and 4% thioglycolic acids at temperatures of 105-110 °C for 48 hours in a CO_2 environment and subsequent evaporation under vacuum at a temperature of 45 °C. Identification of amino acids was performed after grinding the samples, removing fat, and precipitation of protein compounds with 10% trichloroacetic acid **[36]**. For the identification of free amino acids, computer chromatographic processing using the Kodak Digital Science ID software package was used;

‣ structural and mechanical studies were carried out on the universal mechanical test machine "SANS" of the CMT series using special nozzles: Warner-Bretzler for determining the shear force. Calculation of indicators was carried out using the Power Test_DOOE software;
‣the organoleptic evaluation of fermented meat products was carried out by a trained group of nine experts, according to **[37]**. Previously, the samples were kept at 25 ±1 °C in the laboratory. Samples were evaluated by participants in random order.

Description of the Experiment

Sample preparation: The pig skinned-off back muscle *Longissimus dorsi* with a layer of fat no more than 0.5 cm thick, no more than 45 cm long, and about 10 cm wide was used for the balyk to be produced.
3 samples were produced: a control sample – without bacterial drug and experimental samples with starter cultures – SC 1, SC 2. 3 samples were produced: control – without tank preparation and experimental samples with starter cultures - SK 1, SK 2. Starter cultures were added to the brine of the experimental samples in the amount of 0.05% (to the mass of raw materials). Water in the amount of 0.05% (to the mass of raw materials) was added to the control sample instead of starter cultures. From each batch replication, the samples were taken for further analysis right after the salting, after the salting process, and after the ripening and drying in 6 or 9 days.
The starter cultures were added to the brine of experimental samples at 0.05% (to the mass of raw meat materials).
The technological parameters of the balyk production are as follows:
– injection of the brine into the meat in the amount of 30% of its weight;
- massaging of the meat for 4 hours;
– brine aging of the meat at a temperature of 5-10 °C for 72 hours;
– incomplete drying of the meat at a temperature of 18-20 °C for 1 hour, φ = 55%;
– smoking of the meat at a temperature of 40 °C for 1 hour;
– incomplete drying of the meat at a temperature of 18-20 °C for 1 hour, φ = 55%;
– drying of the products in a climate chamber according to the following modes:
the first day: t = 20-22 °C, φ = 88%;
the second day: t = 18-20 °C, φ = 84%;
the third day: t = 16-18 °C, φ = 80%;
the fourth day: t = 14-16 °C, φ = 78%;
the fifth day: t = 12-14 °C, φ = 76%;
from the sixth to the ninth day: t = 11 ±1 °C, φ = 76-74%.

Number of samples analyzed: 54.
Number of repeated analyses: 3.
Number of experiment replication: 3.

Description of the Experiment

Two starter cultures were used for the studies, namely: (starter culture 1) *Lactobacillus plantarum*, *L. rhamnosus,* and *Kocuria rosea* (SC 1) and (starter culture 2) *Staphylococcus carnosus, L. plantarum, L. rhamnosus, L. paracasei* (SC 2). All strains were recovered from domestic dry-cured and raw-smoked meat products produced according to traditional technology. In the products, these strains belonged to the dominant bacteria. The number of viable lactic acid bacteria and micrococci was 3.5×10^{10} CFU/g and 2.9×10^{8} CFU/g, respectively, per 1 g of the starter culture 1. The number of viable lactic acid bacteria and staphylococci was 5.1×10^{10} CFU/g and 3.3×10^{8} CFU/g, respectively, per 1 g of the starter culture 2.
According to traditional technology, three different batches of the balyk were produced in triplicate. These batches were developed according to the added starter culture: C batch (control sample without any starter culture), SC 1 batch (inoculated with *Lactobacillus rhamnosus*, *L. plantarum,* and *Kocuria rosea*), and SC 2 batch (S*taphylococcus carnosus*, *L. plantarum*, *L. rhamnosus*, *L. paracasei*).

After the aseptic skinning, an approximately 10-g piece of the sample was diluted with 90 mL of 0.9% (w/v) sterile NaCl solution in the stomach bag and homogenized in a Lab Blender Stomacher (Seward Medical, London, UK) for 2 min. Decimal dilutions were subsequently prepared and plated on a selective medium (Oxoid, Basingstoke, UK) for specific microbial counting: LAB was counted on de Man–Rogosa–Sharpe agar, and CNC was counted on mannitol-salt agar with added egg yolk emulsion after an incubation at 30 °C for 48 hours; yeast on Sabouraud dextrose agar with 200 mg/l chloramphenicol, after incubation at 30 °C for 72 hours; enterococci were incubated on Slanetz and Bartley medium at 42 °C for 24 hours; *Enterobacteriaceae* on violet-red bile-glucose agar were incubated at 37 °C for 24 hours; *Pseudomonas* were controlled based on Pseudomonas Agar Base with added C-F-C additives and incubated at 30 °C for 48 hours. The analyses were carried out in triplicate at the initial stage (0 days), as well as after 4 and 30 days.

All strains were isolated from domestic raw-cured and raw-smoked meat products produced according to traditional technology. In these products, these strains were among the dominant bacteria. The basis of bacterial preparations for the fermentation of meat raw materials is technologically promising strains that have high productivity and nitrite-reducing activity, possess antagonism towards pathogenic and opportunistic microorganisms and form a significant amount of aromatic compounds.

Physico-chemical studies: Fifty grams of each sample were taken, ground until smooth, and placed in clean, dry containers at 4 °C until the analysis was carried out. Non-protein nitrogen (NPN) was analyzed using the procedure described. The mass fraction of moisture was gravimetrically determined by drying a batch weight of the product in a drying cabinet at 105 °C to a constant weight. The sodium nitrite content was determined by the reaction with N-1-naphthyl ethylenediamine dihydrochloride in an acidic environment with the formation of diazo compounds, the color intensity of which was photometrically measured. The total nitrogen content determined the mass fraction of protein according to the Kjeldahl method followed by ammonia stripping. The water activity (a_w) of the meat samples was measured using a device “aqua lab” of series 3 of the meter group Inc. USA according. The pH value was measured with a pH-meter MR 512 ("Ulab”, China) after mixing 10 g of the sample with 90 ml of distilled water. The amino-acid composition of proteins was studied after hydrolysis of the product samples with a mixture of 6 n. hydrochloric and 4% thioglycolic acids at 105-110 °C for 48 hours in a CO_2 environment and subsequent vacuum evaporation at 45 °C. The amino acids were identified with the use of an automatic amino acid analyzer LC-2000 Biotronik (Germany) after grinding the samples, removing the fat, as well as precipitating the protein compounds with 10% trichloroacetic or sulfosalicylic acids. The computer processing with a chromatograph using the Kodak Digital Science ID software package was used for free amino acids to be identified.

Structural-mechanical studies: The structural-mechanical studies were carried out using a universal mechanical test machine "SANS" of series CMT using special Warner-Bratzler nozzles for shear force to be determined. The parameters were calculated with the use of Power Test_DOOE software. The fermented meat products were organoleptically evaluated by a trained nine experts according to DSTU 4823.2:2007. Sample temperature – 18 ±2 °C, laboratory temperature – 21 ±2 °C, strain gauge – cone height – 10 mm, cone diameter – 10 mm, penetration speed – 20 mm/min, penetration depth – 2 cm. Participants evaluated the samples at random.

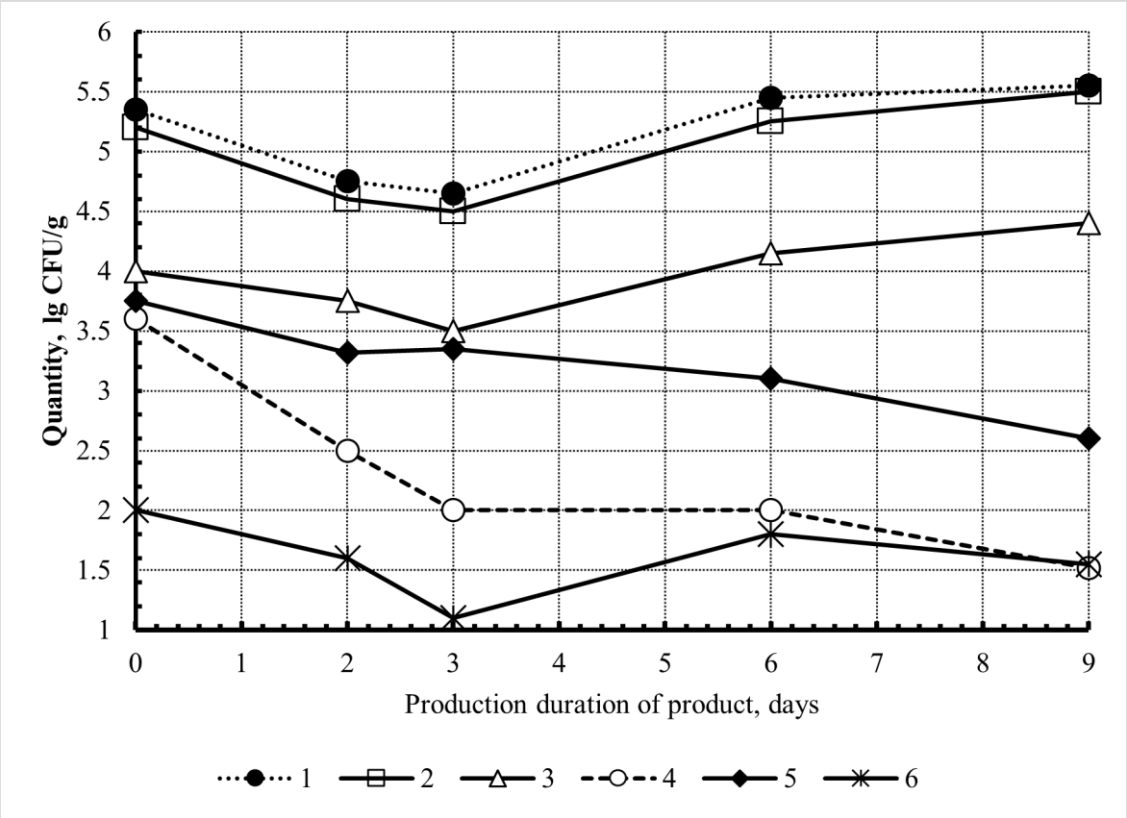
Statistical Analysis

All studies were carried out in triplicate. The data were statistically processed in Excel MS Office 2010 according to the standard methods. The figures show average statistically reliable data for 95% probability.

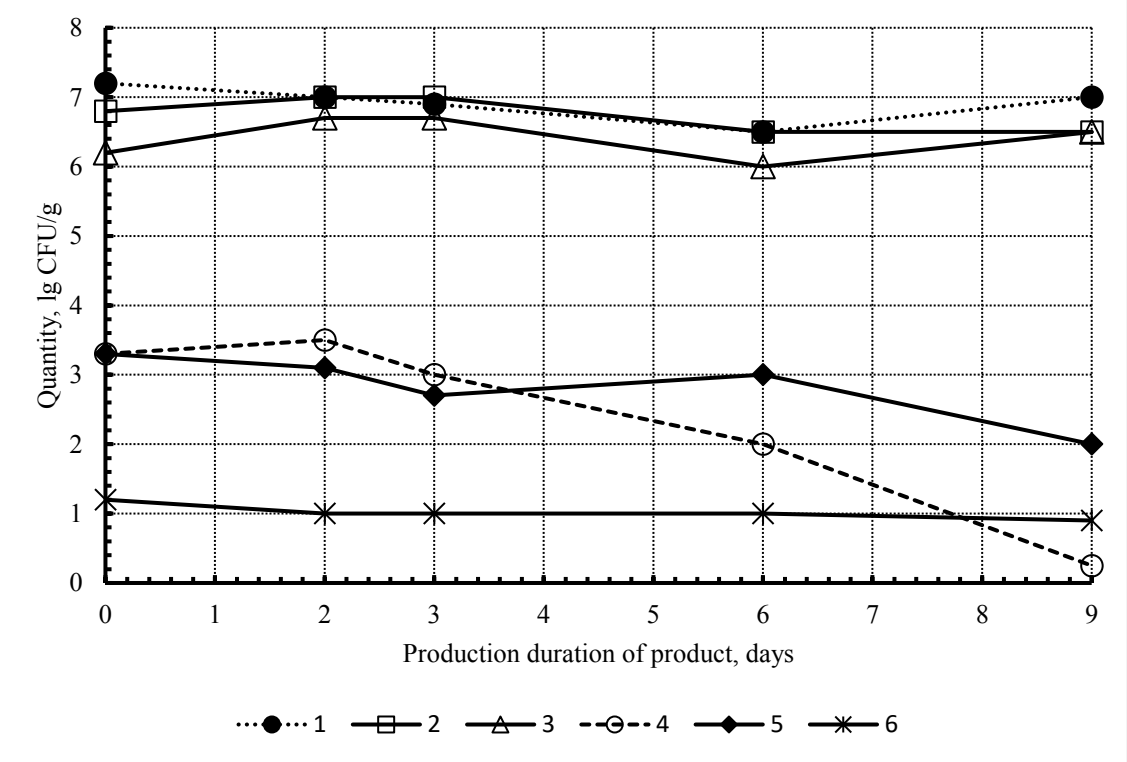
RESULTS AND DISCUSSION

For the guaranteed dominance of the starter cultures to be provided, the inoculant level should be 6.0-7.0 log CFU/g, which is usually two orders of magnitude higher than the level of autochthonous microflora **[38]**.

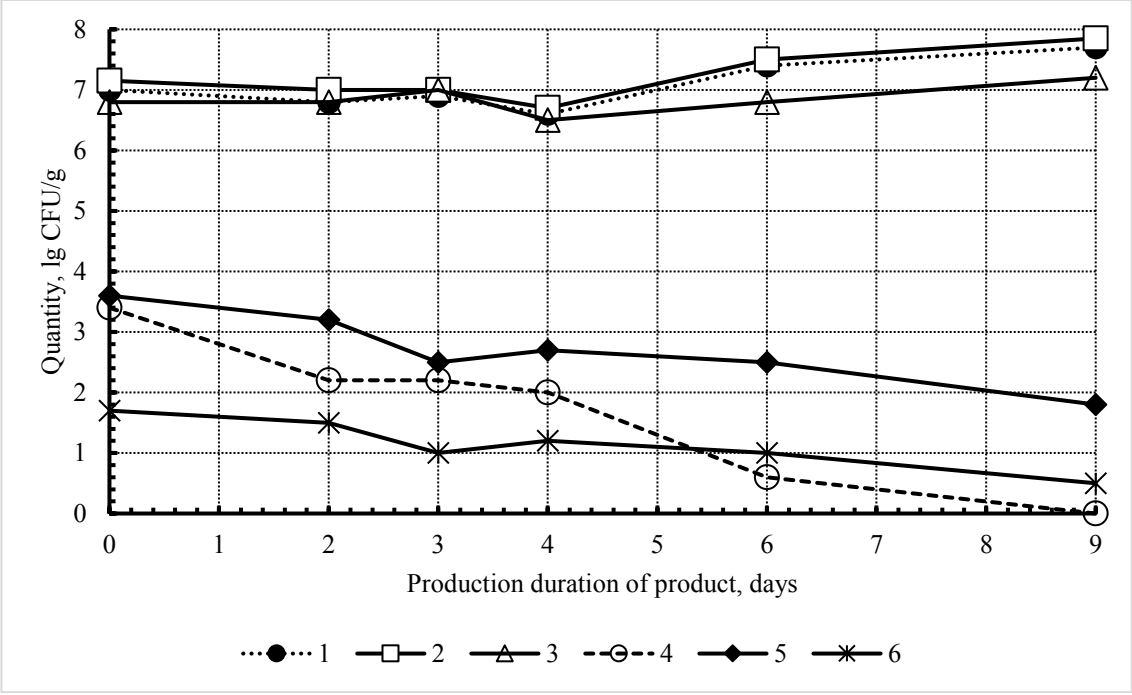
The number dynamics of lactic acid bacteria, the number of micrococci, the number of yeasts and molds, and *Enterobacteriaceae* were studied at all stages of the technological process - after the salting, salting process, ripening and drying in 6, 9 days (Figure 1).


Quantity, lg CFU/g
Production duration of product, days
1
2
3
4
5
6

a)


Quantity, lg CFU/g
Production duration of product, days
1
2
3
4
5
6

b)



c)

Figure 1 Development dynamics of the microbiota of dry-cured balyk during its maturation.
Note: a) the control sample is the product, which is produced without the use of the composition; b) the product with the use of the SC 1 composition; c) the product with the use of the SC 2 composition; 1 is the total number of microorganisms; 2 is the lactic-acid bacteria (LAB); 3 is the micrococci (MC); 4 is the *Enterobacteriaceae*, 5 is the yeast (YE), 6 is the mold (MO).

At the beginning of maturation, the number of spontaneous lactic acid bacteria in the control sample was 1.5×105 CFU/g, which is higher than reported in the papers **[39]**, and at the end of the fermentation, it was increased by 1.74 times to the initial content of cells. The content of micrococci was increased more intensively by 3.5 times compared to the initial amount (1.0×10^4 CFU/g). The yeast was present at the beginning (7.1×10^3 CFU/g) and at the end of the process. Their content during the study was decreased by 18.6 times, and the amount of mold was decreased by 1.6 times compared to the initial amount (1.0×10^2 CFU/g) (Figure 1 a).

It should be noted here that during the entire technological process, the coagulase-positive Staphylococcus ssp. was absent in all variants of the minced meat, indicating that the meat raw materials are of high quality, as well as *Salmonella, L. monocytogenes*, and *Clostridium perfringens* were not found either during the processing of the studied samples or in any of the batches **[40]**.

The number of *Enterobacteriaceae* equal to 4.5×10^3 CFU/g was detected at the beginning of the process. These indicators were gradually decreased; at the end of the experiment, they were decreased by 112.2 times. Similar results were obtained by authors of scientific works **[41]**.

After the 3rd day of the salting, the fermentation microbiota in the studied variants was decreased only by 1.2-1.5 times, and the spontaneous microbiota was decreased by 3.2 times, respectively; this shows the adaptability of the studied compositions to sodium chloride **[42]**.

When the development of the composition microbiota on the 9th day of the fermentation is compared with the 4th day, the greatest increase in viable cells of lactic acid bacteria was observed in the SC 2 composition – by 12.6 times. This indicator in the SC 1 product was increased by 2.5 times. The intensive development of lactic acid bacteria in SC 2 can be explained by the adaptability of the composition to this raw meat material (Figure 1 b, c).

In the studied variants SC 1, and SC 2, the intensive development of micrococci and staphylococci especially occurred during the last 5 days. At the same time, the increase in the number of these microorganisms was recorded by 2.58 and 5.62 times, respectively, compared to their concentration on the 9th day of the drying. These results are consistent with those obtained by authors of scientific works **[43]**.

According to the results of the conducted studies, the SC 2 composition was the best among the studied starter cultures in terms of functioning in pork meat **[44]**.

It should be noted here that the SC 2 and SC 1 compositions had a more intensive influence on the death of *Enterobacteriaceae*, which disappeared on the 9th day of the fermentation. In the control sample, their presence was still observed on this day in the amount of 0.4×10^2 CFU/g. The most intense death occurred in the SC 2

composition (Figure 1). Such results are consistent with those obtained by authors of scientific works **[45]** and confirm the additional effectiveness of the hygiene starter cultures.

At the end of the fermentation, all studied composition variants inhibited the development of yeasts by 30.2-63.1 times more intensively compared to the control sample (by 18.6 times). Still, this process occurred most actively in the variant SC 2. Regarding molds, they died intensively in all studied variants by 3.2-19.9 times compared to the control sample. In the control sample, their number was decreased by only 1.6 times according to the initial concentration of cells. It shows the advisability of the starter-culture compositions for the purity of the fermented meat products to be provided **[46]**.

Therefore, the undesirable foreign microflora in the balyks with the bacterial drug is suppressed earlier, primarily due to the active development of the starter cultures, especially in the first 3-5 days. The active growth of the starter-culture microflora correlates with a more intense increase in the acidity in the balyks with the starter cultures compared to the control samples **[47]**.

The formation of acids, particularly lactic acid, when the meat raw materials are fermented not only prevents the development of putrefactive and pathogenic microflora, thereby increasing the finished product's safety, but also positively influences the technological parameters.

During the first 5 drying days, the acidity increase occurred 2.4-2.5 times more intensively in the variants with the bacterial drugs than without them. During the entire ripening process, the acidity in the balyks with the bacterial drug was higher than in the control sample (Figure 2). At the end of the ripening, it was 5.0-5.2 pH units in the samples SC 1 and SC 2. Pathogenic and opportunistic bacteria of food products do not practically develop at pH values below 4.6 **[48]**, **[49]**.

For the formation process of nitric oxide pigments, the optimum acidity of the medium is 5.4-5.1 pH units; that is, the range close to the optimum bacterial denitrification is approx. 5.5 pH units.

It is known that nitrite is added both for coloring and for protecting the meat products against toxins being formed, which is produced by the anaerobic bacterium Clostridium botulinum, as well as against the development of salmonella and staphylococci when the meat products are ripened **[50]**.

The World Health Organization (WHO) recommends adding sodium nitrite in an amount that does not influence the human body – up to 20 g per 100 kg of the raw meat materials to be salted, but technologically justified dosages are twice as low. In the European Union, sodium nitrite can be used only as an additive to table salt of 0.5 to 0.9% **[51]**.

The concentration of free nitrite is decreased when reducing substances and microorganisms are applied and continues when the finished product is stored at a rate that depends on pH, duration, and storage temperature.

The authors of scientific works **[52]** believe that the optimal concentration of hydrogen ions for nitrite to be bonded is within the range of 5.0-6.2 pH, and the lower the pH, the faster the nitrosation processes, the less residual nitrite remains: 5% of nitrite from its initial concentration is stored in the product at 5.05 pH; 21% – at 5.75 pH; 60% – at 6.2 pH and above.

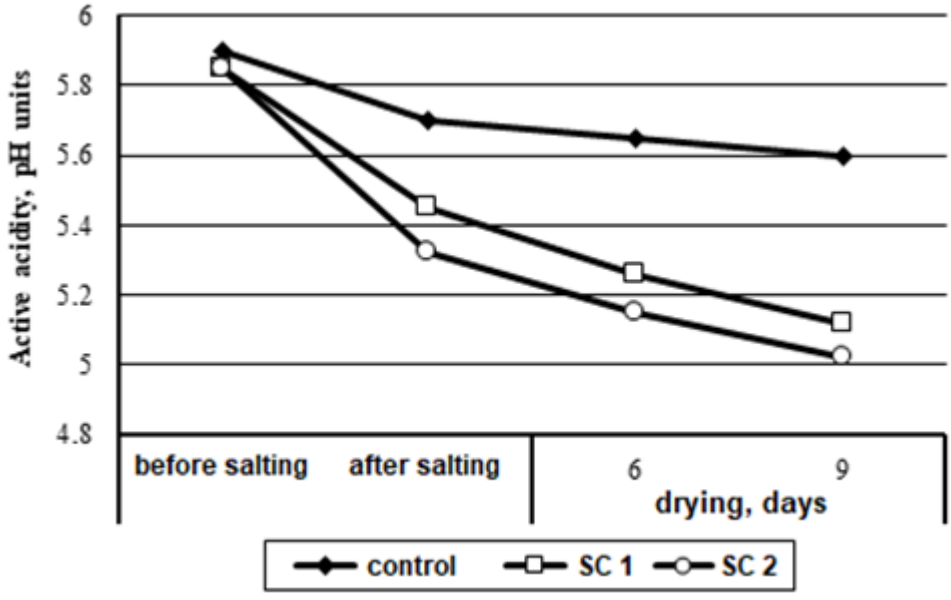


Figure 2 Acidity dynamics when dry-cured balyk is ripened.

Taking the potential danger of sodium nitrite and the regulation complexity of the formation reactions of nitric oxide pigments into consideration, the content of sodium nitrite was studied when dry-cured balyk was ripened (Figure 3).

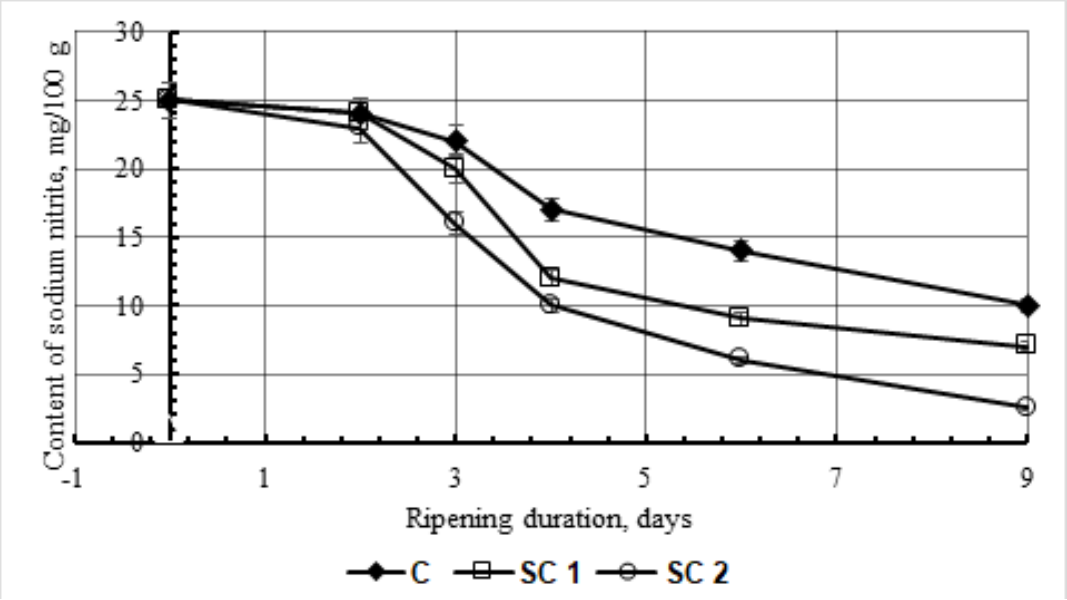


Figure 3 Content of sodium nitrite when dry-cured balyk is ripened.

During the first 3 salting days, the content of sodium nitrite slowly decreased - within the range of 20.5-32.5% in the products that were fermented with the starter-culture compositions. In the control sample, it was 11.3% compared to the initial content of salt, which was 25 mg/100 g of the raw meat materials, which corresponds to the production recipe of the pork balyk **[53]**.

It had been established that in the studied variants, the content of nitrites actively decreased on the 9th ripening day by 70-88% from the initial level. The most active were the compositions SC 1, and SC 2 compared to the control sample (60%) (Figure 3).

The obtained results were consistent with the literature data, which also established the positive role of the bacterial drugs, which include nitrite-reducing microorganisms, for a stable color of the fermented sausages to be formed **[54]**, **[55]**.

The parameter of water activity is of particular importance for the fermented products to be dried, which makes it possible to establish the connection between the state of weakly bound moisture in the product and the possibility of microorganisms developed in it since, as is well-known, microorganisms can consume only active moisture part for their vital activity (Table 1).

Table 1 Parameter of water activity of fermented pork products during their production.

Sample	Meat before salting	Meat after salting	Drying, days	
			4	9
C	0.986	0.978	0.966	0.958
SC 1	0.986	0.978	0.969	0.957
SC 2	0.986	0.976	0.962	0.956

Having analyzed the results according to the quality parameter of water activity, it had been established that the observed dynamics did not show any differences in the starter cultures. The behavior of a_w occurred due to the weight loss, which had no significant differences **[56]**.

The provided study results make it possible to conclude that the use of SC 2 activates the microbiological processes during the salting and drying of the dry-cured meat products and contributes to their dehydration and a sharp decrease of the parameter of water activity in such products.

It is known that the closer the pH value is to the isoelectric point of meat proteins (5.4 units), the lower its ability to bind moisture and, accordingly, the higher the drying speed.

In parallel with the decrease in pH, the content of total moisture decreased (Figure 4) in all samples by an average of 13.65% by the end of the technological process.

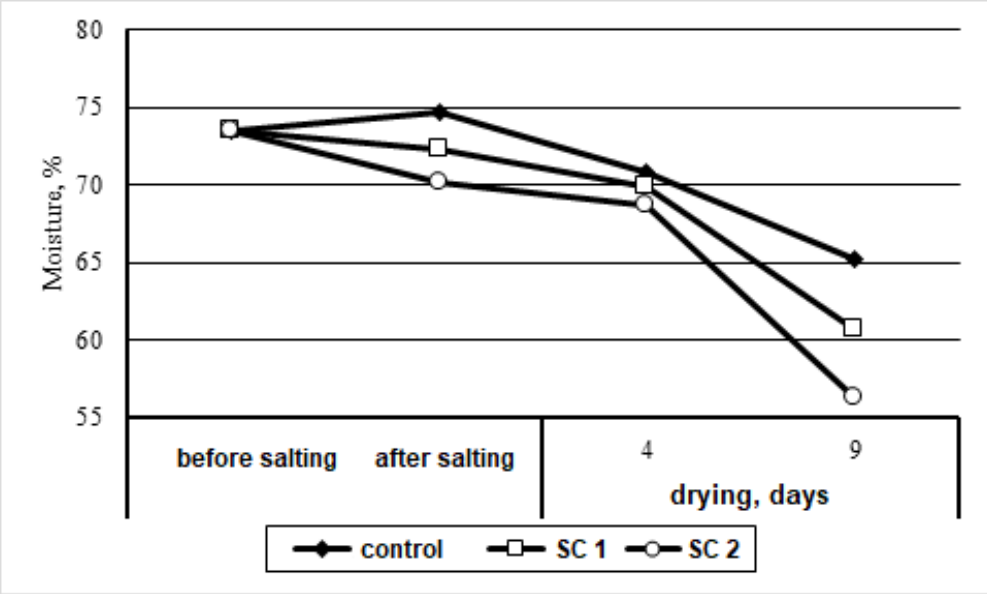


Figure 4 Dynamics of moisture changes of fermented whole-muscle pork products during production.

During the ripening, the protein components of the raw meat materials are substantially changed due to the activity of the tissue enzymes of the meat and microorganisms, which determines the course of biochemical transformations. Free amino acids and other metabolites, associated with the vital activity of microorganisms, have a significant role in the taste-aroma bouquet of the fermented products to be formed.

Since experiments proved that during the fermentation and ripening, the composite cultures dominated in the variants with the bacterial drugs, it can be assumed that it was the starter cultures that determined the specific direction of the biochemical transformation of the proteins of the raw meat materials and the formation of the taste and aroma range of the finished product **[57]**.

Nitrogen-containing substances are transformed when the dry-cured meat products are ripened (Table 2).

Table 2 Content of nitrogen-containing substances in dry-cured meat products.

Nitrogen-containing substances by fractions	Content of nitrogen on a dry matter basis, %			
	Im	C	SC 1	SC 2
	0 days		9 days	
Total nitrogen	12.9*	11.6	11.8	12.0
Non-protein nitrogen	1.1	1.5	1.6	1.7
Protein nitrogen	11.3	10.1	10.3	10.4

Note: *Measurement error does not exceed 0.1 %.

At the beginning of the fermentation, the level of non-protein nitrogen for the raw meat material was 1.1 % of dry matter. On the 9th ripening day, non-protein nitrogen was increased for all samples: SC 1 – by 14.5%, SC 2 – by 15.4%, and control sample – by 13.6%. The protein nitrogen content decreased on the 9th day: SC 1 by 8.8%, SC 2 by 8%. The parameter was slightly higher in the control sample – 10.6%.

Proteolysis greatly influences the quality characteristics of the fermented product, as it is an important source of taste compounds, such as free amino acids **[58]**.

When the dry-cured balyk is ripened, the level of free amino acids is increased, and their spectrum is different in the studied variants with various compositions (Figure 5).

On the 9th fermentation day of the raw meat materials, the concentration of free cyclic amino acids was increased in the studied variants by 1.41-1.57 times and in the control samples – by 1.32 times compared to their initial content (160.28 mg/100 g of dry matter). Regarding the concentration change of acyclic amino acids, in these products, their content was increased by 5.54-7.17 times, in the control sample – only by 4.41 times, compared to the initial raw meat materials. Their initial content was 348.98 mg/100 g of dry matter.

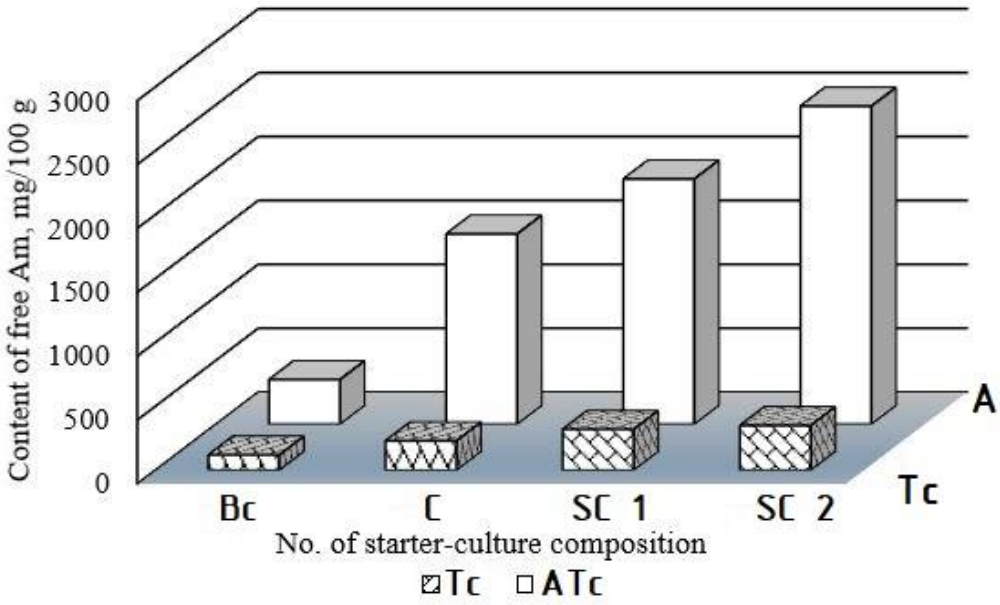


Figure 5 Accumulation of free cyclic and acyclic amino acids in finished dry-cured balyk: Im – initial raw meat materials; C – control sample, produced without the starter culture; SC 1 and SC 2 are the fermented products.

In the products inoculated with the compositions SC 1 and SC 2, the content of acyclic amino acids was increased by 112.4% and 276.0%, respectively, and the content of cyclic amino acids was increased by 8.8% and 24.7%, respectively, compared to their level in the finished control variant (Figure 5).

Having compared the influence of the various starter-culture compositions on pork proteolysis, it had been established that the proteolytic processes occurred more actively in the studied variant SC 2. Casaburi et al. proved that the protein degradation in the sausage inoculated with *Lactobacillus* spp. and *Staphylococcus xylosus* occurs faster during the ripening **[59]**, **[60]**.

The specific sensory quality of fermented meat products is mainly the smell and taste **[61]**. The addition of the bacterial drugs influenced the general aroma and taste of the products (Figure 6).

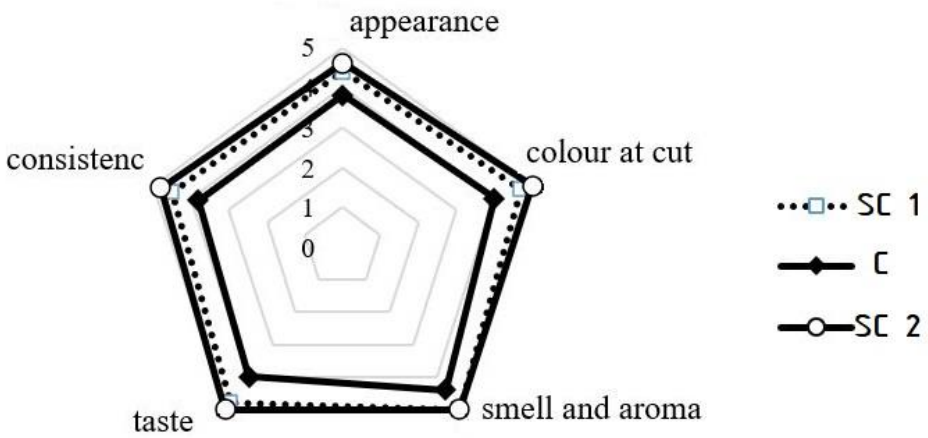


Figure 6 Profile diagram for quality parameters of control and studied (SC 1 and SC 2) product samples.

The sensory properties of fermented meat products (consistency, color, and aroma) depend on many compounds, which are formed during the chemical or biochemical transformations of the raw meat materials, **[62]**.

The differences in the organoleptic characteristics of the dry-cured products with various starter-culture compositions were determined according to the quality evaluation results of the finished product. The finished dry-cured meat products of the variants SC 1 and SC 2 had a beautiful appearance, a cut of red color, an elastic consistency, and a delectable flavor with a characteristic sour after-taste, and for the SC 2. T

here was also a pronounced aroma of dry-curing. Variant C 1 had a good appearance but a slightly darker color and less pronounced taste and aroma. The control variant had a dark-red color, somewhat rubbery consistency, unpronounced taste and aroma, and after-taste of old fat. The aroma and taste of the meat products with the various

bacterial drugs did not differ significantly. Based on the tasting results, the product samples with the addition of the compositions SC 1 and SC 2 received a total score higher than the control sample.

The conducted scientific studies of the influence of starter cultures on the microbiological and physicochemical parameters of dried meat products are an interesting and important topic, therefore, further directions of research can develop in the following directions: research:

Study the influence of different starter cultures on dried products' microbiological composition.

We are optimizing the proportions of different types of bacteria to achieve the best results in production.

Study microbiota dynamics in the production and storage process of dried products.

Analysis of the impact of starter cultures on product shelf life and consumption safety.

Study the effect of starter cultures on textural characteristics and color of dried meat products.

Determination of physicochemical parameters such as moisture, pH, and salt concentration in products depending on the starter cultures used.

Considering the influence of the growing environment of starter cultures on the quality and characteristics of dried products.

Study the influence of seasonal changes and geographical aspects on the microflora structure and product quality.

Development of recommendations for manufacturers regarding optimal production conditions, including temperature, humidity, and process duration.

Study the influence of starter cultures on the organoleptic characteristics of dried products.

Evaluating consumer properties and product taste qualities depends on using different starter cultures.

Therefore, the proposed research directions can be aimed at a detailed study of the influence of starter cultures on various aspects of the quality and safety of various meat products.

CONCLUSION

The *SC 1* starter cultures were tested *based on Lactobacillus plantarum, L. rhamnosus,* and *Kocuria rosea,* and SC 2 based on *Staphylococcus carnosus, Lactobacillus plantarum*, *Lactobacillus rhamnosus and Lactobacillus paracasei.* The influence of the created drugs SC 1 and SC 2 on the main physico-chemical and biochemical parameters of the dry-cured meat products was studied during their production. It had been established that in the studied variants, the content of nitrites actively decreased on the 9th ripening day by 70-88% from the initial level. The most active were the compositions SC 1, and SC 2 compared to the control sample (60%). On the 9th fermentation day of the raw meat materials, the concentration of free cyclic amino acids was increased in the studied variants by 1.41-1.57 times and in the control samples – by 1.32 times compared to their initial content (160.28 mg/100 g of dry matter). The characteristic differences in the biochemical processes in the dry-cured meat products produced without/with the bacterial drug were determined. It had been established that the fermented products have better quality, caused by the action of the starter cultures. The product with the created drug SC 2 had the highest quality parameters, and the death of Enterobacteriaceae occurred on the 9th fermentation day. At the same time, this process in the control sample lasted until the 12th day. It has been shown that the use of the drugs SC 2 and SC 1, when the dry-cured pork balyks are produced, decreases the duration of the technological cycle by 3-4 days, as well as ensures a high degree of sanitary and epidemic safety of the finished product: the absence of pathogenic and opportunistic microorganisms, the low residual content of sodium nitrite of up to 0.003%. The use of the selected starter culture based on the combination of *Staphylococcus carnosus, Lactobacillus plantarum, Lactobacillus rhamnosus,* and *Lactobacillus paracasei* demonstrates a potential interest for its use when the fermented pork product is produced.

REFERENCES

1. Danylenko, S., Naumenko, O., Yemtsev, V., Kryzhska, T., Potemska, O., Tolok, G., Kanishchev, O., Ochkolyas, O., Prokopenko, N., & Omelian, A. (2023). Justification and microbiota compositions development for the fermentation of raw meat. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 405–418). HACCP Consulting. https://doi.org/10.5219/1874
2. Sharma, A., Lee, S., & Park, Y.-S. (2020). Molecular typing tools for identifying and characterizing lactic acid bacteria: a review. In Food Science and Biotechnology (Vol. 29, Issue 10, pp. 1301–1318). Springer Science and Business Media LLC. https://doi.org/10.1007/s10068-020-00802-x
3. Evelyn, & Silva, F. V. M. (2020). Ultrasound-assisted thermal inactivation of spores in foods: Pathogenic and spoilage bacteria, molds and yeasts. In Trends in Food Science & Technology (Vol. 105, pp. 402–415). Elsevier BV. https://doi.org/10.1016/j.tifs.2020.09.020
4. State enterprise "Ukrainian research and training center for problems of standardization, certification and quality". (2007). Meat products. Organoleptic assessment of quality indicators. Part 2. General requirements.

With correction. (DSTU 4823.2:2007). Retrieved from http://online.budstandart.com/ua/catalog/doc-page?id_doc=83084

5. Danylenko, S., Naumenko, O., Ryzhkova, T., Fediaiev, V., Verbytskyi, S., & Korol, Ts. (2022). The amino acid composition of meat products treated with preparations of microbial origin. In Food Science and Technology (Vol. 16, Issue 3). Odesa National University of Technology. https://doi.org/10.15673/fst.v16i3.2416
6. Su, X., Luo, Y., Tian, Z., Yuan, Z., Han, Y., Dong, R., Xu, L., Feng, Y., Liu, X., & Huang, J. (2020). Ctenophore-inspired hydrogels for efficient and repeatable underwater specific adhesion to biotic surfaces. In Materials Horizons (Vol. 7, Issue 10, pp. 2651–2661). Royal Society of Chemistry (RSC). https://doi.org/10.1039/d0mh01344g
7. Amanatidou, A., Bennik, M., Gorris, L., & Smid, E. (2001). Superoxide dismutase plays an important role in the survival of Lactobacillus sake upon exposure to elevated oxygen. In Archives of Microbiology (Vol. 176, Issues 1–2, pp. 79–88). Springer Science and Business Media LLC. https://doi.org/10.1007/s002030100297
8. Ammor, M. S., & Mayo, B. (2007). Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update. In Meat Science (Vol. 76, Issue 1, pp. 138–146). Elsevier BV. https://doi.org/10.1016/j.meatsci.2006.10.022
9. Arnau, J., Guerrero, L., & Sárraga, C. (1998). The effect of green ham pH and NaCl concentration on cathepsin activities and the sensory characteristics of dry-cured hams. In Journal of the Science of Food and Agriculture (Vol. 77, Issue 3, pp. 387–392). Wiley. https://doi.org/10.1002/(sici)1097-0010(199807)77:3<387::aid-jsfa57>3.0.co;2-h
10. Stadnyk, I., Bodnarchuk, O., Kopylova, K., Petrov, P., Bal-Prylypko, L., & Narizhnyy, S. (2021). Modification of the properties of milk-fat emulsions with the phase structure of “oil in water” in the dependence on the mass part of the lipoid and the stabilizing systems. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 741–748). HACCP Consulting. https://doi.org/10.5219/1389
11. Ayhan, K., Kolsarici, N., & Özkan, G. A. (1999). The effects of starter culture on the formation of biogenic amines in Turkish soudjoucks. In Meat Science (Vol. 53, Issue 3, pp. 183–188). Elsevier BV. https://doi.org/10.1016/s0309-1740(99)00046-7
12. Bal’-Prylypko, L. V., Derevyanko, L. P., Slobodyanyuk, N. M., Starkova, E. R., & Androshchiuk, O. S. (2018). Using the Ampullaria glauca snails’ caviar for correction of the effects of the ionizing radiation exposure in a small dose. In Nuclear Physics and Atomic Energy (Vol. 19, Issue 2, pp. 159–165). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/jnpae2018.02.159
13. Barrière, C., Centeno, D., Lebert, A., Leroy-SÃ©trin, S., BerdaguÃ©, J. L., & Talon, R. (2001). Roles of superoxide dismutase and catalase of Staphylococcus xylosus in the inhibition of linoleic acid oxidation. In FEMS Microbiology Letters (Vol. 201, Issue 2, pp. 181–185). Oxford University Press (OUP). https://doi.org/10.1111/j.1574-6968.2001.tb10754.x
14. Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. In AIMS Microbiology (Vol. 4, Issue 4, pp. 665–684). American Institute of Mathematical Sciences (AIMS). https://doi.org/10.3934/microbiol.2018.4.665
15. Montel, M.-C., Reitz, J., Talon, R., Berdagué, J.-L., & Rousset-Akrim, S. (1996). Biochemical activities of Micrococcaceae and their effects on the aromatic profiles and odors of a dry sausage model. In Food Microbiology (Vol. 13, Issue 6, pp. 489–499). Elsevier BV. https://doi.org/10.1006/fmic.1996.0056
16. Bal-Prylypko, L., Yancheva, M., Paska, M., Ryabovol, M., Nikolaenko, M., Israelian, V., Pylypchuk, O., Tverezovska, N., Kushnir, Y., & Nazarenko, M. (2022). The study of the intensification of technological parameters of the sausage production process. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 27–41). HACCP Consulting. https://doi.org/10.5219/1712
17. Charmpi, C., Van Reckem, E., Sameli, N., Van der Veken, D., De Vuyst, L., & Leroy, F. (2020). The Use of Less Conventional Meats or Meat with High pH Can Lead to the Growth of Undesirable Microorganisms during Natural Meat Fermentation. In Foods (Vol. 9, Issue 10, p. 1386). MDPI AG. https://doi.org/10.3390/foods9101386
18. Comi, G., Muzzin, A., Corazzin, M., & Iacumin, L. (2020). Lactic Acid Bacteria: Variability Due to Different Pork Breeds, Breeding Systems, and Fermented Sausage Production Technology. In Foods (Vol. 9, Issue 3, p. 338). MDPI AG. https://doi.org/10.3390/foods9030338
19. Barbieri, F., Montanari, C., Gardini, F., & Tabanelli, G. (2019). Biogenic Amine Production by Lactic Acid Bacteria: A Review. In Foods (Vol. 8, Issue 1, p. 17). MDPI AG. https://doi.org/10.3390/foods8010017

20. Mixers in Biodiesel Production. In Lecture Notes in Mechanical Engineering (pp. 197–207). Springer International Publishing. https://doi.org/10.1007/978-3-031-06044-1_19
21. Hughes, M. C., Kerry, J. P., Arendt, E. K., Kenneally, P. M., McSweeney, P. L. H., & O'Neill, E. E. (2002). Characterization of proteolysis during the ripening of semi-dry fermented sausages. In Meat Science (Vol. 62, Issue 2, pp. 205–216). Elsevier BV. https://doi.org/10.1016/s0309-1740(01)00248-0
22. Chen, Q., Kong, B., Sun, Q., Dong, F., & Liu, Q. (2015). Antioxidant potential of a unique LAB culture isolated from Harbin dry sausage: In vitro and a sausage model. In Meat Science (Vol. 110, pp. 180–188). Elsevier BV. https://doi.org/10.1016/j.meatsci.2015.07.021
23. Cruxen, C. E. dos S., Funck, G. D., Haubert, L., Dannenberg, G. da S., Marques, J. de L., Chaves, F. C., da Silva, W. P., & Fiorentini, Â. M. (2019). Selection of native bacterial starter culture in the production of fermented meat sausages: Application potential, safety aspects, and emerging technologies. In Food Research International (Vol. 122, pp. 371–382). Elsevier BV. https://doi.org/10.1016/j.foodres.2019.04.018
24. Essid, I., & Hassouna, M. (2013). Effect of inoculation of selected Staphylococcus xylosus and Lactobacillus plantarum strains on biochemical, microbiological, and textural characteristics of a Tunisian dry fermented sausage. In Food Control (Vol. 32, Issue 2, pp. 707–714). Elsevier BV. https://doi.org/10.1016/j.foodcont.2013.02.003
25. Erkkilä, S., Petäjä, E., Eerola, S., Lilleberg, L., Mattila-Sandholm, T., & Suihko, M.-L. (2001). Flavour profiles of dry sausages fermented by selected novel meat starter cultures. In Meat Science (Vol. 58, Issue 2, pp. 111–116). Elsevier BV. https://doi.org/10.1016/s0309-1740(00)00135-2
26. Simonová, M., Strompfová, V., Marciňáková, M., Lauková, A., Vesterlund, S., Moratalla, M. L., Bover-Cid, S., & Vidal-Carou, C. (2006). Characterization of Staphylococcus xylosus and Staphylococcus carnosus isolated from Slovak meat products. In Meat Science (Vol. 73, Issue 4, pp. 559–564). Elsevier BV. https://doi.org/10.1016/j.meatsci.2006.02.004
27. Mohamed Esivan, S. M., Rashid, R., Jati, A., & Zaharudin, N. A. (2021). Growth of Lactobacillus casei and Propionibacterium jensenii in Different Glucose Concentration and Incubation Temperature. In Proceedings of the 6th International Conference on Fundamental and Applied Sciences (pp. 97–105). Springer Nature Singapore. https://doi.org/10.1007/978-981-16-4513-6_9
28. Acharya, T., & Hare, J. (2022). Sabouraud Agar and Other Fungal Growth Media. In Fungal Biology (pp. 69–86). Springer International Publishing. https://doi.org/10.1007/978-3-030-83749-5_2
29. DSTU EN ISO 6579-1:2022 Microbiology of the food chain. Horizontal method for detection, counting, and serotyping of Salmonella. Part 1. Detection of Salmonella spp.
30. DSTU ISO 11290-2:2003 Microbiology of Food and Animal Feed. Horizontal method for detecting and counting Listeria monocytogenes. Part 2. Counting Method.
31. Dai, C., Füllgrabe, A., Pfeuffer, J., Solovyeva, E. M., Deng, J., Moreno, P., Kamatchinathan, S., Kundu, D. J., George, N., Fexova, S., Grüning, B., Föll, M. C., Griss, J., Vaudel, M., Audain, E., Locard-Paulet, M., Turewicz, M., Eisenacher, M., Uszkoreit, J., … Perez-Riverol, Y. (2021). A proteomics sample metadata representation for multiomics integration and big data analysis. In Nature Communications (Vol. 12, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41467-021-26111-3
32. Ordóñez, J. A., Hierro, E. M., Bruna, J. M., & Hoz, L. de la. (1999). Changes in the Components of Dry-Fermented Sausages during Ripening. In Critical Reviews in Food Science and Nutrition (Vol. 39, Issue 4, pp. 329–367). Informa UK Limited. https://doi.org/10.1080/10408699991279204
33. Perea-Sanz, L., Montero, R., Belloch, C., & Flores, M. (2018). Nitrate reduction in the fermentation process of salt-reduced dry sausages: Impact on microbial and physicochemical parameters and aroma profile. In International Journal of Food Microbiology (Vol. 282, pp. 84–91). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2018.06.004
34. International Organization for Standardization. (2004). Microbiology of food and animal feeding stuff – Determination of water activity. (ISO 21807:2004). Received from: https://www.iso.org/standard/34728.html
35. International Organization for Standardization. (1999). Meat and meat products – Measurement of pH – Reference method. (ISO 2917:1999). Received from: https://www.iso.org/standard/24785.html
36. International Organization for Standardization. (2017). Microbiology of the food chain. Horizontal method for detecting and enumerating Listeria monocytogenes and Listeria spp. Enumeration method. (BS EN ISO 11290-2:2017). Received from: https://www.iso.org/standard/60314.html
37. Kaban, G., & Kaya, M. (2009). Effects of Lactobacillus plantarum and Staphylococcus xylosus on the Quality Characteristics of Dry Fermented Sausage "Sucuk." In Journal of Food Science (Vol. 74, Issue 1). Wiley. https://doi.org/10.1111/j.1750-3841.2008.01014.x

38. Sánchez-Molinero, F., & Arnau, J. (2008). Effect of the inoculation of a starter culture and vacuum packaging (during resting stage) on the appearance and some microbiological and physicochemical parameters of dry-cured ham. In Meat Science (Vol. 79, Issue 1, pp. 29–38). Elsevier BV. https://doi.org/10.1016/j.meatsci.2007.07.028
39. Iacumin, L., Jayasinghe, A. S., Pellegrini, M., & Comi, G. (2022). Evaluation of Different Techniques, including Modified Atmosphere, under Vacuum Packaging, Washing, and Latilactobacillus sakei as a Bioprotective Agent, to Increase the Shelf-Life of Fresh Gutted Sea Bass (Dicentrarchus labrax) and Sea Bream (Sparus aurata) Stored at 6 ± 2 °C. In Biology (Vol. 11, Issue 2, p. 217). MDPI AG. https://doi.org/10.3390/biology11020217
40. Russell, L., Galindo, C. P., Whyte, P., & Bolton, D. (2021). A preliminary study of Salmonella spp., Listeria monocytogenes, Escherichia coli O157, Enterococcus faecalis, and Clostridium spp. in Irish cattle. In Irish Journal of Agricultural and Food Research (Vol. 60, Issue 1). Compuscript, Ltd. https://doi.org/10.15212/ijafr-2020-0122
41. Zheplinska, M., Mushtruk, M., Vasyliv, V., Slobodyanyuk, N., & Boyko, Y. (2021). The Main Parameters of the Physalis Convection Drying Process. In Lecture Notes in Mechanical Engineering (pp. 306–315). Springer International Publishing. https://doi.org/10.1007/978-3-030-77823-1_31
42. Ojha, K. S., Kerry, J. P., Duffy, G., Beresford, T., & Tiwari, B. K. (2015). Technological advances for enhancing the quality and safety of fermented meat products. In Trends in Food Science & Technology (Vol. 44, Issue 1, pp. 105–116). Elsevier BV. https://doi.org/10.1016/j.tifs.2015.03.010
43. Bal'-Prylypko, L. V., Derevyanko, L. P., Slobodyanyuk, N. M., Starkova, E. R., & Androshchiuk, O. S. (2018). Using of the Ampullaria glauca snails' caviar for correction of the effects of the ionizing radiation exposure in small dose. In Nuclear Physics and Atomic Energy (Vol. 19, Issue 2, pp. 159–165). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/jnpae2018.02.159
44. Perea-Sanz, L., Montero, R., Belloch, C., & Flores, M. (2018). Nitrate reduction in the fermentation process of salt reduced dry sausages: Impact on microbial and physicochemical parameters and aroma profile. In International Journal of Food Microbiology (Vol. 282, pp. 84–91). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2018.06.004
45. Pérez-Palacios, T., Ruiz, J., Barat, J. M., Aristoy, M. C., & Antequera, T. (2010). Influence of pre-cure freezing of Iberian ham on proteolytic changes throughout the ripening process. In Meat Science (Vol. 85, Issue 1, pp. 121–126). Elsevier BV. https://doi.org/10.1016/j.meatsci.2009.12.015
46. Bal-Prylypko, L., Nikolaenko, M., Stepasyuk, L., Cherednichenko, O., & Lialyk, A. (2022). Forecasting the selling price of pork in agricultural enterprises. In Agricultural and Resource Economics: International Scientific E-Journal (Vol. 8, Issue 4, pp. 170–187). Institute of Eastern European Research and Consulting. https://doi.org/10.51599/are.2022.08.04.08
47. Rason, J., Martin, J.-F., Dufour, E., & Lebecque, A. (2007). Diversity of the sensory characteristics of traditional dry sausages from the center of France: Relation with regional manufacturing practice. In Food Quality and Preference (Vol. 18, Issue 3, pp. 517–530). Elsevier BV. https://doi.org/10.1016/j.foodqual.2006.07.002
48. Riebroy, S., Benjakul, S., Visessanguan, W., & Tanaka, M. (2004). Physical properties and microstructure of commercial Som-fug, a fermented fish sausage. In European Food Research and Technology (Vol. 220, Issues 5–6, pp. 520–525). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-004-1094-z
49. Sallan, S., Yılmaz Oral, Z. F., & Kaya, M. (2023). A Review on the Role of Lactic Acid Bacteria in the Formation and Reduction of Volatile Nitrosamines in Fermented Sausages. In Foods (Vol. 12, Issue 4, p. 702). MDPI AG. https://doi.org/10.3390/foods12040702
50. Zheplinska, M., Mushtruk, M., Vasyliv, V., Kuts, A., Slobodyanyuk, N., Bal-Prylypko, L., Nikolaenko, M., Kokhan, O., Reznichenko, Y., & Salavor, O. (2021). The micronutrient profile of medicinal plant extracts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 528–535). HACCP Consulting. https://doi.org/10.5219/1553
51. Sriphochanart, W., & Skolpap, W. (2010). The use of selected lactic acid bacteria starter cultures for improved Thai sausage fermentation. In Journal of Food Processing and Preservation (Vol. 35, Issue 3, pp. 291–298). Hindawi Limited. https://doi.org/10.1111/j.1745-4549.2009.004
52. Takeda, S., Matsufuji, H., Nakade, K., Takenoyama, S., Ahhmed, A., Sakata, R., Kawahara, S., & Muguruma, M. (2016). Investigation of lactic acid bacterial strains for meat fermentation and the product's antioxidant and angiotensin-I-converting-enzyme inhibitory activities. In Animal Science Journal (Vol. 88, Issue 3, pp. 507–516). Wiley. https://doi.org/10.1111/asj.12673

53. Toldrá, F. (2006). The role of muscle enzymes in dry-cured meat products with different drying conditions. In Trends in Food Science & Technology (Vol. 17, Issue 4, pp. 164–168). Elsevier BV. https://doi.org/10.1016/j.tifs.2005.08.007

54. Toledano, A. M., Jordano, R., Medina, L. M., & López-Mendoza, M. C. (2018). Behavior and effect of combined starter cultures on microbiological and physicochemical characteristics of dry-cured ham. In Journal of Food Science and Technology (Vol. 56, Issue 1, pp. 122–131). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-018-3465-7

55. Van Reckem, E., Geeraerts, W., Charmpi, C., Van der Veken, D., De Vuyst, L., & Leroy, F. (2019). Exploring the Link Between the Geographical Origin of European Fermented Foods and the Diversity of Their Bacterial Communities: The Case of Fermented Meats. In Frontiers in Microbiology (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fmicb.2019.02302

56. Vilar, I., Garcia Fontan, M. C., Prieto, B., Tornadijo, M. E., & Carballo, J. (2000). A survey on the microbiological changes during the manufacture of dry-cured lacon, a Spanish traditional meat product. In Journal of Applied Microbiology (Vol. 89, Issue 6, pp. 1018–1026). Oxford University Press (OUP). https://doi.org/10.1046/j.1365-2672.2000.01210.x

57. Wang, J., Hou, J., Zhang, X., Hu, J., Yu, Z., & Zhu, Y. (2022). Improving the Flavor of Fermented Sausage by Increasing Its Bacterial Quality via Inoculation with Lactobacillus plantarum MSZ2 and Staphylococcus xylosus YCC3. In Foods (Vol. 11, Issue 5, p. 736). MDPI AG. https://doi.org/10.3390/foods11050736

58. Wang, X., Wang, S., & Zhao, H. (2019). Unraveling microbial community diversity and succession of Chinese Sichuan sausages during spontaneous fermentation by high-throughput sequencing. In Journal of Food Science and Technology (Vol. 56, Issue 7, pp. 3254–3263). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-019-03781-y

59. Holembovska, N., Tyshchenko, L., Slobodyanyuk, N., Israelian, V., Kryzhova, Y., Ivaniuta, A., Pylypchuk, O., Menchynska, A., Shtonda, O., & Nosevych, D. (2021). Use of aromatic root vegetables in the technology of freshwater fish preserves. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 296–305). HACCP Consulting. https://doi.org/10.5219/1581

60. Minorova, A., Romanchuk, I., Verbytskyi, S., Danylenko, S., Krushelnytska, N., Potemska, O., & Narizhnyi, S. (2022). Effect of protein and carbohydrate components upon quality parameters and viable probiotic bacteria content in milk mixtures during their drying and storage. In Journal of Microbiology, biotechnology and food sciences (p. e3778). Slovak University of Agriculture in Nitra. https://doi.org/10.55251/jmbfs.3778

61. Danylenko, S. G., Naumenko, O. V., Onishchenko, A. S., Teterina, S. M., Khonkiv, M. O., & Skrotskyi, S. O. (2021). Biotechnology of Newly Created Bacterial Composition for Siloing Based on Lactic Acid Bacteria. In Mikrobiolohichnyi Zhurnal (Vol. 83, Issue 6, pp. 20–31). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/microbiolj83.06.020

62. Wang, X., Wang, S., & Zhao, H. (2019). Unraveling microbial community diversity and succession of Chinese Sichuan sausages during spontaneous fermentation by high-throughput sequencing. In Journal of Food Science and Technology (Vol. 56, Issue 7, pp. 3254–3263). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-019-03781-y

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Svitlana Danylenko

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Larysa Bal-Prylypko, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Management of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Polkovnika Potekhina, Str.16, 03041 Kyiv, Ukraine,
E-mail: bplv@ukr.net
ORCID: https://orcid.org/0000-0002-9489-8610

Svitlana Danylenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Management of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Polkovnika Potekhina, Str.16, 03041, Kyiv, Ukraine,
E-mail: svet1973@gmail.com
ORCID: https://orcid.org/0000-0003-4470-4643

Olena Mykhailova, State Biotechnological University, Department of Economics and Business State, str. Alchevskikh, 44, 61002, Kharkov, Ukraine,
E-mail: emi030865@gmail.com
ORCID: https://orcid.org/0000-0002-8319-1281

Liana Nedorizanyuk, Institute of Food Resources of NAAS, str. Eugene Sverstyuk (Marina Raskova), 4A, 02002, Kyiv, Ukraine,
E-mail: nediana@gmail.com
ORCID: https://orcid.org/0000-0002-2190-5648

Alla Bovkun, National University of Food Technologies, Institute of Post-Diploma Training, Volodymyrska St, 68, Kyiv, Ukraine,
E-mail: milk_ipdo@ukr.net
ORCID: https://orcid.org/0000-0002-3174-0963

Nataliia Slobodyanyuk, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products Department of technology of meat, fish and marine products, Polkovnyka Potiekhina Str., 16, 03040, Kyiv, Ukraine,
E-mail: slob2210@ukr.net
ORCID: https://orcid.org/0000-0002-7724-2919

Alina Omelian, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technologies of Meat, Fish, and Seafoods, Heroiv Oborony Str., 15, 03040, Kyiv, Ukraine,
E-mail: alina_omelian@nubip.edu.ua
ORCID: https://orcid.org/0000-0001-9004-5250

***Anastasiia Ivaniuta**, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technology of meat, fish and marine products, Polkovnika Potekhina Str., 16, Kyiv, 03040, Kyiv, Ukraine,
E-mail: ivanyta07@gmail.com
ORCID: https://orcid.org/0000-0002-1770-5774

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 4.1.2024
Revised: 23.2.2024
Accepted: 8.3.2024
Published: 12.3.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 331-346
https://doi.org/10.5219/1957
ISSN: 1337-0960 online
www.potravinarstvo.com

Revolutionizing meat processing: a nexus of technological advancements, sustainability, and cultured meat evolution

Anuarbek Suychinov, Dinara Akimova, Aitbek Kakimov, Yerlan Zharykbasov, Assemgul Baikadamova, Eleonora Okuskhanova, Anara Bakiyeva, Nadir Ibragimov

ABSTRACT

This thorough analysis traverses the ever-changing terrain of meat processing, revealing a story intertwined with technological innovations, environmentally friendly methods, and the revolutionary rise of cultured meat production. The amalgamation of sustainable polymers, sophisticated composite coatings, and potent antioxidant agents strikingly demonstrates the sector's dedication to novelty and ecological accountability. Diagrammatic depictions outline tactical approaches to lowering carbon emissions, highlighting the circular economy in terms of material recycling and the creative recycling of agricultural and food waste into environmentally acceptable packaging. Modern meat processing techniques, automation, and smart technology are all explored, emphasising waste minimization, energy efficiency, and sustainable practices.In terms of the future, the assessment offers a peek at how biotechnological developments and uses of nanotechnology will combine to transform how meat is produced. The integration of precision biotechnology, ethical concerns, and sustainability ushers a new era of responsible and creative food production, positioning the meat processing sector as a pathfinder in addressing consumer needs.

Keywords: meat processing, technology innovation, sustainable polymers, composite coatings

INTRODUCTION

Global meat consumption, technology advancements, sustainability concerns, shifting consumer attitudes, the global supply chain, and regulatory frameworks influence the modern meat business. Due to population increase and economic expansion, there is a greater demand for meat globally, which presents both possibilities and problems for sustainable environmental practices, resource use, and production methods. The meat business has benefited from technological advancements in efficiency, quality, and safety regulations, including automation, robots, data analytics, and artificial intelligence. Environmental impact and other sustainability-related issues have brought eco-friendly techniques, waste minimization, and ethical animal husbandry to the forefront of public attention. Demand for slimmer cuts, organic and grass-fed choices, and additive- and preservative-free goods has increased due to shifting customer views. The COVID-19 pandemic showed weaknesses in the sector, indicating that it also confronts issues associated with the global supply chain, which are impacted by trade agreements, geopolitical dynamics, and disease outbreaks. In addition, strict legal frameworks control the processing of beef to guarantee food safety and quality, which calls for constant compliance and adaptation. Additionally, the sector is adjusting to various consumer demands, such as the growing desire for meat, plant-based substitutes, and other cutting-edge protein sources. Industry participants must balance sustainability, manufacturing efficiency, and response to changing customer needs while navigating this complicated terrain. The meat business must integrate economic, social, and environmental factors into its supply chain management practices, which presents obstacles. Maintaining a favorable customer image while being committed to environmental stewardship is critical to the

industry's survival. To adapt to changing customer demands, the business is also looking into novel protein sources like cultured meat **[1]**, **[2]**, **[3]**, **[4]**.

Growing Global Demand for High-Quality Meat Products

There is a noticeable trend in meat consumption toward a greater desire for premium meat products. This shift is driven by growing prosperity, changing eating habits, cultural influences, increased awareness of health and wellbeing, and the idea of "premiumization." With more spending power, consumers seek superior, unique meat alternatives that prioritize flavor, nutritional value, and overall quality. Leaner cuts, organic alternatives, and meats farmed ethically and sustainably are becoming increasingly popular. With customers prepared to pay more for perceived superior quality, "premiumization" has infiltrated the meat industry and resulted in significant development in niche markets like those for organic and grass-fed beef. The demand for high-quality meat products has increased globally due to factors such as the globalization of culinary trends, technical developments in distribution, and accessibility to a wider selection of meat options. The meat industry has to innovate and adapt to satisfy the increasing demand from across the world. It should prioritize ethical and sustainable practices, allocate resources towards quality assurance, and adopt technologies that improve the overall quality of meat products and production efficiency. The necessity for coordinated, multilevel activities involving all stakeholders to shift towards more sustainable meat supply chains has led to an increase in academic focus on sustainability practices and difficulties in the meat supply chain. The sector is experiencing cyclical problems, which have been exacerbated by supply chain management that prioritizes commercial goals. As a result, it's important to evaluate the meat industry's sustainable supply chain management level, paying particular attention to its social, economic, and environmental aspects. Upholding a favorable consumer image while exhibiting a commitment to environmental stewardship is critical to the survival of the global animal protein business. In addition to preserving and enhancing customer trust, livestock producers must increase production and efficiency, improve animal health and welfare, and promote sustainability by using byproducts and innovative feeds, as well as through improvements in climate science. The meat business is now investigating novel protein sources, such as cultured meat, to adapt to changing customer tastes **[3]**, **[4]**, **[5]**.

Importance of Advanced Techniques in Meat Processing and Preservation

The use of sophisticated methodologies in the processing and preserving meat is important to tackle current issues and conform to evolving customer demands. With automated technologies simplifying jobs like cutting and packing, these advancements greatly increase production and efficiency while lowering operating costs and increasing throughput. Furthermore, across the processing chain, these cutting-edge technologies support improved quality assurance and product consistency. Consumer trust is fostered by precision meat grading, real-time monitoring, and advanced quality control techniques that guarantee meat products meet or surpass industry requirements. Modern preservation techniques like High-Pressure Processing (HPP) and Modified Atmosphere Packaging (MAP) increase the shelf life of beef products. This improves the meat industry's overall sustainability while also lowering food waste. By using cutting-edge methods, it is feasible to adjust to changing consumer tastes, such as a need for slimmer cuts, organic alternatives, and goods with fewer chemicals. Being flexible is essential to preserving market competitiveness. The application of intelligent technology, data analytics, and energy-efficient processing techniques must consider sustainability and resource optimization. These approaches not only lessen the environmental effect of meat production but also align with rising consumer concerns about sustainability. Advanced procedures also address the crucial subject of mitigating dangers to food safety. These technologies range from real-time pathogen control methods to strict hygiene procedures **[4]**, **[6]**, **[7]**.

Economic Significance for Stakeholders in the Meat Industry

Adopting cutting-edge methods for meat processing and preservation, known as "Meat 4.0," is crucial to guaranteeing food safety and quality in the sector. These methods provide exact control over operations, regulatory compliance, and improved traceability from farm to fork. They use elements of Industry 4.0 such as robots, Internet of Things, Big Data, and blockchain. Strict hygiene protocols, real-time monitoring, and data-driven quality control procedures are some of the advanced techniques that help manage pathogens and ensure food safety. In addition, they are essential for maintaining customer trust, brand integrity, and quick action in case of a safety issue or product recall. The meat business may enhance processing, preservation, and the analysis of meat quality, safety, and authenticity by using digitalized and automated solutions and integrating business 4.0 technology. These developments lessen food loss and waste and help create high-quality, safe meat products and stop meat fraud **[6]**.

Role in Ensuring Food Safety and Quality

The meat business is undergoing a lot of change right now, with possibilities and challenges reshaping the industry's terrain. The growing focus on sustainability and its connection to environmental issues like deforestation and greenhouse gas emissions is a significant challenge. An further obstacle arises from evolving customer inclinations, such as an increasing fascination with plant-based substitutes, which demands conventional meat producers to adjust and introduce novel ideas. Disruptions to global supply chains are a persistent problem, as demonstrated by pandemics and geopolitical unrest. Sustainable operations depend heavily on the industry's capacity to bounce back and adjust in the face of these disturbances. Furthermore, the strict regulatory framework necessitates constant efforts to guarantee adherence to changing standards for food safety, quality, and ethics, which calls for constant expenditures in procedures and technology. Conversely, the sector faces many possibilities. The emergence of substitute proteins, including those derived from plants and cultured in laboratories, presents an opportunity for market segment expansion and diversity. Constant technology advancements, such as data analytics and automation, offer chances for improved traceability, efficiency, and quality control, helping the sector meet various obstacles and remain competitive. The meat sector has the opportunity to create better product offers, such as slimmer cuts and alternatives that cater to specific dietary needs, thanks to the focus on health and wellness trends. Direct-to-consumer and e-commerce strategies are revolutionizing marketing and distribution by giving manufacturers direct access to customers and opening up new avenues for product differentiation. Furthermore, firms that provide priority to transparent information regarding sourcing, production techniques, and environmental policies stand to benefit greatly from the growing customer desire for openness. Developing trust via open and honest business practices may provide you with a competitive edge in a market where customer expectations are always changing **[2]**, **[6]**, **[8]**.

Technological Enhancements in Carcass Handling for Efficiency

Artificial intelligence (AI) is a major breakthrough transforming operational capabilities, efficiency, and decision-making processes in meat processing lines. AI technologies are becoming more widely used in all phases of the meat production process, providing special benefits and advancing the sector. Applying machine learning algorithms for predictive maintenance is a crucial component of AI integration. AI systems can forecast when machinery will likely need repair by evaluating past data and equipment performance. This allows for proactive interventions to reduce downtime, improve equipment dependability, and maximize overall processing efficiency. AI is also being used in processing lines for computer vision-based quality control. AI algorithms and cutting-edge imaging technologies are utilized to analyze and evaluate beef products in real-time, guaranteeing consistency in quality standards by spotting flaws, irregularities, or processing-related deviations from intended specifications. AI-driven automation significantly improves processing processes by evaluating and interpreting data from multiple sensors and making real-time modifications to factors such as temperature, humidity, and processing durations. This flexibility results from improved product uniformity, decreased waste, and increased operational efficiency. Additionally, by evaluating demand patterns, market trends, and historical sales data, AI integration in processing lines helps inventory management by optimizing inventory levels, coordinating production with real market demands, reducing excess inventory expenses, and averting stockouts. By offering practical insights into production planning, resource allocation, and overall operational strategies, AI-powered decision support systems improve the decision-making abilities of processors and promote agility and responsiveness in a changing market context. By utilizing data from processing lines and beyond, AI also makes the supply chain visible and enhances traceability by following the path of beef products from farm to fork and promptly detecting and resolving any problems that may arise. AI systems must be continuously improved over time to ensure accuracy and relevance. Issues with biases, data quality, and responding to unanticipated occurrences must be resolved to achieve the most possible benefit from AI integration in meat processing. Working together with AI and conventional processing methods will undoubtedly lead to more innovations and efficiency gains in the meat processing sector as technology develops **[9]**.

Integration of Artificial Intelligence in Processing Lines

Artificial intelligence (AI) has a revolutionary effect on the meat processing industry by improving decision-making, operational efficiency, and overall intelligence in processing lines. AI technologies are transforming many aspects of the industry as they are effortlessly integrated into processing activities. AI-powered vision systems evaluate visual data in real-time, using machine learning algorithms to find faults, inconsistencies, and deviations from strict quality standards. This is a crucial use of AI in meat processing, particularly in quality control. This guarantees a better caliber of goods and makes it possible to identify and stop potential problems early on.

AI also uses sophisticated data analytics to provide a predictive component to meat preparation. Artificial intelligence (AI) algorithms examine historical and real-time data to predict possible bottlenecks, improve production schedules, and simplify the processing workflow overall. This leads to increased operational efficiency and less downtime. Artificial Intelligence has a role in autonomous and adaptive decision-making in robotics. AI-enabled robots can adapt their behavior dynamically in response to input and changing circumstances. AI, for example, allows robots to adjust their motions to changes in the texture of meat during the cutting and deboning process, resulting in clean, accurate cuts every time. AI has an impact on processing line monitoring and maintenance as well. Predictive maintenance systems powered by artificial intelligence (AI) foresee equipment failures, organize maintenance tasks at the best times, and reduce unscheduled downtime, improving processing equipment dependability and preserving operating efficiency. Moreover, integrating AI supports sustainable meat processing objectives. Algorithms that save energy maximize equipment performance, which lowers total energy usage. AI-driven waste reduction solutions also enhance sustainability by reducing product loss and optimizing resource usage. The search results provide light on the development of integrated management systems in food production organizations, the influence of processing and preservation technologies on meat quality, and technical improvements in meat processing. These elements support a thorough comprehension of the larger AI integration context **[10]**.

Precision Meat Grading and Quality Control

Artificial intelligence (AI) has brought about a revolutionary change in the meat processing industry by bringing cutting-edge capabilities that significantly improve operational efficiency, decision-making, and overall intelligence in processing lines. Precision in these operations is essential to fulfill regulatory criteria, satisfy customer expectations, and guarantee consistent product quality. One of the primary technologies affecting meat grading is computer vision, which includes using cameras and image processing algorithms to grade various qualities of meat. These technologies can accurately assess characteristics, including color, muscle texture, marbling, and fat content. The grading process is made less variable and subjective by using automated grading based on objective visual criteria, which guarantees a consistent and standardized assessment. In addition to eye examination, spectroscopic methods play a key role in precision meat grading. For example, meat composition may be non-destructively analyzed using near-infrared spectroscopy. By examining light absorption at various wavelengths, this approach yields comprehensive data on the composition of fat, protein, and moisture content. Precision grading plays a major role in yield optimization when it comes to processing beef. Through precise evaluation of meat quality, processors can decide how best to use various cuts. Higher-quality cuts, for example, may be used for premium items, whilst cuts with certain attributes might be used for value-added products or additional processing. Furthermore, these systems' capabilities are improved when artificial intelligence (AI) is used in precision grading. With time, AI algorithms' accuracy can increase because of their ability to continually learn from and adapt to a large quantity of data. Because of this adaptive learning, the system can take into account differences in the properties of the meat and maintain consistency in the grading criteria even when processing circumstances alter. Precision meat grading technologies promote efficiency and transparency in the meat processing sector. With automated grading, there is a uniform and documented rating of meat quality, giving traceability throughout the supply chain. Meat processing quality control techniques go beyond grading to include food safety and following rules and regulations. Microbial detection devices and other advanced sensor technologies allow for real-time monitoring of meat products for any contamination. By stopping the spread of inferior goods, these technologies' quick identification and action improve food safety **[9]**, **[11]**.

Spectroscopic and Imaging Technologies for Grading

With spectroscopic and imaging technologies, the evaluation of meat products has advanced significantly, particularly in safety, quality, and grading. Hyperspectral imaging (HSI) has emerged as a key method for the rapid and non-destructive examination of meat safety because it gives the spatial distribution of spectrum for assessing muscle meals. This method has shown promise in assessing physical, chemical, and biological safety indicators and other associated hazards or toxins when applied to a range of animal products, including fish, chicken, cattle, lamb, and pigs. In addition to HSI, other spectroscopic techniques have been used for the non-destructive investigation of meat composition, such as near-infrared spectroscopy, which has provided extensive information regarding moisture content, protein levels, and fat composition. These methods, which provide objective data to evaluate the qualitative attributes of these meat products, have proved crucial in assessing carcass composition and meat quality in sheep and goats. Additionally, the prediction of lamb loins' eating quality has been investigated by applying Raman spectroscopic technology. This method offers a quick and non-destructive way to evaluate lamb carcasses, enabling the estimation of eating quality based on interactions between biophysical and biochemical processes during processing and cooking. The meat processing sector might

transform with the implementation of new technologies such as HSI and different spectroscopic approaches, which can improve safety evaluation, quality control, and grading procedures. These non-invasive, non-destructive methods provide insightful information on the composition and quality of meat products, which enhances meat processing and food safety regulations overall **[12]**, **[13]**, **[14]**.

The contrast between old and modern approaches shows how meat preservation techniques are still evolving. Meat products may be kept fresher longer by using conventional techniques like smoking, salting, and drying, which lower moisture content, stop microbiological development, and stop spoiling. However, a paradigm change in the preservation of meat has been brought about by contemporary preservation procedures, which are driven by technical breakthroughs. Modern techniques extensively used to preserve meat quality include refrigeration and freezing, which use temperature control to slow down enzyme reactions and microbiological activity. Another modern method that efficiently delays oxidative degradation and microbiological spoiling is vacuum packaging, which eliminates air and produces a controlled atmosphere. Furthermore, high-pressure processing (HPP) involves applying high pressure to meat products to efficiently inactivate germs and pathogens while preserving the meat's nutritional value and flavor. Meat processing has dynamically adapted to changing customer demands and technology improvements by switching from traditional to contemporary preservation procedures. Modern ways provide accuracy, efficiency, and the capacity to achieve exacting quality requirements, while ancient methods remain culturally relevant. The comparison of various approaches highlights the complexity of meat preservation, where various innovative and traditional processes are used to create the wide range of procedures used in the meat processing business **[15]**.

Traditional vs. Modern Preservation Techniques

The search results include information on conventional and contemporary food preservation techniques, including meat. They discuss using several preservation theories and methods on meat and meat products, including hurdle technology, predictive microbiology, and preservatives such as nisin, natamycin, polyphenols, and chitosan. The papers also stress how crucial it is to preserve food to balance tradition and innovation, considering customer preferences, food safety, and quality. The information gleaned from the search results is consistent with the discourse surrounding the constraints of conventional preservation techniques in light of contemporary needs and anticipations. It highlights the necessity for creative conservation techniques and scientific answers to address modern demands for affordable preservation and user satisfaction regarding well-being, dietary needs, and sensory perception. The search results support the assumption that contemporary preservation techniques are more prevalent due to the expansion of the food business, particularly meat processing, even if older preservation methods still hold cultural value. These contemporary techniques overcome the drawbacks of the old ways by utilizing technology-driven solutions that provide accuracy, consistency, and adaptability to the wide range of modern customer preferences **[15]**, **[16]**, **[17]**, **[18]**, **[19]**.

Limitations of Traditional Methods in a Modern Context

The necessity to address modern challenges and customer expectations while maintaining sustainability and efficiency has led to the growth of meat preservation techniques. Due to their shortcomings in uniformity, speed, and environmental effects, traditional processes, including smoking, drying, and salting, have come under review. Eco-friendly methods, consistency, and a decreased need for chemicals are given priority in modern preservation techniques, which demonstrate a dedication to striking a balance between environmental responsibility and gastronomic legacy. To achieve sustainability, the environmental effects of conventional techniques must be addressed. For example, smoking is a prevalent traditional preservation method that exacerbates air pollution and deforestation. On the other hand, modern preservation techniques try to reduce waste production and resource usage. Furthermore, the difficulties in getting consistent outcomes with conventional procedures because of things like individual skill levels and environmental circumstances highlight the need for more dependable and effective preservation strategies. Modern methods use technological breakthroughs to guarantee consistency and satisfy the strict quality and safety requirements imposed by regulatory bodies. Less reliance on chemicals is necessary for efficient and sustainable preservation. Current preservation methods, driven by customer demand for clearer labeling and healthier food alternatives, seek to reduce additives while preserving product safety and quality. The development of preservation methods has also focused on the sensory characteristics of meat products. Modern preservation techniques highly value maintaining the meat products' sensory attributes to satisfy consumers who want natural, minimally processed meals **[20]**.

Evolution towards Sustainable and Efficient Preservation Techniques

The necessity to fulfill the needs of an expanding global population while maintaining sustainability and efficiency has propelled the innovation of meat preservation techniques. Innovative techniques that prioritize

ecological concerns, resource efficiency, and minimizing environmental effects have replaced traditional methods. Contemporary methods like high-pressure processing (HPP) and modified atmosphere packaging (MAP) aim to decrease the need for chemical additives while increasing shelf life. These techniques, including freezing, vacuum packing, and refrigeration, put efficiency, accuracy, and speed first. Artificial intelligence and data analytics are two examples of technological developments that have improved the accuracy and consistency of preservation procedures. The environmental impact of meat products has also been lessened by using eco-friendly packaging materials, such as recyclable and biodegradable materials. The search results provide information on certain contemporary preservation methods, such as using edible coatings made of alginate and essential oils to enhance the quality of thawed lamb meat following prolonged frozen storage. The results demonstrate the continuous innovation in preservation strategies by discussing creating a thermodynamic model and using non-invasive technologies to monitor the hot air-drying process of chicken flesh. Additionally, the creation of a modeling technique to characterize and simulate the pH evolution of meat products under various preservation settings is also highlighted, as is the significance of pH measurement in monitoring the microbial deterioration of fresh meat products **[21]**, **[22]**.

Principles of MAP and Gas Composition Optimization

Meat products can have their shelf lives extended by Modified Atmosphere package (MAP), which alters the gas composition inside the package. Carbon dioxide (CO2), nitrogen (N2), and oxygen (O2) are the main gases used in MAP. Certain meat kinds require oxygen to be maintained in aerobic conditions. In contrast, nitrogen is utilized to displace oxygen and prevent color degradation and carbon dioxide is used to decrease microbial activity. The optimization of gas composition takes into account the target shelf life, kind of meat, cut, and other specifics of the meat product. Gas analyzers and washing systems are examples of advanced technology making it easier to manage the gas composition during MAP precisely. In addition to increasing the shelf life of meat products, this technique reduces waste by using fewer chemicals and preservatives. With the use of MAP, it is possible to customize atmospheric conditions to meet the particular needs of various meat products, resulting in longer shelf lives, less waste, and the provision of consumers with fresh, premium meat. A careful procedure is involved in optimizing the gas composition in MAP, considering elements like the meat's kind, cut, and intended shelf life. A balanced mixture of gases is applied for poultry to reduce microbial development without sacrificing color. Still, a larger amount of carbon dioxide is frequently used for red meat to maintain its vibrant red color. The gas composition is carefully regulated to maintain the meat's texture, color, and nutritional value while maintaining its visual attractiveness. Gas analyzers and washing systems are examples of advanced technology that make it easier to manage the gas composition during MAP precisely. Gas analyzers provide data on gas concentrations in real time by monitoring the environment within the container. Gas flushing systems are adjusted based on this data to maintain ideal conditions. Utilizing inert gases, such as nitrogen, helps to displace oxygen further, reducing the possibility of oxidative rancidity and preserving the meat's freshness. By reducing the need for chemicals and preservatives, MAP increases shelf life and reduces waste. The packaging's regulated environment maintains the meat's natural properties while also improving microbiological safety. MAP appears as a technique that satisfies customer desires for minimally processed and additive-free goods while guaranteeing the availability of premium meat over a longer period of time **[23]**.

Understanding HPP as a Non-Thermal Preservation Method

A non-thermal preservation technique called High-Pressure Processing (HPP) involves applying high hydrostatic pressure—typically between 100 and 600 megapascals (MPa)—to packed food goods. Because HPP doesn't rely on heat to achieve microbial inactivation like standard thermal processing methods do, it's especially good at retaining meat's nutritional value and freshness. The effect of pressure on the cellular architecture of microorganisms is central to the basic idea of HPP. High pressure causes alterations in the cell membranes of bacteria and other pathogens, which impairs their ability to function and ultimately results in cell death in food—improved food safety results from HPP's ability to inactivate bacterial spores and vegetative germs effectively. It works especially well against bacteria like Salmonella, Escherichia coli (E. coli), and Listeria, which are frequent problems in the meat processing industry. Additionally, HPP maintains the meat products' original flavor, texture, and appearance, satisfying customer demands for premium, minimally processed goods. The technique works well with various meat types, such as red meat, chicken, and fish. It is also helpful for producing pre-packaged deli meats, sausages, and other convenience foods, where it is crucial to preserve freshness and lengthen shelf life. It has been demonstrated that applying HPP to meat products may reduce or eliminate harmful microorganisms while maintaining the goods' nutritional value. Adjustments are crucial because HPP produces modest changes in components, such as protein denaturation in milk and fat oxidation in meat. In general, HPP

preservation can prolong the shelf life and enhance the sensory quality of products while posing less risk of harm to the component and sensory qualities than heat processing **[23]**, **[24]**, **[25]**.

Efficacy in Pathogen Reduction and Spoilage Prevention

It is commonly known that High-Pressure Processing (HPP) effectively reduces pathogens and prevents beef products from spoiling. Without the need for high temperatures, HPP has been demonstrated to successfully decrease and eradicate pathogens in meat products, including harmful strains like Salmonella, E. coli, and Listeria, reducing the possibility of the meat's nutritional and sensory qualities being compromised. Furthermore, by deactivating moulds and other spoilage microbes, HPP helps prolong the shelf life of beef products, avoiding decomposition and keeping the product fresh while lowering the possibility of off-flavours, smells, and textural changes during storage. The effectiveness of HPP is largely due to its non-thermal nature, which preserves the meat's original flavor, texture, and color while enabling microbial inactivation without heating the meat to high temperatures. This feature is especially helpful in satisfying customer demands for premium beef products with little to no processing. Moreover, HPP's adaptability to various meat types—such as red meat, poultry, and seafood—makes it useful for various meat forms and convenient for creating products like pre-packaged deli meats and sausages **[25]**.

Pulsed Electric Field (PEF) Technology in Meat Preservation

Food items are subjected to brief, powerful electric field pulses as part of the non-thermal preservation technique known as pulsed electric field (PEF) technology. Destabilizing microorganisms' cell membranes during meat preservation improves food safety by rendering pathogens inactive without the need for heat. The technique has proven effective in inactivating microorganisms, extending shelf life, and lowering the risk of foodborne infections. PEF technology has demonstrated the potential to improve meat tenderization while maintaining the inherent qualities of meat products. It is also acknowledged for being more energy-efficient than conventional thermal processes, which helps meat processing take sustainability into account. However, to get the best outcomes possible with PEF technology, several parameters need to be carefully taken into account, including product qualities, treatment time, and pulse intensity. More studies on PEF's impact on meat and aquatic products are necessary to support industrial applications, despite its promising results **[26]**, **[27]**.

Use of Natural Antimicrobial Agents in Preservation

Due to its potential for efficient and sustainable non-thermal preservation techniques, using natural antimicrobial agents in meat preservation has drawn attention. These agents include plant-based chemicals, bacteriophages, antimicrobial peptides, essential oils, and botanical extracts. Botanical extracts high in polyphenols, such as thymol, oregano oil, and rosemary extract, display antibacterial and antioxidant characteristics, contributing to increased shelf life. Meat product decomposition and microbial contamination can be addressed using bacteriophages and antimicrobial peptides. Furthermore, the antibacterial and antioxidant capabilities of plant-based substances such as polyphenols, which are present in green tea and grape seed extracts, have been investigated in meat preservation. Although there are difficulties in maintaining uniform effectiveness and managing sensory aspects, the use of natural antimicrobial agents corresponds with consumer inclinations towards minimally processed and additive-free products. Utilizing natural antimicrobial agents is a comprehensive strategy that satisfies customers' desire for clean-label and natural products and the industry's environmental aims. Thus, using natural antimicrobial agents to preserve meat offers the meat processing sector a potential approach **[28]**.

Building Consumer Trust through Blockchain Traceability

Indeed, blockchain technology is significantly improving traceability and transparency in the meat processing sector. Blockchain technology makes it possible to track the entire supply chain of beef products, from the farm to the processing facility, distribution, and retail locations. This gives customers accurate and unbiased information about the items they buy. The integrity of data about the procurement, processing, and distribution of meat products is enhanced by the immutability of blockchain records, which guarantees that the information customers access is correct and has not been altered. Blockchain traceability makes it possible to quickly and precisely identify impacted batches in the case of a product recall or crisis, which is essential for consumer protection and public safety. In addition to addressing customer concerns regarding the provenance and quality of meat products, this increased openness and traceability also help to build industry trust and responsibility. It's crucial to remember that while blockchain technology significantly improves traceability in the meat processing sector, customer trust and preferences are impacted by several other factors. For example, customers prefer USDA certificates over blockchain traceability when making meat choices, according to research looking at the use of

blockchain in food traceability for beef in the US. This implies that for industrial ramifications, customer education about product data's value—rather than data management technologies—is crucial **[28]**, **[29]**.

Data Analytics for Process Optimization

Indeed, the meat processing sector increasingly relies on data analytics to drive efficiency and process improvement. Meat processors may improve overall operational performance by extracting essential insights from the massive volumes of data collected during the production cycle using sophisticated analytics. Comprehensive data gathering from all phases of meat processing, including procurement, manufacturing, and distribution, is the first step in the process. This information, which includes variables like temperature, humidity, processing speeds, and equipment performance, provides the basis for insightful analysis that may spot trends, patterns, and areas needing improvement. Processors may make proactive decisions to fix inefficiencies immediately, improve production processes, and allocate resources efficiently by using data analytics to discover operational inefficiencies and bottlenecks inside the processing line.

Moreover, data analytics forecasts maintenance requirements analyze past equipment performance data and minimizes downtime to support predictive maintenance programs and promote a continuous improvement culture in meat processing plants. Data analytics' capabilities are enhanced when it is combined with Internet of Things (IoT) devices and smart technologies. This allows for more complete optimization techniques and a full picture of the processing environment. While data analytics may significantly improve process efficiency and optimization in the meat processing business, it is crucial to prioritize data security and control. Processors must follow industry standards and data protection laws and put strong cybersecurity measures in place to guard sensitive data from tampering or illegal access **[30]**.

Utilizing By-Products for Value-Added Applications

One important tactic that helps reduce waste and promote environmental sustainability in the meat processing industry is the purposeful use of by-products for value-added applications. Meat processors may reduce waste and increase income by using by-products to create high-value goods. By-products of meat processing may extract several useful ingredients, including proteins, lipids, collagen, and gelatin, used in food, medicine, and cosmetics. Additionally, meat processors connect with circular economy concepts by maximizing organic waste's value and energy potential by reusing by-products for pet food, animal feed, bioenergy generation, and industrial applications. By lowering the amount of garbage dumped in landfills, this strategic use of by-products improves the environment and complies with laws supporting resource conservation and waste reduction. To improve the market's impression of the sector and encourage responsible consumption, it is imperative to inform customers about the value-added uses of by-products. Further information about the recovery and recycling of high-value protein from animal processing by-products, the use of cutting-edge non-thermal technologies to recover and value-added products from crustacean processing by-products, and the potential of vegetable processing by-products for the production of meat products with added value can be found in the search results. These studies demonstrate the increasing interest and continued study in the topic of circular economy methods for by-product valuation in several industries, such as the processing of vegetables, meat, and seafood **[31]**, **[32]**, **[33]**.

Innovative Techniques for Minimizing Processing Waste

The search results explain how the meat processing sector implements cutting-edge sustainability and waste reduction methods. These include the recovery and recycling of high-added value protein from animal processing by-products, the application of material flow cost accounting (MFCA) analysis technique in meatball production for waste reduction, and the use of high-pressure processing to compensate for the effects of salt reduction in ready-to-eat meat products. The abovementioned studies underscore the continuous investigation and implementation of inventive approaches to augment sustainability and diminish waste within the meat processing sector. Implementing these cutting-edge methods—like high-value protein recovery, material flow cost accounting analysis, and high-pressure processing to lower salt levels—shows how committed the meat processing industry is to developing sustainable practices and cutting waste **[31]**, **[34]**, **[35]**.

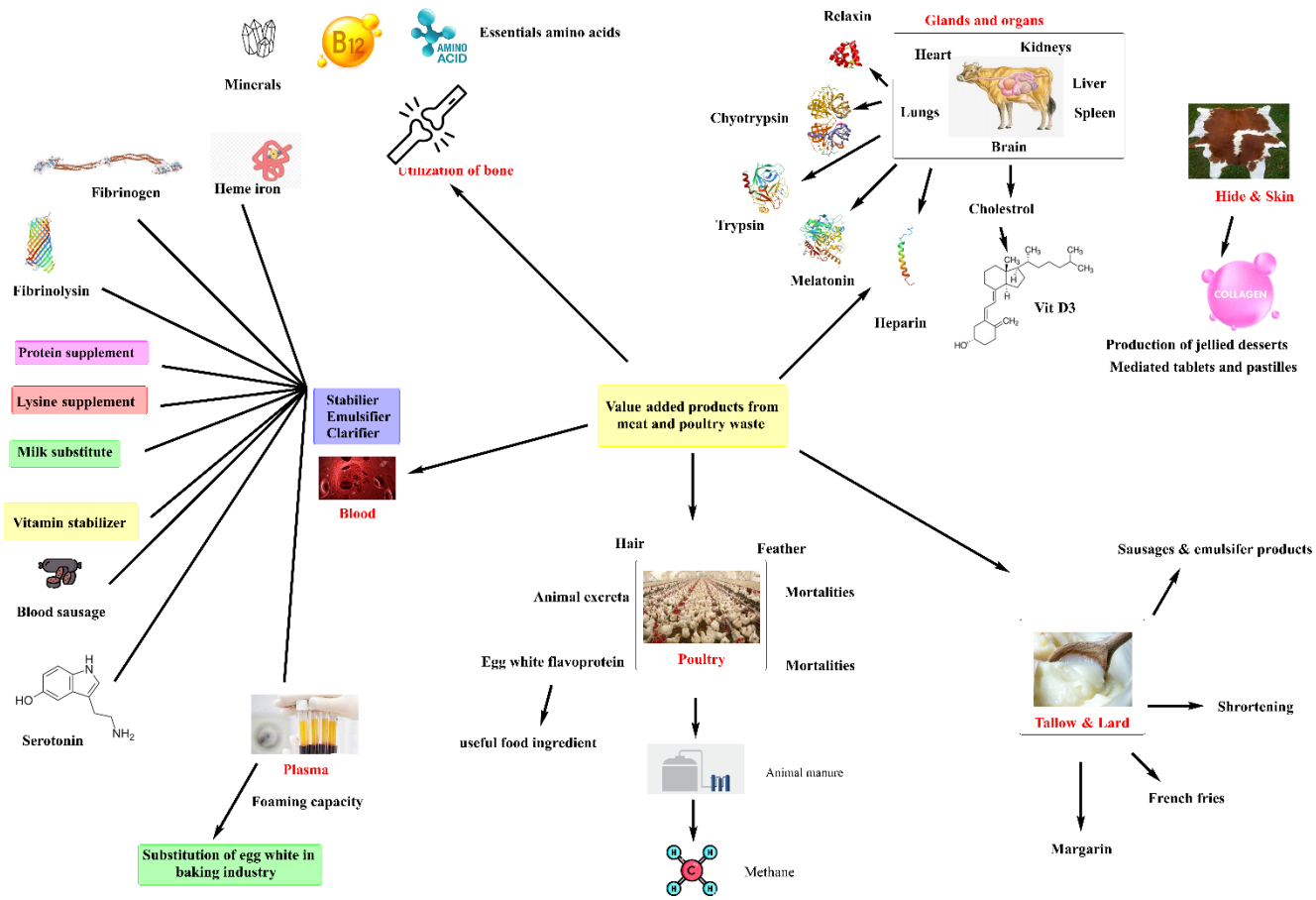


Figure 1 Scheme of various value-added products from meat processes.

Renewable Energy Integration in Meat Processing Facilities

Energy-Efficient Equipment and Technologies

The total energy consumption of meat processing plants is primarily influenced by the operation of their HVAC (heating, ventilation, and air conditioning) systems. These facilities are gradually upgrading to energy-efficient HVAC systems with sophisticated controls, zoning, and optimum ventilation methods to lower energy usage and operating costs while maintaining ideal working conditions. This literature review aims to investigate how energy-efficient HVAC systems, in conjunction with other smart practices and technology, help meat processing plants use less energy.

Energy-efficient HVAC systems have been found to regulate temperatures more efficiently, resulting in reduced energy consumption and operational costs while still maintaining optimal working conditions **[36]**. Refrigeration is essential in beef preparation, and switching to energy-efficient equipment helps lower energy usage. Facilities invest in cutting-edge control systems, high-efficiency compressors, and routine maintenance to maximize refrigeration performance. Energy-efficient refrigeration systems can create a more sustainable processing operation by reducing energy usage and operating expenses **[36]**.

These systems utilize advanced controls, zoning, and optimized ventilation strategies to ensure the temperature is regulated effectively throughout the facility. Doing so minimizes energy waste and provides a comfortable working environment for the staff. Real-time monitoring and management of energy-consuming operations in meat processing plants is made possible by deploying smart sensors and automation technology. These facilities may optimize energy consumption by monitoring and adjusting settings depending on real demand by combining automation systems and sensors. This method improves efficiency and lowers energy waste by fostering a more flexible and adaptable approach to energy management **[37]**. Enhancing energy efficiency in processing plants may also be achieved by improving insulation. The need for excessive heating or cooling is decreased when processing areas have more constant temperatures thanks to upgrades in the insulation of the walls, ceilings, and equipment. Improved insulation lowers energy use while increasing thermal efficiency and giving employees a more comfortable workplace **[38]**. Meat processing requires a lot of water, thus using cutting-edge water-conservation technologies is crucial for energy efficiency. Facilities use low-flow fixtures, water-recycling programs, and water-efficient cleaning systems to reduce water use. By reducing water usage, these technologies also lessen the energy needed to heat and treat water, which adds to the overall sustainability of **[39]**.

Employee Involvement and Awareness

Encouraging a culture of consciousness and accountability among facility personnel is essential for optimizing energy use. Facilities involve staff in energy-saving projects and offer training on energy-saving techniques. Involving employees helps the institution create a sustainable culture by identifying and implementing energy-saving solutions together [39]. With the introduction of next-generation technology that puts efficiency, sustainability, and quality first, the processing and preservation of meat is going through a radical change. These state-of-the-art developments can transform the meat business in several ways, including how goods are processed, stored, and distributed to customers. Utilizing cutting-edge capabilities and enhanced sensing and imaging technologies will improve quality control and monitoring throughout manufacturing. Processing equipment will have high-resolution cameras, spectroscopy, and hyperspectral imaging integrated for real-time meat quality evaluation, guaranteeing accuracy and precision in sorting and grading. Precision biotechnology used to manufacture cultured or lab-grown meat is poised to revolutionize the meat industry. This technique, which produces cattle in controlled settings, replaces the need for conventional livestock farming and provides a resource-efficient and sustainable substitute. Blockchain for Supply Chain Transparency will be essential to maintaining traceability and transparency in the supply chain for beef **[40]**, **[41]**, **[42]**.

Nanotechnology Applications in Meat Industry

The meat business might transform thanks to nanotechnology's ability to help produce new functional meat products and creative packaging. Nanomaterials can target the distribution of bioactive chemicals, increase bioavailability, have antibacterial effects, and improve sensory acceptability in the processing and packaging of meat. However, there are obstacles to using nanomaterials, including unknowns around component manufacture, unstable delivery mechanisms in meat products, and possible health hazards. It is imperative to effectively tackle these obstacles to use nanotechnology in the meat sector. While the most promising area for nanotechnology use is meat packing, long-term impacts on human health and the environment due to nanoparticle migration from the packaging require additional investigation. Ensuring the safety of nanoparticles in food processing and tackling issues related to public acceptance, economics, and legislation are critical to the future of nanotechnology in meat products. The laws that now govern the use of nanomaterials in food items are still developing and vary from place to place. Although there are potential advantages to using nanotechnology in the food sector in terms of enhancing food safety and product quality, there are worries about the safety and legal implications. Nanomaterials in food products—including meat—are continuously being studied and regulated to protect consumer safety and the environment. More investigation is required to identify safe nanomaterial applications that might be commercialized precisely. The regulatory environment around nanomaterials in food items is dynamic and complicated, necessitating a thorough evaluation of the advantages and disadvantages. The laws that now govern the use of nanoparticles in meat products are still developing and range from place to place. The application of nanotechnology in the food business, particularly in meat products, raises concerns about safety and regulatory issues, while it can potentially improve food safety and product quality. To guarantee consumer safety and minimize environmental damage, using nanoparticles in meat products is the subject of continuous study and regulatory oversight. More investigation is required to identify safe nanomaterial applications that might be commercialized precisely. The regulatory environment around nanoparticles in meat products is dynamic and complicated, necessitating a thorough evaluation of the advantages and disadvantages. As a result, it's critical to remain current on the laws and policies that apply to the use of nanoparticles in meat products in a given area. Research and regulatory examination into the possible health hazards related to the use of nanoparticles in meat products is continuing. Although the targeted distribution of bioactive substances, enhanced bioavailability, and antimicrobial properties are some of the advantages of nanotechnology, there are worries about the safety of nanomaterials in food items, particularly meat. The manufacture of components, the stability of the delivery systems in meat products, and the possible health hazards posed by the same attributes that simultaneously provide the advantages present challenges.

Further research is necessary to determine the long-term impacts of nanomaterials on human health and the environment since they can persist, accumulate, and cause toxicity. External validation of research results needs to be improved by the significant diversity in study designs and tested nanomaterials that exist today. More investigation is needed to identify which safe nanomaterial applications will most likely be commercialized. To protect consumer safety and the environment, it is crucial to keep current on the most recent research findings and legal requirements about using nanomaterials in meat products **[43]**, **[44]**, **[45]**, **[46]**.

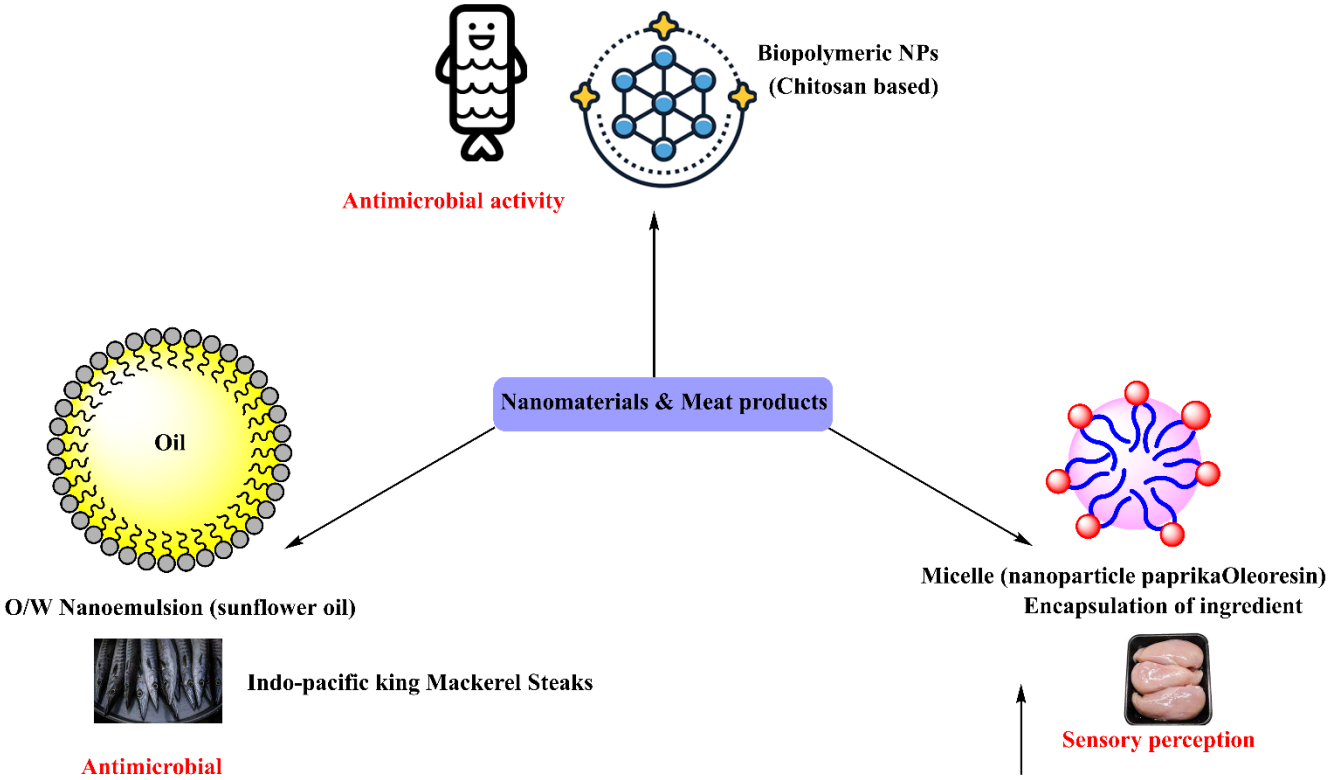


Figure 2 NPs applications in meat industries **[43]**.

Biotechnological Advancements in Cultured Meat Production

Potential advantages of using nanotechnology in the meat sector include increased food safety, longer shelf lives, and better contamination management and detection. However, nanomaterials have obstacles, such as unknowns around component manufacture, unstable delivery mechanisms in meat products, and possible health hazards. Using nanosensors in the bioprocess of cultured beef makes it easier to regulate and evaluate quality across the food supply chain. Although meat packing appears to be the area with the most potential application for nanotechnology, further research is needed to determine the long-term impacts of nanomaterial migration on human health and the environment. The future of meat products using nanotechnology hinges on overcoming obstacles related to public acceptability, economics, and regulation, as well as ensuring the safety of nanomaterials in food processing Poles **[44]**, **[47]**.

Advances in the production of cultured meat can be attributed to the use of biotechnological tools. These tools allow for manipulating cell characteristics to mimic particular characteristics of meat, such as modifying the distribution of muscle fibers, fat content, and protein composition to improve the taste and mouthfeel of products made from cultured meat. Moreover, precise biotechnology makes constructing designer cells with specific nutritional qualities in cultured meat possible, creating goods with improved health advantages. The emergence of intricate tissue architectures in cultured meat is facilitated by bioprinting technology, which raises the variety and realism of items made from cultured meat. Furthermore, biotechnology developments have focused on sustainable production by creating a serum-free culture medium, which lessens dependency on animal-derived ingredients and guarantees cruelty-free and sustainable production of cultured meat. Large-scale bioreactor systems, automation, and improved bioprocessing methods are essential for increasing the output of cultured meat and making it commercially feasible for general consumption. Biotechnological techniques have a special focus on the creation of cultured fat. Lean meat structures are enhanced by manufacturing cultured fat, which is made by integrating produced fat cells into meat products to mimic the marbled effect of conventional meat. This improves the cultured meat's taste, juiciness, and overall sensory experience. Cellular agriculture advances biotechnologically while addressing ethical and sustainable issues. Methods, including cellular dedifferentiation and non-invasive cell sampling, are investigated to meet ethical requirements and sustainability objectives in the cultured meat sector. When taken as a whole, these biotechnology developments solve major obstacles and improve the manufacturing process, which helps the cultured meat sector become more widely accepted. These advancements in research and development help to provide a sustainable and moral substitute for traditional meat production **[48]**, **[49]**.

CONCLUSION

Innovation, sustainability, and ethical considerations drive a revolutionary meat processing and preservation shift. The business is exploring alternate sources, such as cultured meat, and implementing cutting-edge processing technology to meet the demands of a changing global landscape. Notable advancements influencing the future include precise biology, nanotechnology, and biodegradable polymers. Combining biodegradable and renewable polymers solves environmental issues and fits the expanding need for environmentally friendly packaging options. Because of their exceptional stability and barrier qualities, composite films are essential for the long-term storage of meat products, guaranteeing their quality and safety. This shift to environmentally friendly packaging demonstrates a dedication to environmental responsibility. Antioxidants and their constituents are transported via active films, which show a commitment to prolonging the shelf life of meat products and improving consumer health. The actions aimed at lowering the carbon footprint in meat processing are showcased by strategic methods, which are represented schematically through the use of agri-food waste in bio-eco friendly packaging and the recycling of materials for value-added goods. Modern methods of processing and preservation are thoroughly covered, demonstrating the investigation of meat technology. This includes anything from cutting-edge techniques for killing animals to utilizing intelligent technology and environmentally friendly farming methods. The sector is leading the way in adopting innovations that put efficiency, quality, and environmental responsibility first, demonstrating a dedication to comprehensive development. Future trends and prospects for the meat processing industry point to a continual evolutionary path. New technologies have the potential to completely change the meat business, such as the applications of nanotechnology and microbiological developments in the manufacturing of cultured meat. These developments provide answers to problems with food safety, sustainability, and the rising demand for premium beef products throughout the world. A comprehensive strategy is becoming more and more important as the meat business moves toward a future influenced by precise biotechnology, sustainable practices, and innovative processing techniques. It will be essential to strike a balance between environmental responsibility, ethical concerns, and technical innovation in order to build a meat processing business that is both future-ready and robust, capable of meeting the varied requirements of its customers and protecting the environment.

REFERENCES

1. Hötzel, M. J., & Vandresen, B. (2022). Brazilians' attitudes to meat consumption and production: Present and future challenges to the sustainability of the meat industry. In Meat Science (Vol. 192, p. 108893). Elsevier BV. https://doi.org/10.1016/j.meatsci.2022.108893
2. Capper, J. L. (2020). Opportunities and Challenges in Animal Protein Industry Sustainability: The Battle Between Science and Consumer Perception. In Animal Frontiers (Vol. 10, Issue 4, pp. 7–13). Oxford University Press (OUP). https://doi.org/10.1093/af/vfaa034
3. Kot, M. (2022). SUSTAINABLE SUPPLY CHAIN MANAGEMENT IN THE MEAT INDUSTRY IN POLAND. In Acta logistica (Vol. 09, Issue 04, pp. 487–499). 4S go, s.r.o. https://doi.org/10.22306/al.v9i4.356
4. Caccialanza, A., Cerrato, D., & Galli, D. (2023). Sustainability practices and challenges in the meat supply chain: a systematic literature review. In British Food Journal (Vol. 125, Issue 12, pp. 4470–4497). Emerald. https://doi.org/10.1108/bfj-10-2022-0866
5. Zidarič, T., Milojević, M., Vajda, J., Vihar, B., & Maver, U. (2020). Cultured Meat: Meat Industry Hand in Hand with Biomedical Production Methods. In Food Engineering Reviews (Vol. 12, Issue 4, pp. 498–519). Springer Science and Business Media LLC. https://doi.org/10.1007/s12393-020-09253-w
6. Echegaray, N., Hassoun, A., Jagtap, S., Tetteh-Caesar, M., Kumar, M., Tomasevic, I., Goksen, G., & Lorenzo, J. M. (2022). Meat 4.0: Principles and Applications of Industry 4.0 Technologies in the Meat Industry. In Applied Sciences (Vol. 12, Issue 14, p. 6986). MDPI AG. https://doi.org/10.3390/app12146986
7. ur Rahman, U., Sahar, A., Ishaq, A., Aadil, R. M., Zahoor, T., & Ahmad, M. H. (2018). Advanced meat preservation methods: A mini review. In Journal of Food Safety (Vol. 38, Issue 4). Wiley. https://doi.org/10.1111/jfs.12467
8. Zhou, G., Zhang, W., & Xu, X. (2012). China's meat industry revolution: Challenges and opportunities for the future. In Meat Science (Vol. 92, Issue 3, pp. 188–196). Elsevier BV. https://doi.org/10.1016/j.meatsci.2012.04.016
9. Mason, A., Romanov, D., Cordova-Lopez, L. E., Ross, S., & Korostynska, O. (2022). Smart knife: technological advances towards smart cutting tools in meat industry automation. In Sensor Review (Vol. 42, Issue 1, pp. 155–163). Emerald. https://doi.org/10.1108/sr-09-2021-0315

10. Gómez, I., Janardhanan, R., Ibañez, F. C., & Beriain, M. J. (2020). The Effects of Processing and Preservation Technologies on Meat Quality: Sensory and Nutritional Aspects. In Foods (Vol. 9, Issue 10, p. 1416). MDPI AG. https://doi.org/10.3390/foods9101416
11. Devika, G., Priya, Bandaru, C. S. R., Srinivas, R. V., Girdharwal, N., & Devi, M. N. (2023). Real-Time Quality Assurance of Fruits and Vegetables using Hybrid Distance based MKELM Approach. In 2023 International Conference on Sustainable Computing and Smart Systems (ICSCSS). 2023 International Conference on Sustainable Computing and Smart Systems (ICSCSS). IEEE. https://doi.org/10.1109/icscss57650.2023.10169197
12. Pu, H., Wei, Q., & Sun, D.-W. (2022). Recent advances in muscle food safety evaluation: Hyperspectral imaging analyses and applications. In Critical Reviews in Food Science and Nutrition (Vol. 63, Issue 10, pp. 1297–1313). Informa UK Limited. https://doi.org/10.1080/10408398.2022.2121805
13. Silva, S., Guedes, C., Rodrigues, S., & Teixeira, A. (2020). Non-Destructive Imaging and Spectroscopic Techniques for Assessment of Carcass and Meat Quality in Sheep and Goats: A Review. In Foods (Vol. 9, Issue 8, p. 1074). MDPI AG. https://doi.org/10.3390/foods9081074
14. Fowler, S.M., et al. Prediction of eating quality of lamb loin using Raman Spectroscopic technologies. 2021.
15. Ariyamuthu, R., Rupa Albert, V., & Je, S. (2022). An Overview of Food Preservation Using Conventional and Modern Methods. In Journal of Food and Nutrition Sciences (Vol. 10, Issue 3, p. 70). Science Publishing Group. https://doi.org/10.11648/j.jfns.20221003.13
16. Dong-hu, S. (2010). Review on preservation theories and anticorrosive techniques in meats and meat products. Meat Industry.
17. Ben Braïek, O., & Smaoui, S. (2021). Chemistry, Safety, and Challenges of the Use of Organic Acids and Their Derivative Salts in Meat Preservation. In C. Wan (Ed.), Journal of Food Quality (Vol. 2021, pp. 1–20). Hindawi Limited. https://doi.org/10.1155/2021/6653190
18. JANS, C., MULWA KAINDI, D. W., & MEILE, L. (2016). Innovations in food preservation in pastoral zones. In Revue Scientifique et Technique de l'OIE (Vol. 35, Issue 2, pp. 597–610). O.I.E (World Organisation for Animal Health). https://doi.org/10.20506/rst.35.2.2527
19. Kumar, A. (2019). Food Preservation: Traditional and Modern Techniques. In Acta Scientific Nutritional Health (Vol. 3, Issue 12, pp. 45–49). Acta Scientific Publications Pvt. Ltd. https://doi.org/10.31080/asnh.2019.03.0529
20. Rahman, M., Hashem, M., Azad, M., Choudhury, M., & Bhuiyan, M. (2023). Techniques of meat preservation- A review. In Meat Research (Vol. 3, Issue 3). Bangladesh Meat Science Association. https://doi.org/10.55002/mr.3.3.55
21. Castro-Giraldez, M., Tomas-Egea, J. A., Colom, R. J., & Fito, P. J. (2018). Study of the hot air drying process of chicken breast by non-invasive techniques. In Proceedings of 21th International Drying Symposium. 21st International Drying Symposium. Universitat Politècnica València. https://doi.org/10.4995/ids2018.2018.7733
22. Guerrero, A., Ferrero, S., Barahona, M., Boito, B., Lisbinski, E., Maggi, F., & Sañudo, C. (2019). Effects of active edible coating based on thyme and garlic essential oils on lamb meat shelf life after long-term frozen storage. In Journal of the Science of Food and Agriculture (Vol. 100, Issue 2, pp. 656–664). Wiley. https://doi.org/10.1002/jsfa.10061
23. Kandeepan, G., & Tahseen, A. (2022). Modified Atmosphere Packaging (MAP) of Meat and Meat Products: A Review. In Journal of Packaging Technology and Research (Vol. 6, Issue 3, pp. 137–148). Springer Science and Business Media LLC. https://doi.org/10.1007/s41783-022-00139-2
24. Mukhtar, K., Nabi, B. G., Arshad, R. N., Roobab, U., Yaseen, B., Ranjha, M. M. A. N., Aadil, R. M., & Ibrahim, S. A. (2022). Potential impact of ultrasound, pulsed electric field, high-pressure processing and microfludization against thermal treatments preservation regarding sugarcane juice (Saccharum officinarum). In Ultrasonics Sonochemistry (Vol. 90, p. 106194). Elsevier BV. https://doi.org/10.1016/j.ultsonch.2022.106194
25. Adisurya, I. P. K., & Sari, A. R. (2022). Effects of High-Pressure Processing on Milk, Meat, Fruit, and Vegetable: A Review. In Proceedings of the 2nd International Conference on Smart and Innovative Agriculture (ICoSIA 2021). 2nd International Conference on Smart and Innovative Agriculture (ICoSIA 2021). Atlantis Press. https://doi.org/10.2991/absr.k.220305.046
26. Silva, G.O.D., A.T. Abeysundara, and M. Aponso, Impacts of pulsed electric field (PEF) technology in different approaches of food industry: A review. Journal of Pharmacognosy and Phytochemistry, 2018. 7: p. 737-740.

27. Ullah, H., Hussain, Y., Santarcangelo, C., Baldi, A., Di Minno, A., Khan, H., Xiao, J., & Daglia, M. (2022). Natural Polyphenols for the Preservation of Meat and Dairy Products. In Molecules (Vol. 27, Issue 6, p. 1906). MDPI AG. https://doi.org/10.3390/molecules27061906
28. Gopalakrishnan, P. K., & Behdad, S. (2019). A Conceptual Framework for Using Videogrammetry in Blockchain Platforms for Food Supply Chain Traceability. In Volume 4: 24th Design for Manufacturing and the Life Cycle Conference; 13th International Conference on Micro- and Nanosystems. ASME 2019 International Design Engineering Technical Conferences and Computers and Information in Engineering Conference. American Society of Mechanical Engineers. https://doi.org/10.1115/detc2019-97527
29. Shew, A. M., Snell, H. A., Nayga, R. M., Jr, & Lacity, M. C. (2021). Consumer valuation of blockchain traceability for beef in the United States. In Applied Economic Perspectives and Policy (Vol. 44, Issue 1, pp. 299–323). Wiley. https://doi.org/10.1002/aepp.13157
30. Kler, R., Gangurde, R., Elmirzaev, S., Hossain, M. S., Vo, N. V. T., Nguyen, T. V. T., & Kumar, P. N. (2022). Optimization of Meat and Poultry Farm Inventory Stock Using Data Analytics for Green Supply Chain Network. In P. Ghasemi (Ed.), Discrete Dynamics in Nature and Society (Vol. 2022, pp. 1–8). Hindawi Limited. https://doi.org/10.1155/2022/8970549
31. Pérez-Aguilar, H., Lacruz-Asaro, M., & Arán-Ais, F. (2022). Towards a circular bioeconomy: High added value protein recovery and recycling from animal processing by-products. In Sustainable Chemistry and Pharmacy (Vol. 28, p. 100667). Elsevier BV. https://doi.org/10.1016/j.scp.2022.100667
32. De Aguiar Saldanha Pinheiro, A. C., Martí-Quijal, F. J., Barba, F. J., Tappi, S., & Rocculi, P. (2021). Innovative Non-Thermal Technologies for Recovery and Valorization of Value-Added Products from Crustacean Processing By-Products—An Opportunity for a Circular Economy Approach. In Foods (Vol. 10, Issue 9, p. 2030). MDPI AG. https://doi.org/10.3390/foods10092030
33. Cocan, I., Cadariu, A.-I., Negrea, M., Alexa, E., Obistioiu, D., Radulov, I., & Poiana, M.-A. (2022). Investigating the Antioxidant Potential of Bell Pepper Processing By-Products for the Development of Value-Added Sausage Formulations. In Applied Sciences (Vol. 12, Issue 23, p. 12421). MDPI AG. https://doi.org/10.3390/app122312421
34. Nuygen, M., Arvaj, L., & Balamurugan, S. (2022). The use of high pressure processing to compensate for the effects of salt reduction in ready-to-eat meat products. In Critical Reviews in Food Science and Nutrition (pp. 1–15). Informa UK Limited. https://doi.org/10.1080/10408398.2022.2124398
35. Chaiwan, W., Boonmee, C., & Kasemset, C. (2015). Waste Reduction in Meat Processing Industry: The Application of MFCA (ISO 14051). In Toward Sustainable Operations of Supply Chain and Logistics Systems (pp. 183–193). Springer International Publishing. https://doi.org/10.1007/978-3-319-19006-8_12
36. Yousefi, H., Su, H.-M., Imani, S. M., Alkhaldi, K., M. Filipe, C. D., & Didar, T. F. (2019). Intelligent Food Packaging: A Review of Smart Sensing Technologies for Monitoring Food Quality. In ACS Sensors (Vol. 4, Issue 4, pp. 808–821). American Chemical Society (ACS). https://doi.org/10.1021/acssensors.9b00440
37. Aziz, K., Tarapiah, S., Ismail, S. H., & Atalla, S. (2016). Smart real-time healthcare monitoring and tracking system using GSM/GPS technologies. In 2016 3rd MEC International Conference on Big Data and Smart City (ICBDSC). 2016 3rd MEC International Conference on Big Data and Smart City (ICBDSC). IEEE. https://doi.org/10.1109/icbdsc.2016.7460394
38. Sun, B., Luh, P. B., Jia, Q.-S., O'Neill, Z., & Song, F. (2014). Building Energy Doctors: An SPC and Kalman Filter-Based Method for System-Level Fault Detection in HVAC Systems. In IEEE Transactions on Automation Science and Engineering (Vol. 11, Issue 1, pp. 215–229). Institute of Electrical and Electronics Engineers (IEEE). https://doi.org/10.1109/tase.2012.2226155
39. Shahzad, G., Yang, H., Ahmad, A. W., & Lee, C. (2016). Energy-Efficient Intelligent Street Lighting System Using Traffic-Adaptive Control. In IEEE Sensors Journal (Vol. 16, Issue 13, pp. 5397–5405). Institute of Electrical and Electronics Engineers (IEEE). https://doi.org/10.1109/jsen.2016.2557345
40. Kafetzopoulos, D., Stylios, C. D., & Skalkos, D. (2020). Managing Traceability in the Meat Processing Industry: Principles, Guidelines and Technologies. In International Conference on Information and Communication Technologies for Sustainable Agri-production and Environment (p. 302–308). Skiathos, Greece.
41. Kalschne, D. L., Corso, M. P., & Canan, C. (2020). Advances in Meat Processing Technologies: Modern Approaches to Meet Consumer Demand. BENTHAM SCIENCE PUBLISHERS. https://doi.org/10.2174/9789811470196120010 1
42. Singh, M., Novoa Rama, E., Kataria, J., Leone, C., & Thippareddi, H. (2020). Emerging Meat Processing Technologies for Microbiological Safety of Meat and Meat Products. In Meat and Muscle Biology (Vol. 4, Issue 2). Iowa State University. https://doi.org/10.22175/mmb.11180

43. Ramachandraiah, K., Han, S. G., & Chin, K. B. (2014). Nanotechnology in Meat Processing and Packaging: Potential Applications — A Review. In Asian-Australasian Journal of Animal Sciences (Vol. 28, Issue 2, pp. 290–302). Asian Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.14.0607
44. Baltic, M., Z., Boskovic, M., Ivanovic, J., Dokmanovic, M., Janjic, J., Loncina, J., & Baltic, T. (2013). Nanotechnology and its potential applications in meat industry. In Tehnologija mesa (Vol. 54, Issue 2, pp. 168–175). Centre for Evaluation in Education and Science (CEON/CEES). https://doi.org/10.5937/tehmesa1302168b
45. Singh, P. K., Jairath, G., & Ahlawat, S. S. (2015). Nanotechnology: a future tool to improve quality and safety in meat industry. In Journal of Food Science and Technology (Vol. 53, Issue 4, pp. 1739–1749). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-015-2090-y
46. Chelladurai, V., & Jayas, D. S. (2018). Nanoscience and Nanotechnology in Foods and Beverages. CRC Press. https://doi.org/10.1201/9781315153162
47. Balasubramanian, B., Liu, W., Pushparaj, K., & Park, S. (2021). The Epic of In Vitro Meat Production—A Fiction into Reality. In Foods (Vol. 10, Issue 6, p. 1395). MDPI AG. https://doi.org/10.3390/foods10061395
48. Lanzoni, D., Bracco, F., Cheli, F., Colosimo, B. M., Moscatelli, D., Baldi, A., Rebucci, R., & Giromini, C. (2022). Biotechnological and Technical Challenges Related to Cultured Meat Production. In Applied Sciences (Vol. 12, Issue 13, p. 6771). MDPI AG. https://doi.org/10.3390/app12136771
49. Stout, A. J., Arnett, M. J., Chai, K., Guo, T., Liao, L., Mirliani, A. B., Rittenberg, M. L., Shub, M., White, E. C., Yuen, J. S. K., Jr., Zhang, X., & Kaplan, D. L. (2023). Immortalized Bovine Satellite Cells for Cultured Meat Applications. In ACS Synthetic Biology (Vol. 12, Issue 5, pp. 1567–1573). American Chemical Society (ACS). https://doi.org/10.1021/acssynbio.3c00216

Funds:

This research was funded by Ministry of Agriculture of Kazakhstan, grant number IRN BR22884456.

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Anuarbek Suychinov**, Kazakh Research Institute of Processing and Food Industry (Semey Branch), 29 Bairursynov Street, 071410, Semey, Kazakhstan,
Tel.: +7 7222 770026
E-mail: asuychinov@gmail.com
ORCID: https://orcid.org/0000-0003-4862-3293

Dinara Akimova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan; Kazakh Research Institute of Processing and Food Industry (Semey Branch), 29 Bairursynov Street, 071410, Semey, Kazakhstan,
Tel.: + 7 7222 314 602; +7 7222 770026
E-mail: akdilife@mail.ru
ORCID: https://orcid.org/0000-0001-8922-2854

Aitbek Kakimov, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: bibi.53@mail.ru
ORCID: https://orcid.org/0000-0002-9607-1684

Yerlan Zharykbasov, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: erlan-0975@mail.ru
ORCID: https://orcid.org/0000-0001-9707-0539

Assemgul Baikadamova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: asemgul93@yandex.ru
ORCID: https://orcid.org/0000-0003-0062-6997

Eleonora Okuskhanova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: eleonora-okushan@mail.ru
ORCID: https://orcid.org/0000-0001-5139-9291

Anara Bakiyeva, Shakarim University, Department of Technological Equipment and Mechanical Engineering, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: anara_bakieva@mail.ru
ORCID: https://orcid.org/0009-0003-5904-1253

Nadir Ibragimov, Shakarim University, Department of Technological Equipment and Mechanical Engineering, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: ibragimnk@mail.ru
ORCID: https://orcid.org/0000-0001-9607-823X

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 16.1.2024
Revised: 28.2.2024
Accepted: 10.3.2024
Published: 12.3.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 347-365
https://doi.org/10.5219/1962
ISSN: 1337-0960 online
www.potravinarstvo.com

Enzymatic hydrolysis in food processing: biotechnological advancements, applications, and future perspectives

Dinara Akimova, Aitbek Kakimov, Anuarbek Suychinov, Zhumatay Urazbayev, Yerlan Zharykbasov, Nadir Ibragimov, Aigul Bauyrzhanova, Assiya Utegenova

ABSTRACT

In food processing, enzymatic hydrolysis has become a revolutionary biotechnological instrument that provides consistency and sustainability that are unmatched by traditional techniques. This work thoroughly analyzes current developments in enzymatic hydrolysis and examines its uses in various food processing contexts. The biotechnological aspects—such as substrate specificity, enzyme engineering, and sustainable process optimization—are the main focus. The historical background and development of enzymatic hydrolysis in food processing are explored at the study's outset, highlighting the process's transformation from a specialized use to a critical component of contemporary biotechnological food production. A thorough literature review underscores the specificity of enzymes in dissolving various dietary components, offering insights into the biotechnological nuances controlling substrate-enzyme interactions. A careful examination of the many enzymes used in enzymatic hydrolysis and a full assessment of their uses and specificities are provided. Enzymatic hydrolysis selection criteria are outlined, taking regulatory compliance, thermostability, pH sensitivity, and substrate specificity into account. The integration of enzymatic hydrolysis into workflows for food processing is also covered, focusing on compatibility with current infrastructure and processing parameters. The case studies that demonstrate the effective use of enzymatic hydrolysis in various food production situations are the core of the research. These examples illustrate the adaptability and effectiveness of enzymatic processes in improving food quality, from developing gluten-free products to optimizing fermentation in baked goods. In its futuristic conclusion, the article imagines how enzymatic hydrolysis will continue to influence food processing in the years to come. The biotechnological viewpoint strongly emphasizes current research directions, such as integrating enzymatic processes into sustainable food production techniques and engineering enzymes for increased specificity. This biotechnological investigation highlights how enzymatic hydrolysis may completely change the food processing industry by providing accuracy, sustainability, and creativity in pursuing wholesome, nutrient-dense, and aesthetically pleasing food items.

Keywords: enzymatic hydrolysis, food processing, biotechnology, protein hydrolysis, carbohydrate hydrolysis

INTRODUCTION

Using enzymes to convert complex compounds into simpler ones is a process known as enzymatic hydrolysis, and it has a long history and a big influence on the food business. With the discovery of enzymes as biocatalysts with the ability to change particular chemical bonds selectively, the regulated use of enzymatic hydrolysis gained importance in the middle of the 20th century. As a result, important enzymes, including lipases, amylases, and proteases, were systematically included in food processing, changing the sensory and functional characteristics of different food items. Many materials have been subjected to enzymatic hydrolysis, including starch and

hemicellulose. For example, research on the kinetics of hemicellulose hydrolysis has been done, which is crucial for comprehending the process of hemicellulose pyrolysis and expanding its applications. With starch saccharifying enzymes like α-amylase and glucoamylase, enzymatic hydrolysis has also been investigated as a starch hydrolysis alternative to acid hydrolysis. Moreover, enzymatic hydrolysis influences outside of the food sector. Medical applications such as the enzymatic hydrolysis of biodegradable polymers like poly(L-lactide) (PLLA), which is employed in tissue engineering, have also been researched concerning it. The enzymatic hydrolysis rate of polylactic acid (PLLA) is contingent upon its chemical and physical characteristics, with crystallinity being a major determinant of its breakdown trajectory **[1]**, **[2]**, **[3]**, **[4]**.

The development of computational biology and enzyme engineering fueled the customization of enzymes, enabling previously unheard-of control over reaction pathways and specificity. In the late 20th and early 21st centuries, enzymatic hydrolysis gained popularity for meeting new consumer demands, such as those for gluten-free and protein-enriched products. Additional information on particular uses for enzymatic hydrolysis can be found in the search results. For example, ultrasonication has been utilized extensively as a low-temperature, environmentally friendly, non-thermal processing method to support enzymatic hydrolysis, resulting in a notable increase in enzyme hydrolysis efficiency and an increase in the biological activity of substrates **[5]**, **[6]**, **[7]**, **[8]**.

Enzymatic hydrolysis is a vital food processing technique involving enzymes to change the molecular structures of raw food ingredients. Enzymes' specificity makes it possible to regulate and alter proteins, carbohydrates, lipids, and othe vital nutritional ingredients precisely. With its origins in the study of natural enzymatic processes and the subsequent application of these principles in food processing, this procedure has a long history. It has gained special notoriety with the development of contemporary biotechnology. Enzymatic hydrolysis has changed throughout time, going from being a common technology in food formulation to being employed in traditional food operations like dairy production and brewing. A revolution in food production occurred in the middle of the 20th century when scientists started methodically introducing enzymatic hydrolysis, which increased productivity and improved the quality of the final product. Research on the potential of enzymatic hydrolysis to customize food items to particular customer tastes exploded in the following decades, setting the groundwork for its adoption as a common practice. Enzymatic hydrolysis is now an essential part of food processing, demonstrating a dedication to accuracy, sustainability, and satisfying customers' ever-evolving demands for innovative and superior culinary experiences. The search results shed light on several enzymatic hydrolysis-related applications and studies, such as the use of enzymes to get around the challenges of fat biotransformation in the anaerobic digestion of fatty wastes from the food processing industry, the impact of processing decisions and enzymatic hydrolysis on the functionality of pulse proteins, and the in silico enzymatic hydrolysis of soy sauce cake glycinin G4 to reveal bioactive peptides as possible food ingredients **[5-9]**. Figure 1 depicts the enzymes used in food processing and some industrial applications.

Because they can precisely accelerate biological reactions as biocatalysts, enzymes are essential to the biotechnological processes used in food production. They support the development of higher yields, softer processing conditions, and functional ingredients. Because they are extremely focused catalysts, enzymes allow for the precise alteration of raw materials to produce finished goods with desired textures, tastes, and nutritional profiles. Compared to conventional approaches, their catalytic activity enables the tuning of processes, resulting in improved product yields. Furthermore, enzymes function well at lower temperatures and moderate pH levels, which is consistent with the increasing need for food processing that is both sustainable and energy-efficient. Agro-industrial food waste may produce industrially significant enzymes, which provide cost-effective options for commercializing compounds with added value. Microbial enzymes contribute to the sustainable use of agro-industrial food and crop waste by providing cleaner, more environmentally friendly ways to generate fine chemicals and compounds. The production of customized enzymes by genetically modified microorganisms (GEMs) provides special food processing skills. To assure the safety of GEM-produced food components and enzymes, regulatory bodies assess the safety of GEMs and the resultant food substances. This encourages a more coordinated approach to these products' safety evaluation and regulatory control. Scientific guidelines have been created specifically for food enzymes to help applicants prepare applications for food enzyme authorization. This guideline defines the scientific information that should be included in applications for the licensing of food enzymes and addresses the characterization of microorganisms employed as production organisms. Bacteriophage endolysins are lytic enzymes encoded by bacteriophages that are being researched for their potential to replace or enhance antibiotics in clinical and food production settings. This is in addition to their involvement in food production. By breaking down bacterial cell walls, these endolysins have demonstrated promise as antibiotic substitutes and may be developed through bioengineering **[10]**, **[11]**, **[12]**, **[13]**, **[14]**.

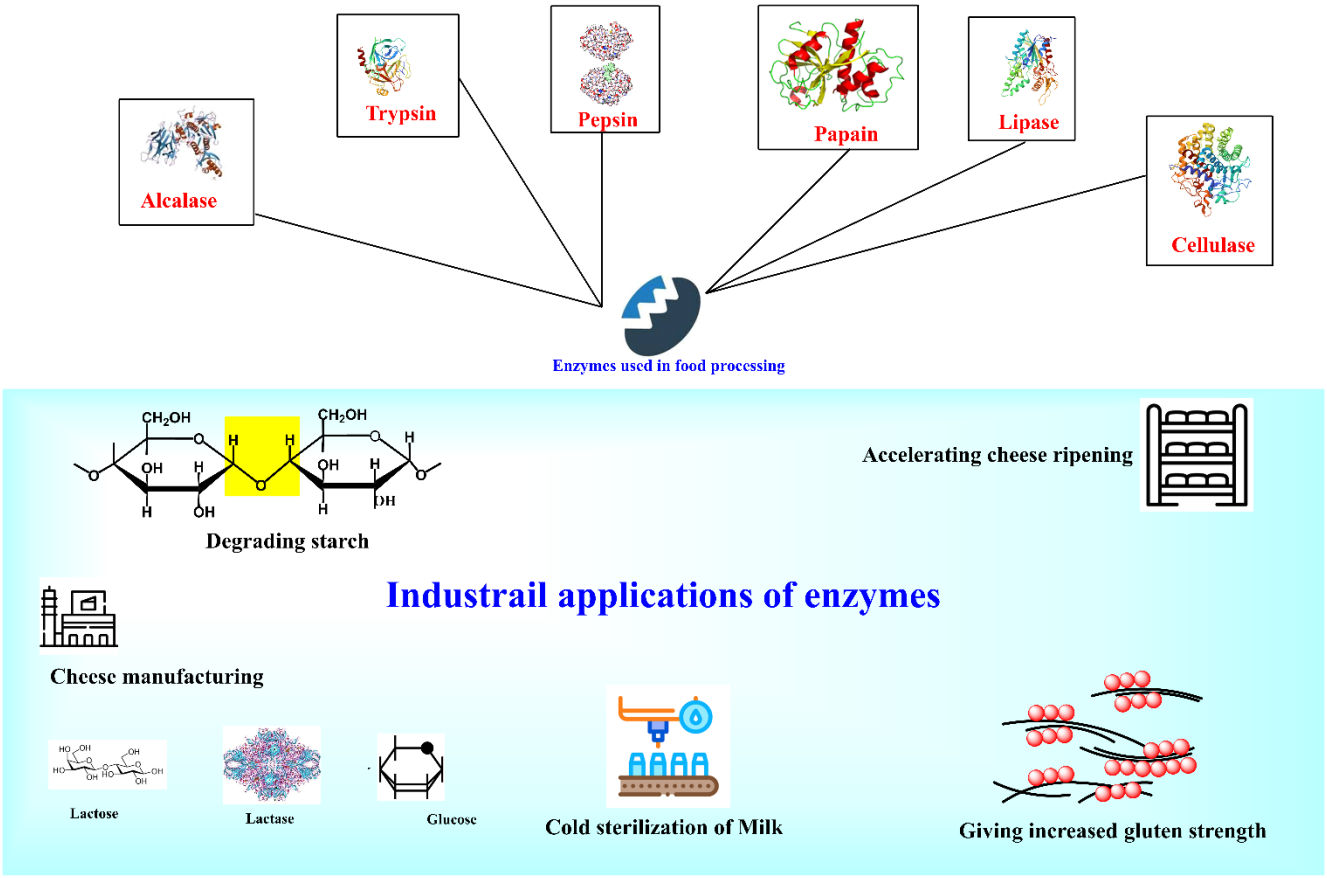


Figure 1 Scheme of industrial applications of enzymes in food processing.

Masterful Precision in Proteolysis: Unveiling the Specificity of Proteases

Proteinases, another name for proteases, are enzymes that cleave peptide links inside proteins with amazing specificity. This specificity, dictated by the protease's affinity for certain amino acid sequences, is the outcome of a molecular conversation between the enzyme and the protein, which serves as its substrate. Protease specificity is not random; it is the outcome of evolutionary adaptation and fine-tuning to match the enzyme's activity with biological requirements. Recent studies have shown that proteases may be reprogrammed to break novel sequences of our choice, opening up new medicinal and biotechnological possibilities. An effective method for producing proteases with custom specificities was established by research that detailed the development of botulinum neurotoxin proteases with reprogrammed specificity. Another work described a method for producing proteases with modified specificities to cleave. An effective method for producing proteases with custom specificities was established by research that detailed the development of botulinum neurotoxin proteases with reprogrammed specificity. Different research described a method for producing proteases with modified specificities to cleave a desired target protein. Protease substrate specificity is a tightly controlled mechanism essential to maintaining cellular homeostasis. Enzymes that deubiquitinate proteins and Ubl-specific proteases (ULPs) are two types of proteases that can identify and extract ubiquitin and proteins that resemble ubiquitin from their substrates. It is yet unclear how precisely these proteases differentiate between various modifiers or between these modifiers' polymeric forms **[15]**, **[16]**, **[17]**.

Chymotrypsin: Precision in Proteolysis

Protease chymotrypsin breaks peptide bonds next to aromatic amino acids, including phenylalanine and tyrosine. The enzyme's evolved molecular structure, which has been fine-tuned by natural selection, is the cause of its targeted specificity. The preference of chymotrypsin for aromatic amino acids is especially important in proteins where these residues are arranged in a certain way, helping to break down the protein under control and produce targeted peptide fragments. Recent studies have demonstrated the unique application of proteases like α-lytic protease for bottom-up proteomics by using orthogonal-specificity proteases to enhance proteome coverage. These alternative proteases improve proteome coverage through cleavage at sequences complementary to trypsin, thereby increasing proteome coverage by 101% relative to trypsin digestion alone. Furthermore, studies have been conducted to characterize the substrate specificity of other proteases, such as metacaspases, which prefer cleaving peptide bonds after arginine or lysine residues at specific sites. Understanding the substrate specificity of these

proteases is relevant for determining the types of proteins they cleave in vivo. Additionally, research has been conducted on the phage-assisted continuous evolution of proteases with altered substrate specificity, establishing a strategy for generating proteases with altered specificities to cleave a target protein of interest **[16]**, **[18]**, **[19]**.

Pepsin

Within the stomach's acidic environment, the protease pepsin demonstrates extraordinary selectivity and versatility in its enzymatic action. Pepsin plays a critical function in the early stages of protein digestion by selectively cleaving peptide bonds in proteins, helping to break down complex protein structures into smaller peptides partially. This adaptability is carefully tailored to the acidic environment. The use of pepsin in scientific and industrial situations has also been studied. One research, for example, highlighted the specialized uses of pepsin that need acidic conditions by optimizing an existing peptic digest process to investigate membrane proteins using bacteriorhodopsin from purple membranes as a reference. Another study utilized pepsin for the limited proteolysis of human growth hormone under acidic solvent conditions. It demonstrated the enzyme's activity at low pH and its role in selective cleavage of peptide bonds. Additionally, the adaptability of pepsin to acidic conditions has been leveraged in industrial processes, such as in the development of an acidic endo-beta-1,4-glucanase with high protease resistance for potential use as a pig feed additive. In summary, pepsin's adaptability to acidic conditions and specificity in cleaving peptide bonds play a pivotal role in the stomach's initial stages of protein digestion. Furthermore, research has demonstrated the diverse applications of pepsin's behavior in acidic environments, ranging from membrane protein analysis to industrial processes **[20]**, **[21]**, **[22]**.

Amylases: Maestros of Carbohydrate Metamorphosis

Amylases are enzymes that break down complex carbohydrates, particularly starches, and glycogen, into simpler sugars. They exhibit a remarkable ability to catalyze the hydrolysis of specific glycosidic bonds, precisely directing the controlled deconstruction of polysaccharides into sugars. There are different classes of amylases, including α-amylase and glucoamylase, widely distributed in bacteria, actinomycetes, and fungi. Microbial α-amylases are the most popular source of industrial α-amylase due to their cost-effectiveness and suitability for industrial demands. The specificity of amylases is a testament to their nuanced enzymatic capabilities. For example, the maltooligosaccharide-forming amylase (MFAse) from *Bacillus stearothermophilus* has been engineered to enhance its specificity for producing maltohexaose from starch, demonstrating the potential for modifying amylases to achieve desired product specificity. The α-amylase is an enzyme with a broad substrate preference and product specificity and is the main representative of family GH13. Still, it is also present in other glycoside hydrolase families such as GH57 and GH119. The α-amylase specificity is present in several subfamilies within family GH13, and these enzymes employ a reaction mechanism giving retention of configuration **[24-26]**.

The precision of α-amylases in cleaving internal α-1,4-glycosidic bonds within starch molecules is a testament to their enzymatic virtuosity. This targeted approach ensures a strategic disassembly of the starch polymer, revealing shorter oligosaccharides as the outcome. The liberation of maltose and maltotriose, resulting from the enzymatic action of α-amylases, marks the initial steps in the breakdown of starch, as these shorter oligosaccharides become the building blocks for subsequent enzymatic actions. The strategic breaking of internal bonds by α-amylases sets the stage for a cascade of molecular transformations, with the liberated maltose and maltotriose molecules becoming substrates for further enzymatic processes. This cascade of transformations acts as a molecular domino effect, with each cleavage event paving the way for subsequent modifications in the carbohydrate landscape. The specificity of α-amylases in cleaving internal bonds results from their remarkable ability to catalyze the hydrolysis of specific glycosidic bonds, precisely directing the controlled deconstruction of polysaccharides into sugars. This specificity is the molecular compass that guides α-amylases to their designated targets within the carbohydrate structures. The engineering of maltooligosaccharide-forming amylases (MFAs) to enhance their specificity for producing specific sugar fragments, such as maltohexaose, demonstrates the potential for tailoring these enzymes for specific industrial applications **[25]**.

The strategic positioning of β-Amylases in enzymatic hydrolysis plays a crucial role in the controlled liberation of maltose molecules from the non-reducing end of starch, contributing to the sequential breakdown of starch into simpler sugars. β-Amylases exhibit focused catalytic action by directing their enzymatic prowess to the non-reducing end of starch. This ensures meticulous control and systematic liberation of maltose molecules, which become integral building blocks in the carbohydrate metamorphosis journey. The specificity of β-Amylases acting on the non-reducing end is pivotal in orchestrating a controlled release of maltose units, unlike indiscriminate cleavage, ensuring a stepwise breakdown of starch into manageable fragments. Each liberated maltose unit becomes a molecular currency, ready to participate in further carbohydrate metamorphosis. The study of the impact of different amylases on the utilization of cornstarch in broiler chickens fed a corn-based diet showed that

the type and concentration of amylase supplementation can affect the digestibility of energy, feed conversion rate, and other physiological parameters in broiler chickens **[27]**, **[28]**.

Lipase

The specificity of lipases, such as pancreatic lipase, plays a crucial role in the hydrolysis of triglycerides, leading to the liberation of essential fatty acids and glycerol. Lipases, including pancreatic lipase, demonstrate distinctive action at the water-lipid interface, allowing them to effectively engage with lipid substrates, illustrating their prowess in molecular domains where hydrophobic and hydrophilic forces coalesce. Lipases' specificity and affinity for triglycerides make them essential in the culinary field, contributing to texture modification and sensory enhancement in lipid-containing foods. Research has also shown the optimization of enzymatic hydrolysis of oils, such as *Moringa oleifera* Lam oil, using lipase-catalyzed hydrolysis, demonstrating the specific affinity of lipases for fatty acids. Additionally, studies have investigated the synergistic effects of combi-lipases in the efficient hydrolysis of soybean oil, highlighting the importance of lipase specificity in the hydrolysis process **[29]**, **[30]**, **[31]**.

The primary dietary fat, triglycerides, are highly specific to pancreatic lipase and essential for hydrolyzing them. Studies have indicated that pancreatic lipase's selectivity for triglycerides stems from its capacity to hydrolyze ester bonds at particular locations along the glycerol backbone. This results in the regulated release of fatty acids and glycerol. This specificity is necessary for the controlled hydrolysis of triglycerides, which releases vital components and aids in the body's absorption of nutrients and energy metabolism. Research has further indicated that the pancreatic lipase's β5-loop and lid domains play a role in its substrate selectivity, underscoring the complex interplay between the structure and function of the enzyme **[32]**, **[33]**, **[34]**. **[35]**, **[36]**.

Cellulase

The ability of cellulases to specifically target cellulose, the primary constituent of plant cell walls, enables the controlled hydrolysis of cellulose into glucose units, with significant ecological and industrial applications. Cellulases, such as endoglucanases, exoglucanases, and β-glucosidases, have a remarkable affinity for cellulose, which allows them to act on the ends of cellulose chains, hydrolyze cellobiose into individual glucose units, and break internal β-1,4-glycosidic linkages. This selectivity is crucial in the regulated breakdown of cellulose chains, which release glucose units. This metabolic unit gives microorganisms a direct source of energy and aids in the synthesis of biofuel. methods used by fungi to access and break down cellulose. Furthermore, studies have demonstrated that the effectiveness of biological deconstruction of polymer materials is enhanced by the spatiotemporally coordinated action of cellulases that have a synergistic function in polymer chain depolymerization. Thus, the cellulose-specificity of cellulases reveals a metabolic symphony with revolutionary potential for many businesses, including the manufacture of biofuel and the environmentally friendly uses of immobilized cellulases **[36]**, **[37]**, **[38]**.

Endoglucanases & Exoglucanases

One of the most distinctive features of endoglucanases' enzymatic activity is their specialization in cleaving internal β-1,4-glycosidic linkages inside the cellulose structure, which starts the regulated disintegration of cellulose chains and produces shorter cellulose fragments. The release of glucose units is the final result of the operations of β-glucosidases and exoglucanases, which are set in motion by this regulated fragmentation. As demonstrated by research, the efficiency of biological deconstruction of polymer materials is enhanced by the spatiotemporally coordinated action of enzymes with synergistic function in polymer chain depolymerization. This emphasizes the significance of endoglucanases' cooperative role with other cellulases in the cellulolytic orchestra. Further research is required to better understand the catalytic mechanisms of endoglucanases and their importance in cellulose deconstruction. Studies have also shown that endoglucanases, especially those from glycoside hydrolase family 48 (GH48), are essential components of natural lignocellulose-degrading systems and greatly synergize with complementary endocellulases in free cellulase systems or cellulosome systems. Thus, endoglucanases' internal accuracy in cleaving β-1,4-glycosidic linkages is crucial for optimizing cellulose accessibility and maximizing the effectiveness of the cellulolytic ensemble, emphasizing the grace of enzymatic processes in nature's grand bio-fabrication **[37]**, **[39]**, **[40]**.

As virtuosos in the cellulolytic ensemble, exoglucanases exhibit dexterity at the extremities of cellulose chains, systematically releasing cellobiose units and aiding in the cellulose polymer's methodical unravelling. Their tailored activities guarantee harmonic development in the cellulolytic orchestra by preparing the released cellobiose units as substrates for the last enzymatic acts in the cellulolytic symphony. Studies have demonstrated that the efficient biological breakdown of polymer materials is enhanced by the spatiotemporally coordinated action of enzymes with synergistic roles in polymer chain depolymerization. This highlights the significance of exoglucanases' cooperative role with other cellulases in the cellulolytic orchestra. Furthermore, research has

demonstrated the vital function of exoglucanases in the orderly disintegration of the cellulose polymer and the production of cellobiose units for the latter phases of cellulose hydrolysis, highlighting the grace and effectiveness of nature's enzymatic artistry. Thus, exoglucanases, under their accuracy at the ends of cellulose chains, are essential for the systematic disintegration of the cellulose polymer, which enhances the effectiveness of cellulose deconstruction and readys cellobiose units for the last phases of cellulose hydrolysis **[37]**, **[39]**, **[40]**.

Enzymes known as β-glucosidases are essential for the last phase of cellulose breakdown. Their area of expertise is the breakdown of cellobiose, a disaccharide produced by previous enzymatic procedures. This process breaks down cellobiose into separate glucose units, signifying the successful completion of cellulose breakdown. The many uses of glucose in energy metabolism, the synthesis of biofuels, and other metabolic pathways make the release of glucose by β-glucosidases important. According to research, β-glucosidases, like the cellulolytic component C1 of Trichoderma koningii, function as beta-1,4-glucan cellobiohydrolases. They release terminal cellobiose units from cellulose and work closely with cellobiase to prolong cellulose hydrolysis and release glucose. Studies on the real-time monitoring of cellobiose and glucose synthesis during enzymatic biomass hydrolysis have also been carried out, underscoring the need to precisely measure these products to optimize the industrial degradation process.

Additionally, the hydrolysis process of cellobiose to glucose by β-glucosidase has been tested using computational fluid dynamics (CFD) simulations, showing the possibility of immobilizing β-glucosidase on an appropriate substrate to enhance its catalytic activity. The release of glucose by β-glucosidases is vital because glucose is a treasure trove of energy and a crucial building block for the synthesis of biofuels. It has been demonstrated that immobilizing β-glucosidases on appropriate substrates enhances their catalytic activity, which may have consequences for a range of industrial uses, such as the generation of biofuel **[41]**, **[42]**, **[43]**.

Metabolic Symphony Unveiled by Cellulases

The term "metabolic symphony" describes cellulose's sequential and well-coordinated breakdown into glucose by many cellulase types. This technique is compared to a well-balanced composition significantly affecting several sectors and the natural world. How endoglucanases, exoglucanases, and β-glucosidases work together to cleave cellulose into glucose units demonstrates the specificity of cellulases. Cellulases play a subtle symphony of sequential cleavage in the biochemical orchestra that is nature. They are like virtuosos in the orchestra. This orchestration results from several cellulases working together, each having a specific function in converting cellulose to glucose. In the metabolic symphony of cellulases, endoglucanases initiate the process by cleaving internal bonds, and exoglucanases control the process in a choreographed manner. The last note of cellulose hydrolysis is played by β-glucosidases, which break down cellobiose into individual glucose units. The release of glucose, the primary component of nature's biochemical repertory, has profound effects on the metabolism of energy, the synthesis of biofuels, and other vital biological functions. Within the industrial domain, the metabolic symphony facilitated by cellulases presents both transformational possibilities and long-term fixes. It creates opportunities for the production of biofuel, sustainable bioprocessing, and the development of biotechnology. The ability of cellulases to be particular serves as a light in sustainable bioprocessing, providing practical and eco-friendly methods for turning biomass into valuable products. Unlocking the energy held in cellulose and opening the door for sustainable practices, the "metabolic symphony" of cellulases echoes across the complexities of nature as well as the opportunities inside industrial environments **[43]**, **[44]**.

Glucose as Metabolic Currency

The most common polymer on Earth, cellulose is vital in maintaining soil nutrients and renewable energy sources. The enzymes known as cellulases, which are generated by various microbes, are essential in the breakdown of cellulose into glucose units, which can then be directly used as an energy source by bacteria and creatures that break down cellulose. Turning cellulose into glucose demonstrates how valuable and versatile glucose is. The manufacture of biofuels has excellent potential because of cellulases' ability to break down cellulose into glucose units under regulated conditions. The released glucose transforms from the inflexible walls of plant cells into tanks of sustainable biofuels, serving as a crucial precursor for bioethanol synthesis. The precise enzymatic activities of cellulases provide an ecologically favorable substitute for conventional fossil fuels and contribute to sustainable energy solutions. Cellulases, including β-glucosidases, endoglucanases, and exoglucanases, turn cellulose into a metabolic symphony that demonstrates the complex biochemical processes that support life and have the power to revolutionize several sectors. Microorganisms such as *Bacillus licheniformis* can produce cellulase, including glucosidase and endo-glucanase, which are crucial for the breakdown of cellulose **[45]**, **[46]**.

Pectinase

Pectinases are crucial enzymatic artisans who are vital to the processing of fruits and vegetables. They include pectin esterases, polygalacturonases, and pectin lyases. During processing, these enzymes precisely control texture, modifying the physical characteristics of fruits and vegetables. By altering the structural characteristics of pectin, they also support efforts to extend processed foods' shelf life by preserving their quality and freshness for longer. Furthermore, food processors can customize products to suit a wide range of preferences thanks to the specificity of pectinases, which ensures that every innovation reflects a keen understanding of the changing tastes and expectations of the modern consumer **[47]**, **[48]**, **[49]**, **[50]**. A class of enzymes known as pectinases is dedicated to degrading the complex material known as pectin. This group of enzymes includes pectin lyases, polygalacturonases, and esterases specific to different bonds in the pectin molecule. Pectinases are crucial to the food business, especially when processing fruits and vegetables, since their specific activities affect the quality and sensory qualities of the end product. Pectinases' enzymatic actions, such as de-esterification and depolymerization, help processed food items have a different texture, better quality, and longer shelf lives. Pectinases are derived from higher plants and microbes and are widely employed in the food sector, especially in the extraction of fruit juice **[47]**, **[48]**.

Pectin esterase

The enzymes known as pectin esterases are essential to the enzymatic conversion of pectin because they break down ester bonds found in the structure of the protein. This specific activity helps to de-esterify pectin, which modifies its structural characteristics and affects how processed fruits and vegetables feel. Pectin esterases demonstrate catalytic accuracy in hydrolyzing bonds by selectively targeting particular sites and cleaving ester links. Pectin esterase-mediated de-esterification significantly impacts fruit and vegetable texture during processing, changing the extent of esterification and causing differences in the firmness, viscosity, and overall mouthfeel of processed foods. Pectin esterases are essential in cooking because they affect the texture and flavor of processed fruits and vegetables and are becoming increasingly common. Pectin esterases facilitate de-esterification, which improves mouthfeel and consistency in processed fruits and vegetables. This balance in texture control allows for the modification of culinary attributes. Pectin esterases are the master builders of pectin hydrolysis; they meticulously manipulate the structure of ester bonds and the texture of fruits and vegetables to produce finished foods that are not only aesthetically pleasing but also showcase the intricate artistry of enzymatic science **[48]**, **[49]**.

Polygalacturonase

Enzymes known as polygalacturonases are adept at breaking bonds involving galacturonic acid, a crucial part of pectin. Pectin is depolymerized due to this enzymatic cleavage, which affects the quality and consistency of processed fruit and vegetable products. Polygalacturonases target galacturonic acid precisely, resulting in targeted cleavage and thorough dissection of the structural details of pectin. Pectin's molecular structure changes due to the galacturonic acid bond's enzymatical breaking by polygalacturonases, which starts the depolymerization process. Polygalacturonases play a vital role in the depolymerization process, which determines the final culinary creations' quality by affecting the consistency of processed fruit and vegetable products. Polygalacturonases are essential in the culinary arts because they affect the sensory attributes and characteristics of processed fruits and vegetables. They may also be a creative tool for food scientists and processors. The depolymerization of pectin by polygalacturonases is an essential process that shapes the texture of processed fruit and vegetable products. This process enhances the overall quality of processed goods by customising culinary features to suit a wide range of customer tastes. Polygalacturonases play a vital role in the depolymerization process, which determines the final culinary creations' quality by affecting the consistency of processed fruit and vegetable products. Polygalacturonases are essential in the culinary arts because they affect the sensory attributes and characteristics of processed fruits and vegetables. They may also be a creative tool for food scientists and processors. The depolymerization of pectin by polygalacturonases is an essential process that shapes the texture of processed fruit and vegetable products. This process enhances the overall quality of processed goods by enabling the customization of culinary features to suit a wide range of customer tastes **[48]**, **[50]**, **[51]**.

Pectin lyases

Pectin lyases are enzymes that catalyze elimination reactions within the pectin structure, forming unsaturated products. This enzymatic transformation modifies pectin's physical properties, impacting processed fruits and vegetables' texture and shelf life. Pectin lyases exhibit enzymatic precision in catalyzing elimination reactions, selectively cleaving specific bonds within the pectin structure, and creating unsaturated products. The formation of unsaturated products initiates a molecular rearrangement within the pectin structure, impacting its physical

properties. Pectin lyases catalyze an enzymatic change that affects processed fruits and vegetables' texture, hardness, and general structural integrity, affecting how long they last on the shelf. Pectin lyases affect the shelf life of processed goods, change the texture of processed fruits and vegetables, and foster culinary creativity by bringing variety to the sensory characteristics of processed meals **[52]**, **[53]**. During processing, pectinases are essential for maintaining the color and taste of fruits and vegetables. Their focused efforts help to improve the overall quality of food items by preventing undesired changes in texture and appearance.

Furthermore, pectinases influence the structural integrity of pectin, which helps to modulate shelf stability and increase the shelf life of processed goods. This enzymatic alteration helps preserve the quality and freshness of fruits and vegetables throughout time, demonstrating the accuracy and importance of pectinases at the nexus of enzymatic science and culinary artistry. Pectinases' accuracy as enzymatic instruments in the food processing toolset is reflected in their specialization in targeting different bonds within the pectin structure. Pectinases, the builders of pectin hydrolysis, are essential because they bridge the gap between enzymatic science and culinary creativity by enhancing fruit and vegetable products' palatability and aesthetic appeal **[54]**, **[55]**.

The search results may include further details on the significance of pectin degradation in the environment and industry. Pectinases, which comprise pectin esterases, polygalacturonases, and pectin lyases, are essential for breaking down plant cell walls, and pectin is an integral part of building cell walls. Pectinases are used in industry to enhance the quality of fruit juices and can also be employed to break down tea leaves or coffee cherries. These enzymes could also help lessen the negative effects of cotton and paper on the environment. The search results also highlight the importance of comprehending microbial pectin degradation, particularly in species phylogenetically different from Aspergillus and Enterobacteriaceae, as it may decrease the expense and increase the efficiency of plant cell wall breakdown for biofuel or chemical production and help identify novel enzymes with other industrial uses **[56]**.

Pectinases are essential for processing fruits and vegetables because they improve the overall quality of the final product by preserving taste and refining texture. By carefully breaking apart certain bonds in pectin to break down the plant cell wall, these enzymes function as molecular architects, molding the texture and keeping the original tastes of plant-based goods. Pectinases also help improve product quality by prolonging the shelf life of processed foods and preserving the nutritional value of fruits and vegetables over time. Their practical uses in the food processing sectors, such as oil extraction, juice processing, and alcoholic beverage processing, enhance output levels and product quality. Pectinases, including polygalacturonases, are widely utilized in various food processing sectors, including wine clarifying, fruit juice extraction, and jam and jelly manufacturing. Pectinases are, therefore, necessary to convert unprocessed plant materials into the delectable works of art that adorn our dinner tables **[57]**, **[58]**, **[59]**.

Nucleases

As genetic sculptors in enzymatic hydrolysis, nucleases are essential for breaking down nucleic acids like DNA and RNA and for developing tastes in various culinary applications, including the maturation of cheese. Their ability to precisely hydrolyze nucleic acids releases a symphony of tastes, improving food items' overall flavor character. Nucleases play a crucial role in the ripening of cheese for the following reasons:

1. Development of flavor: Nucleases break nucleic acids, which release bound flavors and improve the cheese's overall flavor. 2. Production of aroma: Flavor chemicals like 3-methyl butanol and 1-pentanol, which give the cheese its distinct flavor, can be formed by the hydrolysis of nucleic acids. 3. Smoother and creamier texture: Enzymatic hydrolysis breaks down proteins to give cheese a smoother, creamier texture. 4. Less allergens: Cheese that has had its milk proteins hydrolyzed has fewer allergens, making it better for those with a milk allergy. To get the ideal flavor and texture in cheese, optimizing the parameters for enzymatic hydrolysis, including temperature, enzyme concentration, and hydrolysis duration is essential.

For instance, one study discovered that employing bromelain to hydrolyze cockle meat wash water precipitate produced a product with a nitrogen concentration of 0.6% and a hydrolysis degree of 48%. These circumstances have been demonstrated to result in a hydrolysate containing flavorings in oysters and clams. Nucleases have been used in food in ways other than cheese, such as the hydrolysis of fish bone protein to enhance the taste and antioxidant capacity of the resulting hydrolysates. To recover proteins from fish backbones, steam explosion-assisted extraction has been compared to hot-pressure extraction, and the functional characteristics and IgG/IgE-binding capability of skimmed cow milk have been investigated using enzymatic hydrolysis **[60]**, **[61]**, **[62]**, **[63]**.

Reaction Mechanisms: Navigating the Specificity Terrain

The active sites of enzymes display selectivity, essential for molecule recognition and catalysis in enzymatic hydrolysis activities. The interaction of chemical fingerprints and geometric geometries determines the specificity of active sites, allowing enzymes to identify the substrates they are meant to bind to precisely. Enzymes and

substrates have extraordinary geometric and chemical complementarity, fitting together like a jigsaw. The active site pockets' particular configuration of atoms, charges, and functional groups ensures that substrates locate their binding partners and start the chain reaction of catalysis. Knowing the specificity of active sites reveals the beauty of molecular recognition in enzymatic hydrolysis. It offers insights into the molecular level of reaction orchestration, where specificity and accuracy are paramount **[64]**, **[65]**, **[66]**.

Lock-and-Key vs. Induced Fit Models: A Choreography of Molecular Recognition

Lock-and-Key Model

According to the lock-and-key concept, the substrate (the key) and the enzyme (the lock) must fit precisely. According to this paradigm, the substrate and the active site of the enzyme exhibit predetermined complementarity that enables smooth binding. The active site pockets' particular configuration of atoms, charges, and functional groups ensures that substrates locate their binding partners and start the chain reaction of catalysis. Adding a dynamic aspect to the chemical interaction, the induced fit model adds flexibility to the lock-and-key model's rigidity. According to this hypothesis, conformational changes occur in both the enzyme and the substrate during binding. Because of its flexibility, the active site's shapes may be adjusted to meet the substrate's structure and guarantee a snug fit. The dance between the substrate and the enzyme changes in reaction to their interaction, growing more intimate as they adjust to each other's structural peculiarities. The induced fit model's intrinsic flexibility becomes essential in the complex world of enzymatic hydrolysis. Different substrates pose different structural difficulties, and the dance between the substrate and the enzyme needs to consider this diversity. With its elegant adaptability, the induced fit model enables enzymes to accept substrates of different sizes and shapes, resulting in a flexible and efficient molecular stage performance **[67]**, **[68]**, **[69]**.

In the context of enzymatic hydrolysis, the search results shed light on the catalytic processes of several enzyme types, including proteases and carbohydrate-binding modules (CBMs). The results highlight the wide range of molecular tools that different enzyme classes use, which helps explain why different enzyme classes approach different substrates in enzymatic hydrolysis with customized accuracy. The first two search results provide insight into the lock-and-key and induced fit processes involved in the interaction between an enzyme and its substrate by discussing the selectivity and conformational changes in protease active sites. These findings demonstrate the adaptive usefulness of the lock-and-key mechanism in promoting high efficiency in enzymatic reactions by offering a thorough knowledge of the molecular interactions and conformational changes that influence future reactions with substrates. The function of CBMs in the enzymatic hydrolysis of complex carbohydrates is the subject of the following two findings. They demonstrate how CBMs, which are physically and functionally independent of the catalytic domains they are linked with, contribute to the hydrolysis of certain substrates via unique methods, highlighting the customized accuracy with which enzymes tackle the hydrolysis of complex carbohydrates **[66]**, **[69]**, **[70]**, **[71]**.

Co-factors and Coenzymes: Catalysts' Trusted Allies

In enzymatic hydrolysis, co-factors and coenzymes are crucial partners that are crucial to catalytic mastery. Non-protein molecules called co-factors attach to enzymes to increase their catalytic activity and improve the accuracy and efficiency of enzymatic hydrolysis. They facilitate biological reactions and function as molecular bridges by bridging molecules between enzymes and their substrates. As a subclass of co-factors, coenzymes are organic compounds that improve other enzymes' activity and participate actively in the catalytic dance. They experience reversible chemical changes during catalysis and shuttle back and forth between the enzyme and the substrate, enabling key stages in the reaction pathway. Comprehending the function of these indispensable associates is paramount to precisely crafting enzymatic procedures, as they offer indispensable perspectives into the enzymatic choir, directing enzymes through the complexities of catalysis and exhibiting the musical interaction that underpins the grace of enzymatic processes **[69]**.

Competitive and Non-competitive Inhibition: A Molecular Chessboard

Enzyme inhibition that is not competitive occurs when an inhibitor attaches to the enzyme at a position other than the active site, changing the enzyme's structure and lowering its activity. The substrate concentration cannot be increased to overcome this inhibition. Non-competitive inhibitors are frequently employed in the creation of medicinal treatments for a range of illnesses. For the treatment of neuropathic pain and chronic inflammation, for example, studies on non-competitive inhibition of enzymes like fatty acid amide hydrolase (FAAH) have been carried out **[72]**, **[73]**, **[74]**. **[75]**, **[76]**.

Non-competitive inhibition is based on the molecular mechanism of the inhibitor attaching itself to the complex of enzyme and substrate, hence reducing the enzyme's turnover rate. This kind of inhibition is

distinguished by its capacity to change the enzyme's Vmax and its independence from the concentration of the substrate. It is essential to comprehend the molecular mechanisms of non-competitive inhibition to design therapeutic interventions that work and to understand biological systems better. The listed publications and research articles shed light on the possible biological and therapeutic uses of non-competitive inhibition and its molecular underpinnings. These discoveries enhance the comprehension of enzyme control and the creation of innovative therapy approaches for various ailments.

One study that clarifies the role of non-competitive inhibitors in managing neuropathic pain and chronic inflammation is the molecular basis of the non-competitive inhibition of fatty acid amide hydrolase (FAAH). Furthermore, studies on the supramolecular interaction between β-galactosidase and a molecular cage demonstrate how a non-competitive inhibitor affects enzyme activity and how it may be used for antimicrobial activity. Because of these results, the strategic dynamics of enzyme inhibition and its consequences for many biological and therapeutic contexts are better understood **[72]**, **[76]**.

Inhibitors can contribute to the enzymatic hydrolysis process through competitive and non-competitive inhibition. Non-competitive inhibitors attach to a location different from the enzyme's active site, causing conformational changes, whereas competitive inhibitors imitate the substrate and compete for the active site. It is essential to comprehend these pathways to maximize enzymatic hydrolysis. Research on the enzymatic hydrolysis of proteins in red tilapia viscera revealed that competitive inhibitors, such as lipids, may significantly compete with genuine substrates for the enzyme's active site. Conversely, non-competitive inhibitors, like certain catalysts, might cause the enzyme to alter conformation, which can impact the catalytic process as a whole **[77]**, **[78]**, **[79]**. **[80]**, **[81]**.

Orchestrating Precision: Allosteric Ballet in Enzymatic Hydrolysis

Like a choreographed masterpiece, the complex process of allosteric control in enzymatic hydrolysis introduces a subtle ballet of molecular interactions that extends beyond the boundaries of the active site. Allosteric regulation presents a dance at a separate stage—the allosteric site—in contrast to the direct involvement observed in competitive and non-competitive inhibition. This is where an allosteric effector molecule enters the picture. It binds to a location other than the active site, changing the enzyme's shape and affecting its catalytic activity. The balletic precision required to regulate enzymatic activities precisely is provided by allostery regulation. The allosteric effector regulates the enzyme's activity, which also sets the speed and tempo of the catalytic performance. Enzymatic hydrolysis may be precisely regulated by this subtle control, guaranteeing that it proceeds with the elegance and dexterity of a well-practiced process. Allosteric control brings a symphony of molecular motions to the big spectacle of enzymatic hydrolysis, where the far-off murmurs at the allosteric site reverberate throughout the enzyme. A smooth integration of regulatory cues is produced by the active site and allosteric site's perfect synchronization, reminiscent of a ballet's timed movements. The designers of enzymatic systems must comprehend the fundamentals of allosteric control. Scientists may create choreographies that enable dynamic and responsive enzymatic hydrolysis by carefully arranging molecules that act as allosteric effectors. This ensures that the dance of chemical changes unfolds with grace and accuracy **[82]**, **[83]**.

Awakening the Catalysts: Unveiling the Secrets of Enzyme Activation

Enzyme activation is an essential first step in the complex realm of enzymatic hydrolysis, marking the beginning of the biochemical process. Deciphering the elements that cause an enzyme to activate is like unlocking the latent potential of an old ritual by knowing which cues to use. Among the crucial elements of enzyme activation are:

1. Cofactors as Vitalizing Liquids: Cofactors, organic molecules, or metal ions are frequently needed to activate enzymes. These cofactors give enzymes life and cause them to emerge from dormancy **[84]**, **[85]**.

2. Post-Translational Magic: Enzymes can undergo post-translational modifications, such as phosphorylation, acetylation, or glycosylation, which can change them and set off a series of events that lead to the enzyme's activation **[85]**.

3. Harmony in Activation: Cofactor availability, post-translational modification accuracy, and molecular cue alignment are just a few of the variables that play a subtle role in orchestrating enzyme activation. This harmonic interaction aroused enzymes to a crescendo of catalytic frenzy **[86]**.

4. Harmonizing the Resurrection: Like contemporary magicians, scientists practice precisely controlling the activation of enzymes. They acquire the ability to precisely control enzymes' waking by learning the cofactors' language and the subtleties of post-translational modifications. Thanks to this fine-tuning, enzymatic hydrolysis will proceed with the elegance of a well-rehearsed show **[86]**.

Criteria for Selecting Enzymes in Enzymatic Hydrolysis

Many parameters should be taken into account while choosing enzymes for enzymatic hydrolysis. It is essential to examine the characteristics of the substrate, including proteins, carbohydrates, and fats, to select enzymes that have a natural affinity for the intended substrate. Because enzymes have preferences for particular bonds, knowing the desired transformation helps the selection process so that the selected enzymes coincide with the required bonds. Choosing the proper enzyme turns becomes a calculated move, much like picking the appropriate explorer for a certain area. The functional impact of enzymatic hydrolysis on pulse proteins varies significantly depending on the enzyme, substrate, heat treatment, level of hydrolysis, and pH during processing. Cellulases' substrate specificity was established concerning the fibers of genetically distinct cotton lines **[87]**, **[88]**.

Fine-Tuning the Hydrolytic Symphony: Optimal pH and Temperature Choreography

Temperature and pH levels are critical factors in determining the rhythm and harmony of the catalytic process during enzymatic hydrolysis. Like virtuoso musicians, enzymes resonate at a certain pH, producing a harmonic symphony of catalysis. Enzymes exhibit their highest effectiveness at the sweet spot, which is the ideal pH range. The hydrolytic ballet's beat is set by temperature, an understated yet powerful maestro in the enzymatic ensemble. Enzymes function best and work at their peak in a range of temperatures. The design of enzymatic hydrolysis processes involves strategic considerations such as selecting enzymes that grow best in specific pH and temperature ranges and maintaining these parameters during hydrolysis. Enzymes may show their catalytic virtuosity in an environment where pH and temperature are precisely controlled. This leads to ideal substrate transformation and the production of culinary marvels. The hydrolytic symphony is shaped by temperature and pH, which catalyze the story to a crescendo. The effectiveness of enzymatic hydrolysis is determined by their subtle artistic influence on enzyme activity, which goes beyond plain technical details. Enzymes convert substrates into carefully planned pH sensitivity and temperature responsiveness culinary compositions in this choreographed dance. This demonstrates the intricate interactions between variables that raise enzymatic hydrolysis to the level of a symphony of culinary brilliance **[89]**.

Criteria for Selecting Enzymes in Enzymatic Hydrolysis

The choice of enzymes for enzymatic hydrolysis is an important stage that greatly impacts the procedure's effectiveness and outcome. When selecting enzymes for a particular hydrolysis application, a few important factors are to consider. Some requirements are the ideal pH and temperature ranges, substrate selectivity, enzyme fortitude (chemical stability and thermostability), and thoughtful regulation of pH and temperature. Regarding the kind of substrate and the targeted linkages, enzymes show selectivity. The enzyme selection should align with the target substrate to achieve effective hydrolysis. For example, various substrates are best suited for different specificities of proteases, carbohydrates, and lipases. The temperature and pH levels greatly impact how well enzymes hydrolyze materials. Enzymes function best in particular pH and temperature ranges. To maximize substrate transformation efficiency, the pH and temperature of the enzymatic milieu must align with the selected enzymes' ideal ranges. Choosing the right enzymes for enzymatic hydrolysis requires careful consideration of chemical stability and thermostatability. Enzymes that are thermostatable continue to function in high-temperature environments, which enhances efficiency. Enzyme resistance to denaturation or inactivation caused by different additives, processing aids, and chemicals within the food matrix is ensured by chemical stability **[88]**. When developing gluten-free goods, enzymatic hydrolysis is essential for overcoming difficulties arising from the special characteristics of gluten proteins. In this context, the advantages of enzymatic hydrolysis are clear from several applications. By breaking down gluten proteins into smaller peptides, enzymatic proteolysis uses proteases from bacteria or fungi to provide cohesive and stretchy characteristics. Complex networks are broken down in this process, which lessens the natural stiffness of gluten-free dough and gives it a softer, more malleable feel akin to its conventional gluten-containing counterparts. Because of their capacity to hydrolyze starches, amylases play a major role in enhancing dough handling and water absorption. Amylases in gluten-free flours hydrolyze complex carbohydrates to release simpler sugars that improve the flour's ability to retain water. Thus, the consistency and workability of the dough is improved. Enzymes like amylases and proteases reduce the bitterness in gluten-free recipes. These enzymes work on proteins and starches, respectively, to lessen the bitter flavor of some gluten-free foods. This is achieved by dissolving certain peptide linkages and complex carbohydrates, improving the overall palatability of gluten-free compositions. Enzymes, such as transglutaminases, are essential for improving the texture and structure of fermented foods free of gluten. Transglutaminases catalyze cross-link formation between proteins, improving structural cohesiveness and taste better. The process of enzymatic hydrolysis is useful for increasing the bioavailability of nutrients. Vital minerals are released by identifying and eliminating antinutritional elements like phytates and protease inhibitors found in gluten-free grains, improving overall nutritional value by increasing their availability for absorption in the digestive tract. Proteases and other

pertinent enzymes aid in the enzymatic modification process, which is essential for increasing the amount of gluten-free baked goods. Enhancing the gas retention qualities of gluten-free flours helps create an environment more conducive to gas retention, increasing the amount of baked goods free of gluten. Similarly, enzymatic modification using amylases and proteases changes the structure and content of gluten-free foods to improve the texture and crunchiness of gluten-free snacks. As a consequence, customers get a more pleasurable sensory experience. These uses highlight how adaptable and successful enzymatic hydrolysis addresses obstacles in creating gluten-free products. It is feasible to produce gluten-free goods with the appropriate texture and mouthfeel and a sensory experience that is on par with their conventional, gluten-containing equivalents by carefully using enzymes **[90]**, **[91]**, **[92]**, **[93]**. Enzymatic adjustments are essential for improving the quality of products that are free of gluten. Enzymes such as amylases, transglutaminases, proteases, and others play a role in modifying the structure of gluten-free substitutes to enhance their attributes. Amylases, for example, work on complex starch structures to improve the end product's texture, malleability, and leavening by affecting dough handling, water absorption, and gas retention. Transglutaminases help proteins cross-link, strengthening the protein network, improving gas retention, and improving the texture of baked goods devoid of gluten. Furthermore, to improve the quality of gluten-free goods, enzymes, including phytases, proteases, and hemicellulases, alter non-gluten polysaccharides, lessen bitterness, and improve nutrient accessibility. These enzymatic procedures provide answers to problems relating to gluten-free baking, including managing dough, texture, volume, and sensory qualities. Enzymatic modification research is still ongoing, and it might lead to future improvements in the creation of goods devoid of gluten **[94]**, **[95]**, **[96]**, **[97]**.

CONCLUSION

To sum up, enzymatic hydrolysis has become a revolutionary and adaptable method for creating gluten-free goods, providing creative answers to the problems caused by the lack of gluten. The applications concerning many facets of the production of gluten-free products demonstrate the important influence of enzymatic interventions on texture, flavor, and nutritional value. The crunchiness problem in gluten-free snacks can be solved using enzymatic modification made possible by amylases and proteases. This method alters the structure and makeup of gluten-free components, adding to a more satisfying sensory experience evocative of classic gluten-containing treats. Additionally essential to enhancing nutritional bioavailability in gluten-free goods is enzymatic hydrolysis. Amylases and phytases work in tandem to break down complex carbohydrates and phytic acid, respectively. This increases the amount of energy available during digestion and improves the absorption of vital minerals. Enzymatic hydrolysis is a useful method for reducing the bitterness of gluten-free food. Amylases, lipases, and proteases collaborate to convert bitter molecules into smaller, less noticeable peptides. This tactical move improves the sensory experience, giving customers a more appealing choice. Furthermore, the use of amylases and hemicellulases for enzymatic modification of gluten-free flours is beneficial in customizing rheological characteristics, resolving issues related to water absorption, and enhancing the general consistency of dough. Protease-based enzymatic hydrolysis in gluten-free snacks and convenience meals demonstrates the potential of this process to improve texture and other sensory aspects. These enzymes help produce gluten-free foods with improved crispiness and mouthfeel, satisfying customer expectations for quality by carefully altering protein structures. Essentially, the combined data from various uses highlights the accuracy and versatility of enzymatic hydrolysis in addressing the intrinsic constraints of gluten-free recipes.

REFERENCES

1. Yuan, Q., Liu, S., Ma, M.-G., Ji, X.-X., Choi, S.-E., & Si, C. (2021). The Kinetics Studies on Hydrolysis of Hemicellulose. In Frontiers in Chemistry (Vol. 9). Frontiers Media SA. https://doi.org/10.3389/fchem.2021.781291
2. Arola, S., Malho, J., Laaksonen, P., Lille, M., & Linder, M. B. (2013). The role of hemicellulose in nanofibrillated cellulose networks. In Soft Matter (Vol. 9, Issue 4, pp. 1319–1326). Royal Society of Chemistry (RSC). https://doi.org/10.1039/c2sm26932e
3. Malek, M.I.A., A.S. Azmi, and N.I.M. Puad. Review on applications of sago starch processing effluent. 2016.
4. You, W., Duan, B., Wang, M., & Lam, W. (2011). Isothermal and Non-isothermal Crystallization Kinetics of Poly(L-Lactide)/Carbonated Hydroxyapatite Nanocomposite Microspheres. In Advances in Diverse Industrial Applications of Nanocomposites. InTech. https://doi.org/10.5772/14715
5. Qian, J., Chen, D., Zhang, Y., Gao, X., Xu, L., Guan, G., & Wang, F. (2023). Ultrasound-Assisted Enzymatic Protein Hydrolysis in Food Processing: Mechanism and Parameters. In Foods (Vol. 12, Issue 21, p. 4027). MDPI AG. https://doi.org/10.3390/foods12214027

6. Dent, T., & Maleky, F. (2022). Pulse protein processing: The effect of processing choices and enzymatic hydrolysis on ingredient functionality. In Critical Reviews in Food Science and Nutrition (Vol. 63, Issue 29, pp. 9914–9925). Informa UK Limited. https://doi.org/10.1080/10408398.2022.2070723
7. Wubshet, S. G., Måge, I., Böcker, U., Lindberg, D., Knutsen, S. H., Rieder, A., Rodriguez, D. A., & Afseth, N. K. (2017). FTIR as a rapid tool for monitoring molecular weight distribution during enzymatic protein hydrolysis of food processing by-products. In Analytical Methods (Vol. 9, Issue 29, pp. 4247–4254). Royal Society of Chemistry (RSC). https://doi.org/10.1039/c7ay00865a
8. Pascale, N. C., Chastinet, J. J., Bila, D. M., SantAnna, G. L., Jr, Quitério, S. L., & Vendramel, S. M. R. (2018). Enzymatic hydrolysis of floatable fatty wastes from dairy and meat food-processing industries and further anaerobic digestion. In Water Science and Technology (Vol. 79, Issue 5, pp. 985–992). IWA Publishing. https://doi.org/10.2166/wst.2018.508
9. Lim, J. Y., Chai, T.-T., Lam, M. Q., Ng, W. J., & Ee, K. Y. (2022). In silico enzymatic hydrolysis of soy sauce cake glycinin G4 to reveal the bioactive peptides as potential food ingredients. In Journal of Food Measurement and Characterization (Vol. 16, Issue 5, pp. 3477–3487). Springer Science and Business Media LLC. https://doi.org/10.1007/s11694-022-01433-y
10. Sharma, V., Tsai, M.-L., Nargotra, P., Chen, C.-W., Kuo, C.-H., Sun, P.-P., & Dong, C.-D. (2022). Agro-Industrial Food Waste as a Low-Cost Substrate for Sustainable Production of Industrial Enzymes: A Critical Review. In Catalysts (Vol. 12, Issue 11, p. 1373). MDPI AG. https://doi.org/10.3390/catal12111373
11. Hanlon, P., & Sewalt, V. (2020). GEMs: genetically engineered microorganisms and the regulatory oversight of their uses in modern food production. In Critical Reviews in Food Science and Nutrition (Vol. 61, Issue 6, pp. 959–970). Informa UK Limited. https://doi.org/10.1080/10408398.2020.1749026
12. EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Silano, V., Barat Baviera, J. M., Bolognesi, C., Brüschweiler, B. J., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mortensen, A., Rivière, G., Steffensen, I., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Herman, L., … Chesson, A. (2019). Characterisation of microorganisms used for the production of food enzymes [JB]. EFSA Journal, 17(6). https://doi.org/10.2903/j.efsa.2019.5741
13. EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (EFSA CEP Panel), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Glandorf, B., Herman, L., … Chesson, A. (2021). Scientific Guidance for the submission of dossiers on Food Enzymes [JB]. EFSA Journal, 19(10). https://doi.org/10.2903/j.efsa.2021.6851
14. Love, M., Bhandari, D., Dobson, R., & Billington, C. (2018). Potential for Bacteriophage Endolysins to Supplement or Replace Antibiotics in Food Production and Clinical Care. In Antibiotics (Vol. 7, Issue 1, p. 17). MDPI AG. https://doi.org/10.3390/antibiotics7010017
15. Blum, T. R., Liu, H., Packer, M. S., Xiong, X., Lee, P.-G., Zhang, S., Richter, M., Minasov, G., Satchell, K. J. F., Dong, M., & Liu, D. R. (2021). Phage-assisted evolution of botulinum neurotoxin proteases with reprogrammed specificity. In Science (Vol. 371, Issue 6531, pp. 803–810). American Association for the Advancement of Science (AAAS). https://doi.org/10.1126/science.abf5972
16. Packer, M. S., Rees, H. A., & Liu, D. R. (2017). Phage-assisted continuous evolution of proteases with altered substrate specificity. In Nature Communications (Vol. 8, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41467-017-01055-9
17. Ronau, J. A., Beckmann, J. F., & Hochstrasser, M. (2016). Substrate specificity of the ubiquitin and Ubl proteases. In Cell Research (Vol. 26, Issue 4, pp. 441–456). Springer Science and Business Media LLC. https://doi.org/10.1038/cr.2016.38
18. Meyer, J. G., Kim, S., Maltby, D. A., Ghassemian, M., Bandeira, N., & Komives, E. A. (2014). Expanding Proteome Coverage with Orthogonal-specificity α-Lytic Proteases. In Molecular & Cellular Proteomics (Vol. 13, Issue 3, pp. 823–835). Elsevier BV. https://doi.org/10.1074/mcp.m113.034710
19. Nguyen, L. M., & Fox, K. M. (2019). Enzyme Kinetic Characterization and Substrate Specificity of Schizophyllum commune Metacaspases. In The FASEB Journal (Vol. 33, Issue S1). Wiley. https://doi.org/10.1096/fasebj.2019.33.1_supplement.781.12
20. Rietschel, B., Bornemann, S., Arrey, T. N., Baeumlisberger, D., Karas, M., & Meyer, B. (2009). Membrane protein analysis using an improved peptic in-solution digestion protocol. In PROTEOMICS (Vol. 9, Issue 24, pp. 5553–5557). Wiley. https://doi.org/10.1002/pmic.200900532
21. Spolaore, B., Polverino de Laureto, P., Zambonin, M., & Fontana, A. (2004). Limited Proteolysis of Human Growth Hormone at Low pH: Isolation, Characterization, and Complementation of the Two Biologically Relevant Fragments 1−44 and 45−191. In Biochemistry (Vol. 43, Issue 21, pp. 6576–6586). American Chemical Society (ACS). https://doi.org/10.1021/bi049491g

22. Bai, Y., Wang, J., Zhang, Z., Shi, P., Luo, H., Huang, H., Feng, Y., & Yao, B. (2010). Extremely Acidic β-1,4-Glucanase, CelA4, from Thermoacidophilic Alicyclobacillus sp. A4 with High Protease Resistance and Potential as a Pig Feed Additive. In Journal of Agricultural and Food Chemistry (Vol. 58, Issue 3, pp. 1970–1975). American Chemical Society (ACS). https://doi.org/10.1021/jf9035595
23. Lim, S. J., & Oslan, S. N. (2021). Native to designed: microbial α-amylases for industrial applications. In PeerJ (Vol. 9, p. e11315). PeerJ. https://doi.org/10.7717/peerj.11315
24. Kumari, N., Sushil, Malik, K., & Avtar, R. (2019). Microbial amylases: An overview on recent advancement. In Journal of entomology and zoology studies (Vol. 7, p. 198–205). Society of Agricultural Research and Social Development.
25. Xie, X., Ban, X., Gu, Z., Li, C., Hong, Y., Cheng, L., & Li, Z. (2020). Structure-Based Engineering of a Maltooligosaccharide-Forming Amylase To Enhance Product Specificity. In Journal of Agricultural and Food Chemistry (Vol. 68, Issue 3, pp. 838–844). American Chemical Society (ACS). https://doi.org/10.1021/acs.jafc.9b07234
26. Janeček, Š., Svensson, B., & MacGregor, E. A. (2013). α-Amylase: an enzyme specificity found in various families of glycoside hydrolases. In Cellular and Molecular Life Sciences (Vol. 71, Issue 7, pp. 1149–1170). Springer Science and Business Media LLC. https://doi.org/10.1007/s00018-013-1388-z
27. Rittenauer, M., Gladis, S., Gastl, M., & Becker, T. (2021). Gelatinization or Pasting? The Impact of Different Temperature Levels on the Saccharification Efficiency of Barley Malt Starch. In Foods (Vol. 10, Issue 8, p. 1733). MDPI AG. https://doi.org/10.3390/foods10081733
28. Yuan, J., Wang, X., Yin, D., Wang, M., Yin, X., Lei, Z., & Guo, Y. (2017). Effect of different amylases on the utilization of cornstarch in broiler chickens. In Poultry Science (Vol. 96, Issue 5, pp. 1139–1148). Elsevier BV. https://doi.org/10.3382/ps/pew323
29. Barbosa, M. S., Freire, C. C. C., Almeida, L. C., Freitas, L. S., Souza, R. L., Pereira, E. B., Mendes, A. A., Pereira, M. M., Lima, Á. S., & Soares, C. M. F. (2019). Optimization of the enzymatic hydrolysis of Moringa oleifera Lam oil using molecular docking analysis for fatty acid specificity. In Biotechnology and Applied Biochemistry (Vol. 66, Issue 5, pp. 823–832). Wiley. https://doi.org/10.1002/bab.1793
30. Souza, G. P. R., Correia, T. B. A., Reis, W. S. M., Bredda, E. H., Da Rós, P. C. M., & Pereira, E. B. (2022). Enzymatic Hydrolysis of Waste Cooking Oil by Lipase Catalysis: Simplex Mixture Design Optimization. In Catalysis Letters (Vol. 153, Issue 3, pp. 689–697). Springer Science and Business Media LLC. https://doi.org/10.1007/s10562-022-04025-z
31. Qiao, H., Zhang, F., Guan, W., Zuo, J., & Feng, D. (2016). Optimisation of combi-lipases from Aspergillus niger for the synergistic and efficient hydrolysis of soybean oil. In Animal Science Journal (Vol. 88, Issue 5, pp. 772–780). Wiley. https://doi.org/10.1111/asj.12718
32. HIRAYAMA, O. (1964). Glyceride Structure and Biosynthesis of Natural Fats. In Agricultural and Biological Chemistry (Vol. 28, Issue 4, pp. 193–200). Oxford University Press (OUP). https://doi.org/10.1271/bbb1961.28.193
33. Jensen, R. G., Sampugna, J., & Pereira, R. L. (1964). Intermolecular Specificity of Pancreatic Lipase and the Structural Analysis of Milk Triglycerides. In Journal of Dairy Science (Vol. 47, Issue 7, pp. 727–732). American Dairy Science Association. https://doi.org/10.3168/jds.s0022-0302(64)88753-1
34. Lim, S. Y., Steiner, J. M., & Cridge, H. (2022). Lipases: it's not just pancreatic lipase! In American Journal of Veterinary Research (Vol. 83, Issue 8). American Veterinary Medical Association (AVMA). https://doi.org/10.2460/ajvr.22.03.0048
35. Xiao, X., & Lowe, M. E. (2015). The β5-Loop and Lid Domain Contribute to the Substrate Specificity of Pancreatic Lipase-related Protein 2 (PNLIPRP2). In Journal of Biological Chemistry (Vol. 290, Issue 48, pp. 28847–28856). Elsevier BV. https://doi.org/10.1074/jbc.m115.683375
36. Qamar, S. A., Qamar, M., Bilal, M., Bharagava, R. N., Ferreira, L. F. R., Sher, F., & Iqbal, H. M. N. (2021). Cellulose-deconstruction potential of nano-biocatalytic systems: A strategic drive from designing to sustainable applications of immobilized cellulases. In International Journal of Biological Macromolecules (Vol. 185, pp. 1–19). Elsevier BV. https://doi.org/10.1016/j.ijbiomac.2021.06.079
37. Zajki-Zechmeister, K., Kaira, G. S., Eibinger, M., Seelich, K., & Nidetzky, B. (2021). Processive Enzymes Kept on a Leash: How Cellulase Activity in Multienzyme Complexes Directs Nanoscale Deconstruction of Cellulose. In ACS Catalysis (Vol. 11, Issue 21, pp. 13530–13542). American Chemical Society (ACS). https://doi.org/10.1021/acscatal.1c03465
38. Berlemont, R. (2022). The Potential for Cellulose Deconstruction in Fungal Genomes. In Encyclopedia (Vol. 2, Issue 2, pp. 990–1003). MDPI AG. https://doi.org/10.3390/encyclopedia2020065
39. Resch, M. G., Donohoe, B. S., Baker, J. O., Decker, S. R., Bayer, E. A., Beckham, G. T., & Himmel, M. E. (2013). Fungal cellulases and complexed cellulosomal enzymes exhibit synergistic mechanisms in cellulose

deconstruction. In Energy & Environmental Science (Vol. 6, Issue 6, p. 1858). Royal Society of Chemistry (RSC). https://doi.org/10.1039/c3ee00019b

40. You, C., Liu, Y.-J., Cui, Q., & Feng, Y. (2023). Glycoside Hydrolase Family 48 Cellulase: A Key Player in Cellulolytic Bacteria for Lignocellulose Biorefinery. In Fermentation (Vol. 9, Issue 3, p. 204). MDPI AG. https://doi.org/10.3390/fermentation9030204

41. Halliwell, G., & Griffin, M. (1973). The nature and mode of action of the cellulolytic component C1 of Trichoderma koningii on native cellulose. In Biochemical Journal (Vol. 135, Issue 4, pp. 587–594). Portland Press Ltd. https://doi.org/10.1042/bj1350587

42. Venezia, V., Califano, V., Pota, G., Costantini, A., Landi, G., & Di Benedetto, A. (2020). CFD Simulations of Microreactors for the Hydrolysis of Cellobiose to Glucose by β-Glucosidase Enzyme. In Micromachines (Vol. 11, Issue 9, p. 790). MDPI AG. https://doi.org/10.3390/mi11090790

43. Yepes, C., Estévez, J., Arroyo, M., & Ladero, M. (2022). Immobilization of an Industrial β-Glucosidase from Aspergillus fumigatus and Its Use for Cellobiose Hydrolysis. In Processes (Vol. 10, Issue 6, p. 1225). MDPI AG. https://doi.org/10.3390/pr10061225

44. Wei, H., Wang, W., Alper, H. S., Xu, Q., Knoshaug, E. P., Van Wychen, S., Lin, C.-Y., Luo, Y., Decker, S. R., Himmel, M. E., & Zhang, M. (2019). Ameliorating the Metabolic Burden of the Co-expression of Secreted Fungal Cellulases in a High Lipid-Accumulating Yarrowia lipolytica Strain by Medium C/N Ratio and a Chemical Chaperone. In Frontiers in Microbiology (Vol. 9). Frontiers Media SA. https://doi.org/10.3389/fmicb.2018.03276

45. Wang, J., Liu, S., Li, Y., Wang, H., Xiao, S., Li, C., & Liu, B. (2017). Central carbon metabolism influences cellulase production in Bacillus licheniformis. In Letters in Applied Microbiology (Vol. 66, Issue 1, pp. 49–54). Oxford University Press (OUP). https://doi.org/10.1111/lam.12813

46. Sultana, S., Sawrav, Md. S. S., Das, S. R., Alam, M., Aziz, Md. A., Hossain, Md. A.-A., & Haque, Md. A. (2022). Isolation and Biochemical Characterization of Cellulase Producing Goat Rumen Bacteria. In Proceedings of International Conference on Emerging Trends in Engineering and Advanced Science. International Conference on Emerging Trends in Engineering and Advanced Science. AIJR Publisher. https://doi.org/10.21467/proceedings.123.12

47. Singh Jadaun, J. (2018). Pectinase: A Useful Tool in Fruit Processing Industries. In Nutrition & Food Science International Journal (Vol. 5, Issue 5). Juniper Publishers. https://doi.org/10.19080/nfsij.2018.05.555673

48. Adapa, V., Ramya, L. N., Pulicherla, K. K., & Rao, K. R. S. S. (2014). Cold Active Pectinases: Advancing the Food Industry to the Next Generation. In Applied Biochemistry and Biotechnology (Vol. 172, Issue 5, pp. 2324–2337). Springer Science and Business Media LLC. https://doi.org/10.1007/s12010-013-0685-1

49. Satapathy, S., Rout, J. R., Kerry, R. G., Thatoi, H., & Sahoo, S. L. (2020). Biochemical Prospects of Various Microbial Pectinase and Pectin: An Approachable Concept in Pharmaceutical Bioprocessing. In Frontiers in Nutrition (Vol. 7). Frontiers Media SA. https://doi.org/10.3389/fnut.2020.00117

50. Haile, S., & Ayele, A. (2022). Pectinase from Microorganisms and Its Industrial Applications. In S. Rodriguez-Couto (Ed.), The Scientific World Journal (Vol. 2022, pp. 1–15). Hindawi Limited. https://doi.org/10.1155/2022/1881305

51. Wormit, A., & Usadel, B. (2018). The Multifaceted Role of Pectin Methylesterase Inhibitors (PMEIs). In International Journal of Molecular Sciences (Vol. 19, Issue 10, p. 2878). MDPI AG. https://doi.org/10.3390/ijms19102878

52. Biz, A., Farias, F. C., Motter, F. A., de Paula, D. H., Richard, P., Krieger, N., & Mitchell, D. A. (2014). Pectinase Activity Determination: An Early Deceleration in the Release of Reducing Sugars Throws a Spanner in the Works! In V. N. Uversky (Ed.), PLoS ONE (Vol. 9, Issue 10, p. e109529). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0109529

53. Wu, H.-C., Bulgakov, V. P., & Jinn, T.-L. (2018). Pectin Methylesterases: Cell Wall Remodeling Proteins Are Required for Plant Response to Heat Stress. In Frontiers in Plant Science (Vol. 9). Frontiers Media SA. https://doi.org/10.3389/fpls.2018.01612

54. Yadav, S., Yadav, P. K., Yadav, D., & Yadav, K. D. S. (2009). Pectin lyase: A review. In Process Biochemistry (Vol. 44, Issue 1, pp. 1–10). Elsevier BV. https://doi.org/10.1016/j.procbio.2008.09.012

55. Biz, A., Farias, F. C., Motter, F. A., de Paula, D. H., Richard, P., Krieger, N., & Mitchell, D. A. (2014). Pectinase Activity Determination: An Early Deceleration in the Release of Reducing Sugars Throws a Spanner in the Works! In V. N. Uversky (Ed.), PLoS ONE (Vol. 9, Issue 10, p. e109529). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0109529

56. Toy, J. Y. H., Lu, Y., Huang, D., Matsumura, K., & Liu, S.-Q. (2020). Enzymatic treatment, unfermented and fermented fruit-based products: current state of knowledge. In Critical Reviews in Food Science and

Nutrition (Vol. 62, Issue 7, pp. 1890–1911). Informa UK Limited. https://doi.org/10.1080/10408398.2020.1848788

57. Keggi, C., & Doran-Peterson, J. (2020). The Homogalacturonan Deconstruction System of Paenibacillus amylolyticus 27C64 Requires No Extracellular Pectin Methylesterase and Has Significant Industrial Potential. In E. R. Master (Ed.), Applied and Environmental Microbiology (Vol. 86, Issue 12). American Society for Microbiology. https://doi.org/10.1128/aem.02275-19

58. Zhao, Li-li., Tian, CH-r., Hua, J. I., & Shuang, L. (2007). Research Actuality of Microbe Pectinases and its Application in Fruits and Vegetables Processing. In Progress in Modern Biomedicine (Issue 6, pp. 951–953). Ministry of Science and Technology of China Statistic.

59. Duvetter, T., Sila, D. N., Van Buggenhout, S., Jolie, R., Van Loey, A., & Hendrickx, M. (2009). Pectins in Processed Fruit and Vegetables: Part I—Stability and Catalytic Activity of Pectinases. In Comprehensive Reviews in Food Science and Food Safety (Vol. 8, Issue 2, pp. 75–85). Wiley. https://doi.org/10.1111/j.1541-4337.2009.00070.x

60. Rafique, N., Bashir, S., Khan, M. Z., Hayat, I., Orts, W., & Wong, D. W. S. (2021). Metabolic engineering of Bacillus subtilis with an endopolygalacturonase gene isolated from Pectobacterium. carotovorum; a plant pathogenic bacterial strain. In K. S. Ahmad (Ed.), PLOS ONE (Vol. 16, Issue 12, p. e0256562). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0256562

61. Hashim, H., Maskat, M. Y., Mustapha, W. A. W., Mamot, S., & Saadiah, I. (2013). Optimization of enzymatic hydrolysis of cockle (Anadara Granosa) meat wash water precipitate for the development of seafood flavor. In International Food Research Journal (Vol. 20, Issue 6, pp. 3053–3059). Universiti Putra Malaysia.

62. Dong, Y., Yan, W., Zhang, X.-D., Dai, Z.-Y., & Zhang, Y.-Q. (2021). Steam Explosion-Assisted Extraction of Protein from Fish Backbones and Effect of Enzymatic Hydrolysis on the Extracts. In Foods (Vol. 10, Issue 8, p. 1942). MDPI AG. https://doi.org/10.3390/foods10081942

63. Liang, X., Qian, G., Yang, H., Chen, N., Ai, Z., Xing, Y., Huang, W., Xu, L., Li, M., Wang, Z., Zheng, Y., & Yue, X. (2023). Evaluation of IgG/IgE-binding capacity and functional properties of enzymatic hydrolysis in skimmed cow milk system. In Journal of Food Science (Vol. 88, Issue 7, pp. 2780–2795). Wiley. https://doi.org/10.1111/1750-3841.16579

64. Fang, Fu-y., Miao, Y-l., & Song, W-d. (2009). Compound Enzymatic Hydrolysis of Paphia undulate Meat and Development of Its Functional Oral Liquid. In Food Science (Vol. 30, Issue 18, pp. 412–415). China Food Publishing Co.

65. Izadyar, L., Friboulet, A., Remy, M. H., Roseto, A., & Thomas, D. (1993). Monoclonal anti-idiotypic antibodies as functional internal images of enzyme active sites: production of a catalytic antibody with a cholinesterase activity. In Proceedings of the National Academy of Sciences (Vol. 90, Issue 19, pp. 8876–8880). Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.90.19.8876

66. Lu, J., Jiang, X., Zhen, L., Luan, H.-F., Chen, H., Wang, X.-J., & Ching, C.-B. (2010). Cloning, expression, purification and characterization of the carboxylesterase Yeig from Escherichia coli k12. In African Journal of Microbiology Research (Vol. 4, Issue 9, pp. 757–765). Academic Journals.

67. Inagami, T., & Mitsuda, H. (1964). The Mechanism of the Specificity of Trypsin Catalysis. In Journal of Biological Chemistry (Vol. 239, Issue 5, pp. 1388–1394). Elsevier BV. https://doi.org/10.1016/s0021-9258(18)91326-8

68. Navanietha Krishnaraj, R., David, A., & Sani, R. K. (2017). Fundamentals of Enzymatic Processes. In Extremophilic Enzymatic Processing of Lignocellulosic Feedstocks to Bioenergy (pp. 5–29). Springer International Publishing. https://doi.org/10.1007/978-3-319-54684-1_2

69. Lingenfelder, M., Tomba, G., Costantini, G., Colombi Ciacchi, L., De Vita, A., & Kern, K. (2007). Tracking the Chiral Recognition of Adsorbed Dipeptides at the Single-Molecule Level. In Angewandte Chemie International Edition (Vol. 46, Issue 24, pp. 4492–4495). Wiley. https://doi.org/10.1002/anie.200700194

70. Pan, R., Zhang, X.-J., Zhang, Z.-J., Zhou, Y., Tian, W.-X., & He, R.-Q. (2010). Substrate-induced Changes in Protease Active Site Conformation Impact on Subsequent Reactions with Substrates. In Journal of Biological Chemistry (Vol. 285, Issue 30, pp. 22950–22956). Elsevier BV. https://doi.org/10.1074/jbc.m110.103549

71. Venditto, I., Najmudin, S., Luís, A. S., Ferreira, L. M. A., Sakka, K., Knox, J. P., Gilbert, H. J., & Fontes, C. M. G. A. (2015). Family 46 Carbohydrate-binding Modules Contribute to the Enzymatic Hydrolysis of Xyloglucan and β-1,3–1,4-Glucans through Distinct Mechanisms. In Journal of Biological Chemistry (Vol. 290, Issue 17, pp. 10572–10586). Elsevier BV. https://doi.org/10.1074/jbc.m115.637827

72. Venditto, I., Najmudin, S., Luís, A. S., Ferreira, L. M. A., Sakka, K., Knox, J. P., Gilbert, H. J., & Fontes, C. M. G. A. (2015). Family 46 Carbohydrate-binding Modules Contribute to the Enzymatic Hydrolysis of

Xyloglucan and β-1,3–1,4-Glucans through Distinct Mechanisms. In Journal of Biological Chemistry (Vol. 290, Issue 17, pp. 10572–10586). Elsevier BV. https://doi.org/10.1074/jbc.m115.637827

73. Morgillo, C. M., Lupia, A., Deplano, A., Pirone, L., Fiorillo, B., Pedone, E., Luque, F. J., Onnis, V., Moraca, F., & Catalanotti, B. (2022). Molecular Basis for Non-Covalent, Non-Competitive FAAH Inhibition. In International Journal of Molecular Sciences (Vol. 23, Issue 24, p. 15502). MDPI AG. https://doi.org/10.3390/ijms232415502

74. Kisselev, A. F., Akintola, O., & Smith, J. (2021). Abstract 1396: Non-competitive inhibition of proteasome by kinase inhibitors. In Cancer Research (Vol. 81, Issue 13_Supplement, pp. 1396–1396). American Association for Cancer Research (AACR). https://doi.org/10.1158/1538-7445.am2021-1396

75. Sanchez, O. F., Lee, J., Yu King Hing, N., Kim, S.-E., Freeman, J. L., & Yuan, C. (2017). Lead (Pb) exposure reduces global DNA methylation level by non-competitive inhibition and alteration of dnmt expression. In Metallomics (Vol. 9, Issue 2, pp. 149–160). Oxford University Press (OUP). https://doi.org/10.1039/c6mt00198j

76. Cole, R. N., Chen, W., Pascal, L. E., Nelson, J. B., Wipf, P., & Wang, Z. (2022). (+)-JJ-74–138 is a Novel Noncompetitive Androgen Receptor Antagonist. In Molecular Cancer Therapeutics (Vol. 21, Issue 4, pp. 483–492). American Association for Cancer Research (AACR). https://doi.org/10.1158/1535-7163.mct-21-0432

77. Mondal, A., Bhat, I. A., Karunakaran, S., & De, M. (2021). Supramolecular Interaction of Molecular Cage and β-Galactosidase: Application in Enzymatic Inhibition, Drug Delivery and Antimicrobial Activity. In ChemBioChem (Vol. 22, Issue 11, pp. 1955–1960). Wiley. https://doi.org/10.1002/cbic.202100008

78. Sampedro, L. J. G., Grimaldos, N. A. G., Pereañez, J. A., & Montoya, J. E. Z. (2019). Lipids as competitive inhibitors of subtilisin carlsberg in the enzymatic hydrolysis of proteins in red tilapia (oreochromis sp.) viscera: insights from kinetic models and a molecular docking study. In Brazilian Journal of Chemical Engineering (Vol. 36, Issue 2, pp. 647–655). FapUNIFESP (SciELO). https://doi.org/10.1590/0104-6632.20190362s20180346

79. Brazdausks, P., Godina, D., & Puke, M. (2023). Phosphorus-Containing Catalyst Impact on Furfural and Glucose Production during Consecutive Hydrothermal Pretreatment and Enzymatic Hydrolysis. In Fermentation (Vol. 9, Issue 9, p. 803). MDPI AG. https://doi.org/10.3390/fermentation9090803

80. ZHANG, R.-H., HU, Q.-Z., KANG, Q., QI, L.-B., PANG, Y.-P., & YU, L. (2021). Research on Competitive Enzymatic Hydrolysis-Assisted Liquid Crystal-based Acetylcholine Sensor. In Chinese Journal of Analytical Chemistry (Vol. 49, Issue 2, pp. e21014–e21019). Elsevier BV. https://doi.org/10.1016/s1872-2040(20)60081-0

81. Padron-Regalado, E., Mejía-Olvera, E. E., Marruffo-Carmona, A. M., Solís-Suárez, J. C., Peralta-Agapito, D., & Medina-Rivero, E. (2023). Efficient enzymatic hydrolysis for enriching the antioxidant content of agro-food industrial residues. In JSFA reports (Vol. 3, Issue 9, pp. 420–428). Wiley. https://doi.org/10.1002/jsf2.145

82. Areeya, S., Tawai, A., & Sriariyanun, M. (2020). Model-Based Control for Fed-Batch Enzymatic Hydrolysis Reactor of Lignocellulosic Biomass. In 2020 6th International Conference on Control, Automation and Robotics (ICCAR). 2020 6th International Conference on Control, Automation and Robotics (ICCAR). IEEE. https://doi.org/10.1109/iccar49639.2020.9108066

83. Baranwal, J., Lhospice, S., Kanade, M., Chakraborty, S., Gade, P. R., Harne, S., Herrou, J., Mignot, T., & Gayathri, P. (2019). Allosteric regulation of a prokaryotic small Ras-like GTPase contributes to cell polarity oscillations in bacterial motility. In A. M. Stock (Ed.), PLOS Biology (Vol. 17, Issue 9, p. e3000459). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pbio.3000459

84. Guzmán-Ortiz, F. A., Castro-Rosas, J., Gómez-Aldapa, C. A., Mora-Escobedo, R., Rojas-León, A., Rodríguez-Marín, M. L., Falfán-Cortés, R. N., & Román-Gutiérrez, A. D. (2018). Enzyme activity during germination of different cereals: A review. In Food Reviews International (Vol. 35, Issue 3, pp. 177–200). Informa UK Limited. https://doi.org/10.1080/87559129.2018.1514623

85. Chang-po, S. (2013). Study on the factors to affect the activity of zearalenone degrading enzyme ZLHY6. In Science and Technology of Cereals, Oils and Foods. National Food Authority only non-profit research institutions - Academy of State Grain Administration.

86. BravoRodriguez, V., JuradoAlameda, E., MartinezGallegos, J. F., ReyesRequena, A., & GarciaLopez, A. I. (2006). Enzymatic Hydrolysis of Soluble Starch with an α-Amylase from Bacillus licheniformis. In Biotechnology Progress (Vol. 22, Issue 3, pp. 718–722). Wiley. https://doi.org/10.1021/bp060057a

87. Donovan, J. W., & Hansen, L. U. (1971). The β-n-acetylglucosaminidase activity of egg white. 1. Kinetics of the Reaction and Determination of the Factors Affecting the Stability of the Enzyme in Egg White. In

Journal of Food Science (Vol. 36, Issue 1, pp. 174–177). Wiley. https://doi.org/10.1111/j.1365-2621.1971.tb02063.x

88. Nazarov, K., Sagdiyev, X., Shukurxonova, M., & Karimova, K. (2022). Features of enzymatic hydrolysis of fibers of genetically different cotton lines. In S. Sadullozoda, Z. Usmanov, & A. Gibadullin (Eds.), International Conference on Remote Sensing of the Earth: Geoinformatics, Cartography, Ecology, and Agriculture (RSE 2022). SPIE. https://doi.org/10.1117/12.2643189
89. Vogelsang-O'Dwyer, M., Sahin, A. W., Arendt, E. K., & Zannini, E. (2022). Enzymatic Hydrolysis of Pulse Proteins as a Tool to Improve Techno-Functional Properties. In Foods (Vol. 11, Issue 9, p. 1307). MDPI AG. https://doi.org/10.3390/foods11091307
90. Saini, R., Patel, A. K., Saini, J. K., Chen, C.-W., Varjani, S., Singhania, R. R., & Di Dong, C. (2022). Recent advancements in prebiotic oligomers synthesis via enzymatic hydrolysis of lignocellulosic biomass. In Bioengineered (Vol. 13, Issue 2, pp. 2139–2172). Informa UK Limited. https://doi.org/10.1080/21655979.2021.2023801
91. Sharikov, A., Amelyakina, M., Serba, E., Ivanov, V., Polivanovskaya, D., & Abramova, I. (2022). Steam Extraction System Use in the Gluten-Free Cereal Snacks Technology. In Food Industry (Vol. 7, Issue 4, pp. 6–14). Ural State University of Economics. https://doi.org/10.29141/2500-1922-2022-7-4-1
92. Sharikov, A. Yu., Ivanov, V. V., Sokolova, E. N., Amelyakina, M. V., & Serba, E. (2023). Technological aspects of obtaining gluten-free cereal products based on biocatalytic and hydrothermomechanical processes. In Proceedings of the 1st International Congress "The Latest Achievements of Medicine, Healthcare, and Health-Saving Technologies." I International Congress "The Latest Achievements of Medicine, Healthcare, and Health-Saving Technologies." Kemerovo State University. https://doi.org/10.21603/-i-ic-152
93. Medvid, I., Shydlovska, O., & Dotsenko, V. (2019). The use of sunflower lecithin in the technology on gluten-free bread with enzymatic modification of flour starch. In Journal of Faculty of Food Engineering (Vol. 17, Issue 4, pp. 352–362). Stefan cel Mare University of Suceava.
94. Kroghsbo, S., Andersen, N. B., Rasmussen, T. F., Jacobsen, S., & Madsen, C. B. (2014). Acid Hydrolysis of Wheat Gluten Induces Formation of New Epitopes but Does Not Enhance Sensitizing Capacity by the Oral Route: A Study in "Gluten Free" Brown Norway Rats. In N. J. Mantis (Ed.), PLoS ONE (Vol. 9, Issue 9, p. e107137). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0107137
95. Dotsenko, V., Medvid, I., Shydlovska, O., & Ishchenko, T. (2019). Studying the possibility of using enzymes, lecithin, and albumen in the technology of gluten-free bread. In Eastern-European Journal of Enterprise Technologies (Vol. 1, Issue 11 (97), pp. 42–51). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2019.154957
96. Espinoza-Herrera, J., Martínez, L. M., Serna-Saldívar, S. O., & Chuck-Hernández, C. (2021). Methods for the Modification and Evaluation of Cereal Proteins for the Substitution of Wheat Gluten in Dough Systems. In Foods (Vol. 10, Issue 1, p. 118). MDPI AG. https://doi.org/10.3390/foods10010118
97. Wang, J., Bai, H., Zhang, R., Ding, G., Cai, X., Wang, W., Zhu, G., Zhou, P., & Zhang, Y. (2023). Effect of a Bacterial Laccase on the Quality and Micro-Structure of Whole Wheat Bread. In Journal of Microbiology and Biotechnology (Vol. 33, Issue 12, pp. 1671–1680). Korean Society for Microbiology and Biotechnology. https://doi.org/10.4014/jmb.2305.05008

Funds:

This research was funded by Ministry of Agriculture of Kazakhstan, grant number IRN BR22884456.

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Dinara Akimova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan; Kazakh Research Institute of Processing and Food Industry (Semey Branch), 29 Bairursynov Street, 071410, Semey, Kazakhstan,
Tel.: + 7 7222 314 602; +7 7222 770026
E-mail: akdilife@mail.ru
ORCID: https://orcid.org/0000-0001-8922-2854

Aitbek Kakimov, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: bibi.53@mail.ru
ORCID: https://orcid.org/0000-0002-9607-1684

***Anuarbek Suychinov**, Kazakh Research Institute of Processing and Food Industry (Semey Branch), 29 Bairursynov Street, 071410, Semey, Kazakhstan,
Tel.: +7 7222 770026
E-mail: asuychinov@gmail.com
ORCID: https://orcid.org/0000-0003-4862-3293

Zhumatay Urazbayev, Kazakh Research Institute of Processing and Food Industry, Gagarin Ave., 238 "G", 050060, Almaty, Kazakhstan,
Tel.: +7 7222 770026
E-mail: sb.info@rpf.kz
ORCID: https://orcid.org/0000-0003-1898-0564

Yerlan Zharykbasov, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: erlan-0975@mail.ru
ORCID: https://orcid.org/0000-0001-9707-0539

Nadir Ibragimov, Shakarim University, Department of Technological Equipment and Mechanical Engineering, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: ibragimnk@mail.ru
ORCID: https://orcid.org/0000-0001-9607-823X

Aigul Bauyrzhanova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: bauyrzhanova@shakarim.kz
ORCID: https://orcid.org/0009-0007-5031-8411

Assiya Utegenova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: asia_aksu@mail.ru
ORCID: https://orcid.org/0000-0003-3378-6815

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 4.2.2024
Revised: 20.3.2024
Accepted: 28.3.2024
Published: 3.4.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 366-385
https://doi.org/10.5219/1961
ISSN: 1337-0960 online
www.potravinarstvo.com

Quality assessment of different polyethylene-packaged groundwater in the Ilorin metropolis of Nigeria for compliance with standards

Mutiat Balogun, Tawakalitu Aruna, Olaide Akintayo, Rasheedat Imam, Olayinka Shukurat, Oluseye Lala, Fausat Kolawole, Christianah Gbadebo

ABSTRACT

There is a notable proliferation of sachet water brands in the Ilorin Metropolis of Nigeria. However, more information regarding their safety for human consumption is required. This study aimed to determine the quality attributes of sachet water produced within the Ilorin metropolis of Nigeria to ascertain their conformity with regulatory standards. Three sachet water companies were each selected from three Local Government Areas within the study area. Physicochemical and microbial analyses were conducted on the collected water samples within 24 hours of production and monthly during 4-month storage. The results showed that temperature (25.10-30.13 °C) and turbidity (0.37-2.84 NTU) were within permissible limits. Total hardness (36.0-136.33 mg/L) and pH (6.42-8.86) significantly ($p \leq 0.05$) increased during storage and were above standards in some samples. There were significant ($p \leq 0.05$) variations in chlorides (2.83-8.57 mg/L), nitrates (0.15-0.64 mg/L), sulphates (4.70-7.12 mg/L), and sodium contents (10.16-18.74 mg/L) but all were within standards. While five of the nine sachet water brands complied (2-100 CFU/mL) with the limit of 100 CFU/mL for the total viable count, all the samples failed the zero-tolerance requirement for coliform. Possible fecal contamination was concluded as the main concern with the sachet water brands produced within Ilorin Metropolis, suggesting their unfitness for human consumption. The water brands may, therefore, pose severe health hazards to consumers and threaten public health. In conclusion, the study demonstrated the likely unfitness of some sachet water brands in the Ilorin Metropolis of Nigeria for human consumption, owing to fecal coliform. This underscores the need for regulatory agencies to intensify the certification process of prospective sachet water factories. Furthermore, regular inspections of certified factories and their practices and products are recommended to ensure continued compliance with regulatory standards.

Keywords: sachet water, physicochemical, microbial, regulatory standards, conformity

INTRODUCTION

Water is essential for various aspects of life, such as drinking, domestic, industrial, and agricultural purposes **[1]**. Therefore, a reliable supply of clean, wholesome water is vital to promote healthy living in any community. According to a review **[2]**, drinking up to 8 glasses of water may prevent certain chronic diseases. However, models for delivering safe drinking water in developed countries need to be improved, partly due to poverty **[3]**. The World Health Organisation (WHO) and the United Nations International Children's Emergency Fund (UNICEF) jointly reported that about 66 million people lack access to improved drinking water in Nigeria **[4]**. Thus, there is an increasing search for low-cost but safe drinking water schemes in the country, a typical example of which is polyethylene-packaged water (subsequently referred to as sachet water). While this strategy is

recommended to ensure sustainable access to potable water in rural and peri-urban settings of developing Nations **[5]**, its effectiveness would depend on consumers' prior awareness of the potential health risks associated with unsafe drinking water **[6]**.

Water pollution seriously threatens public health due to already established contamination sources and emerging ones **[7]**. While the typical water pollutants may be agricultural, industrial, or environmental, there are growing concerns about evolving chiral pollutants from pharmaceutical and agrochemical residues **[8]**, **[9]**. Some of these pollutants could significantly impact the safety of the environment and water resources, increasing their associated risks of carcinogenicity and antifertility to humans **[10]**. As a result, the suitability of ground and surface waters for human use, especially for drinking purposes, becomes doubtful. Meanwhile, groundwater is a common source of sachet water production in Nigeria.

The consumption of sachet water is prevalent in Nigeria. The innovation, popularly known as 'pure water' in local parlance, represents a direct response to the yearnings of many Nigerians for potable water **[11]**. Akinsola et al. **[12]** described sachet water as Nigeria's most ubiquitous form of water marketing. Since its introduction in the 1990s, it has constituted one of the fastest-growing industries in the country **[6]**. During hot weather conditions, consumers typically desire chilled water. To meet this demand, vendors sell chilled sachet water on roadsides and in traffic build-ups **[11]**. The popularity of sachet water in Nigeria may also be associated with its affordability and perceived hygienic quality. However, the sachet water sector has become very attractive to would-be small-scale investors whose interests lie in making quick profits, leaving much to be desired regarding product quality **[11]**. To safeguard the safety of sachet water, the National Agency for Food and Drug Administration and Control (NAFDAC) scrutinizes intending manufacturers and their facilities before certification and registration numbers are granted. After approval, the agency conducts follow-up regulatory checks, mainly by end-product monitoring, to ensure that the companies comply with WHO's standards. This approach was, however, deemed insufficient to ensure the safety of sachet water and guarantee public health **[5]**.

According to some past studies, the quality of sachet water brands in some major cities of Nigeria is still being determined. For instance, Yusuf et al. **[13]** reported that all sachet water samples analyzed in Zaria, Nigeria, were biologically unfit based on the WHO standard for permissible coliform count (0 per 100 mL). More recently, a similar study conducted in one of the Nigerian Federal Universities revealed that two brands of sachet water failed coliform tests **[14]**. On the other hand, available literature suggests that Nigerian sachet water may generally comply more with physicochemical standards than microbiological standards. Notwithstanding, reports indicated that a notable number of sachet water brands in the country exceeded regulatory limits in some physicochemical parameters, including temperature, pH, Iron, and total hardness **[6]**, **[11]**, **[15]**.

Moreover, prolonged storage of sachet water beyond 12 weeks could result in non-compliance with chemical standards **[16]**. While these reports present valuable data about the quality of sachet water in some famous cities of Nigeria, their relevance may go only as far as the peculiarities of the studied regions. Due to geographical differences, several agents may influence water quality in a large country like Nigeria. Some reports on the quality of packaged water in Ilorin, Nigeria, are available. However, these were not focused on polyethylene-based packaging, nor were freshly produced samples directly from the production sources considered. For example, Olayemi **[17]** reported that 40% of bottled and cellophane-packaged water being hawked in Ilorin was unfit for drinking. The author found *Pseudomonas* predominant in the water samples and emphasized the need to institutionalize the drinking water supply system, which was regarded as informal then.

Similarly, 10% of bottled water obtained from different outlet centers within Ilorin did not pass total coliform tests **[18]**. Furthermore, the researchers concluded that 15% of the bottled water was unfit for drinking from the standpoints of bacteriological and physicochemical qualities. Considering the high demand and popularity of sachet water in Ilorin, the current need for more information concerning the physicochemical and microbiological safety of the product calls for more research. This identified gap underlies the novelty of the present study, which sought to provide insights into the extent of compliance of sachet water brands within the study area to regulatory standards set for safe drinking water.

Therefore, this study aimed to determine the physicochemical and microbiological attributes of different brands of sachet water produced in Ilorin metropolis of Nigeria. The water brands were studied within 24 hours of production and after every month during 4-month storage. This investigation was to ascertain their conformity with the safe drinking water standards of regulatory agencies. The research was conducted between 2020 and 2021 in Ilorin, Kwara State, Nigeria.

The three Local Government Areas under investigation are Ilorin East, Ilorin West, and Ilorin South, all located within Ilorin, the largest Metropolitan City of Kwara State, Nigeria (Figure 1). Ilorin is located within the Latitudes 8°24′ N and 8°36′ N, and Longitude 4°10′ E and 4°36′ E **[19]**. The city's topography was 257 m and 431 m above sea level, receiving water from the South Northward flow of the Oyun and Asa rivers **[20]**. In terms of climate, Ilorin is characterized by both wet and dry seasons, from April to October and November to April,

respectively **[20]**. Its mean annual rainfall is around 1222 mm, typically heaviest between June and September **[21]**. While high temperatures generally prevail between November and January (33-35 °C), as well as between February and April (35-37 °C) **[20]**, relative humidity in Ilorin is average of 70% during the rainy season and 80% during the wet season **[21]**. The locations of the Local Government Areas being currently studied are as follows: 8°61′ N, 4°79′ E (Ilorin East), 8°49′ N, 4°51′ (Ilorin West) and 8°43′ N, 4°67′ E (Ilorin South).

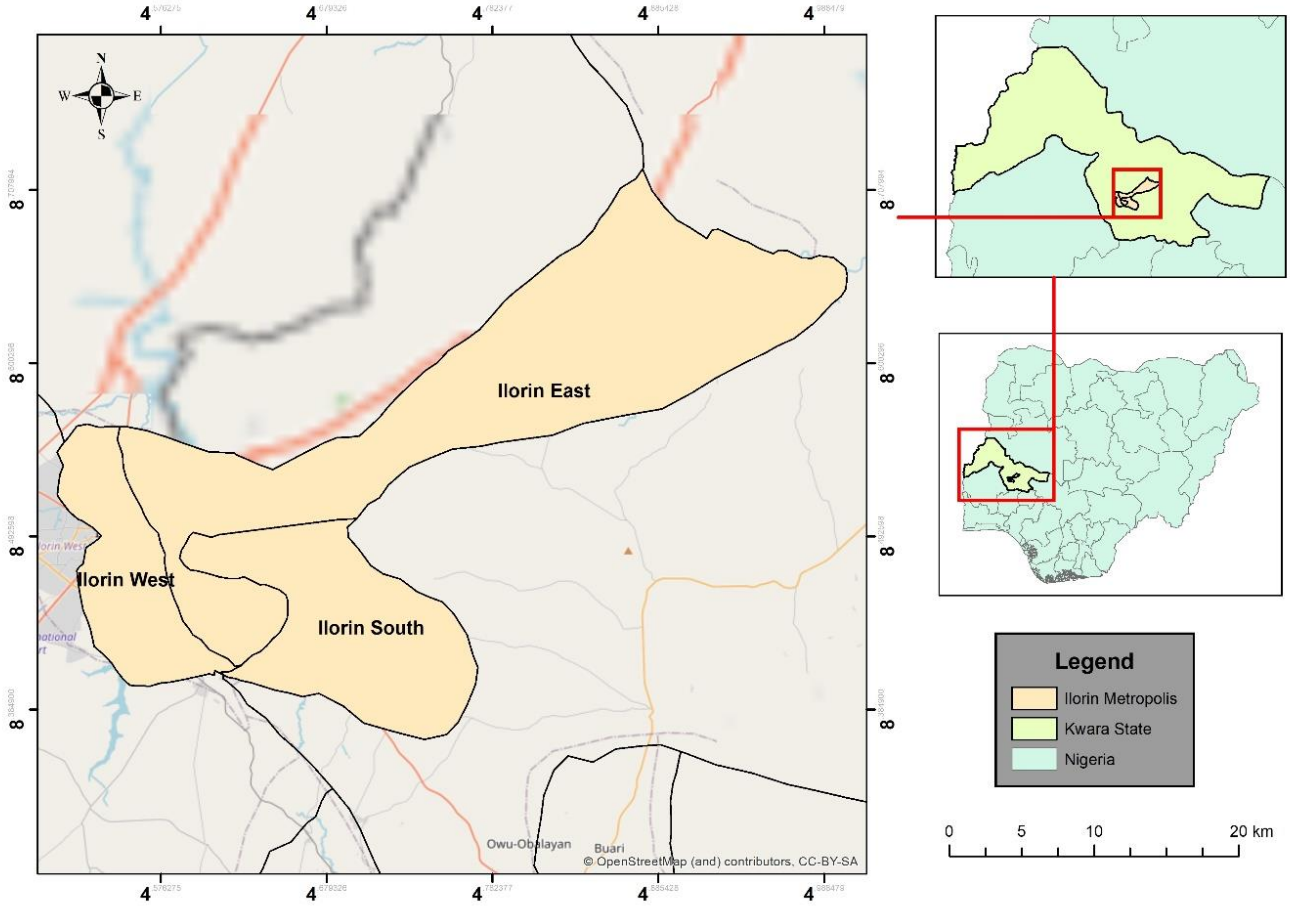


Figure 1 Map of Ilorin, Nigeria, showing the study areas.

Scientific Hypothesis

The compliance of some Nigerian sachet water brands to regulatory standards is still being determined. We expect changes in collected sachet water brands' physicochemical and bacterial properties during ambient storage.

MATERIAL AND METHODOLOGY

Samples

Nine brands of NAFDAC-certified sachet water, sourced from groundwater, were collected within 24 hours of production from nine randomly selected pure water companies in the three local government areas. Samples collected were labelled ILM1, ILM2, ILM3, ILM4, ILM5, ILM6, ILM7, ILM8, and ILM9.

Typically, the production of the various sachet water brands involves pumping groundwater from a confined source (bore-hole), followed by its transfer into a storage tank subjected to biological and chemical treatments. In line with the report of Ajala et al. **[2]**, these treatments include chlorination, sedimentation, flocculation, and filtration. On the other hand, the packaging process is automated, i.e., treated water is filled into a sterile polyethylene packaging material by an electronic machine and then sealed by the same machine. Next, the water is manually packed, 20 sachets per bag for distribution. The water treatment and filling occur at the same sites (the factories) where the water is sourced.

Chemicals

All reagents and chemicals used were of analytical-grade quality. Culture media (TVC and MacConkey) were from Hi-media (Mubai, India).

Instruments

The were mercury-in-glass thermometer, Hach portable colorimeter (Model DR/890, Hach Corporation, USA), multipurpose water quality tester (model EZ-9908, Jinan Runjie Electronic Technology, China), spectrophotometer (Hach DR/2000, Hach Corporation, USA), flame photometer (Systronics Flame Photometer 128, Systronics Ltd, India), and atomic absorption spectrophotomter (model 210 VGP, Buck Scientific incorporation, USA).

Laboratory Methods

A mercury-in-glass thermometer was used to determine the temperature of the water samples. The nephelometric method was used to determine the turbidity of the water samples. Before turbidity measurement, the Hach portable colorimeter was first zeroed with 10 mL of deionized water in a cuvette. Each of the water samples (10 mL) was placed in the cell compartment of the Hach portable colorimeter, and the, program 95‘ was pressed to measure turbidity. Turbidity was expressed in the Nephelometric Turbidity Unit (NTU) **[22]**. For pH, total dissolved solids, and electrical conductivity, the multipurpose water quality tester was calibrated with Buffer solutions of pH 4, 7, and 10, after which the electrode was rinsed with deionized water. The electrode was then immersed in 50 mL of each of the water samples and allowed to stabilize for 1-2 min. pH, total dissolved solids (mg/L), and electrical conductivity (µS/cm) were then recorded appropriately.

Total Alkalinity was determined by titration **[23]**. Two drops of phenolphthalein indicator were added to the 50 mL of water sample in a 250 mL conical flask. This was titrated against 0.2 N H_2SO_4 until the pink colour disappeared. Next, 2 drops of methyl orange were added and the mixture was further titrated with 0.2 N H_2SO_4 until a reddish-orange colour appeared indicating the endpoint.

$$\text{Total alkalinity (mg/L)} = \frac{\text{Titre value (mL) x Normality of acid x 50.00x1000}}{\text{Volume of sample (mL)}}$$

Total hardness was assessed using the ethylenediamine tetraacetic acid (EDTA) titrimetric method **[23]** as follows: The water sample (50 mL) was mixed with 1 mL of NH_4Cl-NH_4OH buffer solution and 2 mL of Eriochrome Black T. This was then titrated against 1 M ethylenediamine tetraacetic acid (EDTA) until a blue end point was reached.

$$\text{Total hardness } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{Titre value (mL) x Normality of EDTA x 1000}}{\text{Volume of sample (mL)}}$$

Test for the concentration of nitrates was done using the spectrophotometric sodium salicylate method described by Onweluzo and Akuagbazie **[24]**. Standard solutions of potassium nitrates (0-5 mg/L) were each mixed with 1 mL of 1% sodium salicylate solution and 2 mL of 96% sulphuric acid and allowed to rest for 15 min. A calibration curve of the working standards was obtained by plotting absorbance values taken at 420 nm in a spectrophotometer against concentrations. To each of the water samples, 15 mL of deionized water and 15 mL of sodium tartrate and absorbance read at 420 nm. Nitrate concentrations in the water samples were then extrapolated from the calibration curve of the standards.

Previously recommended methods by Baird et al. **[22]** were adopted for chloride (Argentometric), sulphate (turbidimetric), sodium (flame emission photometric), magnesium, lead, iron, and copper (atomic absorption spectrophotometric). To test for chlorides, 100 ml of each sample was measured in a conical flask and three drops of 10% potassium chromate indicator were added. The mixture was titrated against 0.014 N $AgNO_3$ until a pinkish-yellow colour endpoint. The reagent blank was established using distilled water. The concentration of chloride was calculated using the equation:

$$Cl^- \text{ (mg/L)} = \frac{(A-B) \text{ x N x } 35.45}{\text{Volume of sample}}$$

Where:
A = Titre value of sample; B = Titre value of distilled water; N = Normality of $AgNO_3$.

For sulphates, 100 ml of sample was mixed with 20 ml of buffer solution (30 g/L $MgCl_2 \cdot 6H_2O$, 5 g/L $CH_3COONa \cdot 3H_2O$, 1 g/L KNO_3, and 20 mL/L acetic acid) in a conical flask. While stirring, a spoonful of $BaCl_2$ crystals was added and allowed to mix for 60 s. The turbidity of the mixture developed after 5 min was then read using the spectrophotometer at 420 nm. A sample blank was also analysed using deionized water but without adding $BaCl_2$. Sulphate concentration was estimated using the calibration curve plotted from different concentrations (0-40 mg/L) of sulphate standards. Concerning sodium, serial dilutions (0 to 10 mg/L) from stock

sodium standard were prepared. Emmison intensities of the standards were then measured at 589 nm in a flame photometer and used to plot a calibration curve. The established calibration curve was used to determine the sodium concentrations in the water samples. As for magnesium, lead, iron, and copper, serial dilutions of 0-4 ppm were prepared from commercial annular grade their respective standard stock solutions. The absorbance values of the working standard solutions were then measured at 285.2, 283.3, 248.3, and 324 nm for magnesium, lead, iron, and copper, respectively, using an atomic absorption spectrophotometer. Calibration curves plotted from the working standard concentrations against absorbance values were used to determine the mineral concentrations in the water samples.

The pour plate method previously described **[25]**, **[26]** was adapted for the isolation and enumeration of heterotrophic bacteria. From each water sample, 0.1 mL was aseptically transferred using a sterile micropipette onto Nutrient agar in sterile Petri dishes. The nutrient agar had been previously sterilised (121 °C, 0.15 mPa, 15 min) and cooled. The petri dishes were then swirled gently to mix the sample and the media before incubation at 30 °C for 24 hr. After incubation, developed colonies were counted, and the total viable count (TVC) was expressed in colony-forming units per mL (CFU/mL) of the water sample tested. For total coliform analysis, the most probable number method involving presumptive, confirmatory, and complete tests, was carried out according to the method described by Sutton **[27]**. Briefly, three sets of test tubes in three places (making a total of nine) were used to prepare. The presumptive test was carried out by adding 10 mL of double-strength, 5 mL of single-strength, and 5 mL of double-strength lactose broth into the first, second, and third sets of the test tubes, respectively. This was followed by the placement of inverted Durham tubes in all the test tubes, then sterilization at 121 °C and 0.15 mPa for 15 min. After being cooled to 40 °C, the sterilized test tubes were inoculated with the varied volumes of water sample: 10 mL in double-strength lactose broth, 1 mL in single-strength lactose broth, and 0.1 mL in single-strength lactose broth. These were incubated at 37 °C for 48 hr to examine acid and gas formation as indicated by the colour change of the broth. For a confirmatory test, a loopful from each of the positive fermentation tubes was inoculated in McConkey broth (containing an inverted Durham's tube) and incubated at 37 °C for 48 hr. The tubes with gas production were considered positive. Finally, the complete test was conducted on the positive tubes from the confirmatory stage. This was done by streaking the inocula from the positive tubes in Eiosine Methyle Blue followed by incubation at 37 °C for 24 hr for the growth of *Escherichia coli.*

Description of the Experiment

Sample preparation: Bags (packs) of the labeled sachet water were immediately conveyed to the laboratory, where subsamples were subjected to physicochemical and microbial analyses. From each pack, a sachet was taken, and cut open from the edge using a pair of sterilized scissors. The content of the sachet was then poured into a sterilized beaker just before analyses.

Number of repeated analyses: Analyses were conducted in three replicates except where otherwise specified.

Number of samples analyzed: 18 samples (i.e., 2 from each brand) were analysed.

Number of experiment replication: The experiment was conducted twice. This involved sample preparation before analyses, as already manufactured sachet water brands were studied.

Design of the experiment: The experiment was designed to determine the compliance of sachet water produced within the study area with physicochemical and microbial standards. First, selected samples were collected within 24 hrs of production and tested for physicochemical and microbial parameters. Next, unopened samples were stored for 4 months, and the same physicochemical and microbial parameters were monitored. Results obtained were evaluated against the permissible limits stipulated by the World Health Organisation and Nigerian Industrial Standards.

Statistical Analysis

Statistical Package for Social Sciences (SPSS, version 20) was used for the statistical analysis. Physicochemical and microbial parameters were statistically subjected to the Analysis of Variance (ANOVA) and the Duncan Multiple Range Comparison Test. Results were reported as means of triplicate determinations ± SD. Means were considered significantly different at 5%.

RESULTS AND DISCUSSION

The temperature of the water samples significantly ($p \leq 0.05$) varied from 25.10 to 27.40 °C before storage and from 26.41 to 30.13 °C after four months of storage (Table 1). This is in line with the findings of Unegbu **[28]**, who also observed a temperature rise while storing sachet water samples in their study. The predominant increase in temperature may be attributed to metabolic activities **[27]**, such as the growth of mesophilic microbes within the optimal temperature range of 20-45 °C **[29]**. On the other hand, the general temperature drop observed in most

of the samples after the first month could be attributed to some external influences from the storage ambient temperatures **[29]**. According to NIS, the water temperature should not exceed that of the environment (ambient) in which it is stored **[30]**. The ambient temperature of the storage environment (27 ±3 °C) in this study is similar to the range of temperature values recorded for the various water brands.

The pH of the sachet water samples ranged from 6.42 to 7.65 during the 24 hours of production (Table 2). The majority of the brands satisfied the WHO recommended limits (6.5-8.5), but the significantly ($p \leq 0.05$) lower pH of ILM1 and ILM9 may impact an acidic taste. pH increased as the storage period progressed, probably due to microbial activities and changing chemical components. However, the pH of all the sachet water samples, except ILM4 and ILM5, remained within the acceptable range throughout the storage period. While a relationship exists between pH and other chemical properties of water, such as copper and sulphates, the interaction is somewhat complicated and varies with different water sources **[31]**. Therefore, it may not be immediately clear to directly link the changing pH to a particular chemical or physicochemical property, except if such a property is studied in isolation **[31]**. The simultaneous influence of microbial activity is another factor that may complicate any attempt to predict the relationship between water's changing chemical components and pH. Nonetheless, the pH values obtained showed that most of the sachet water brands were slightly alkaline, which could potentially cause acidosis. The pH results from the present research are consistent with an earlier study by Duru et al. **[16]** on sachet water brands studied in Owerri metropolis of Nigeria. According to the authors, most of the water samples had pH in the range of 6.85-7.37, with a steady increase during storage. The results are also in line with what was reported by Unegbu **[28]**. The pH of drinking water is critical to its sensory properties as pH outside the WHO/NIS acceptable range of 6.5-8.5 may constitute an undesirable taste **[32]**. Water with an acidic pH can be corrosive to metallic pipes **[14]**.

Table 1 Temperature of selected sachet water brands produced in Ilorin Metropolis over a 4-month storage.

Sample	Storage period (month)				
	0	**1**	**2**	**3**	**4**
ILM_1	27.30 ±0.00^{c}	25.60 ±0.01^{d}	27.37 ±0.05^{f}	28.33 ±0.05^{f}	29.35 ±0.01^{b}
ILM_2	27.07 ±0.06^{d}	25.53 ±0.05^{e}	27.60 ±0.00^{e}	28.40 ±0.00^{e}	28.35 ±0.01^{d}
ILM_3	27.60 ±0.00^{a}	25.80 ±0.01^{c}	27.80 ±0.00^{d}	28.90 ±0.00^{b}	30.08 ±0.00^{a}
ILM_4	26.80 ±0.00^{e}	25.80 ±0.01^{c}	28.00 ±0.00^{c}	28.80 ±0.00^{c}	28.29 ±0.09^{e}
ILM_5	27.00 ±0.00^{e}	25.40 ±0.01^{f}	27.60 ±0.00^{e}	28.40 ±0.00^{e}	26.41 ±0.01^{f}
ILM_6	27.40 ±0.00^{b}	26.40 ±0.01^{b}	28.10 ±0.00^{b}	28.50 ±0.00^{d}	28.50 ±0.01^{c}
ILM_7	25.10 ±0.00^{g}	26.80 ±0.01^{a}	27.30 ±0.00^{g}	29.20 ±0.00^{a}	30.13 ±0.02^{a}
ILM_8	26.40 ±0.00^{f}	26.40 ±0.01^{b}	28.40 ±0.00^{a}	27.80 ±0.00^{g}	28.45 ±0.01^{c}
ILM_9	27.38 ±0.06^{b}	26.78 ±0.04^{a}	28.39 ±0.03^{a}	28.62 ±0.03^{e}	29.33 ±0.04^{b}
LIMITS	**Ambient °C (WHO/NIS)**				

Note: Values are Means ±SD of triplicate determinations (n = 3); Means with different superscripts along the same column are significantly ($p \leq 0.05$) different.

Table 2 pH of selected sachet water brands produced in Ilorin Metropolis over a 4-month.

Sample	Storage period (month)				
	0	**1**	**2**	**3**	**4**
ILM_1	6.42 ±0.03^{b}	7.83 ±0.04^{c}	7.55 ±0.05^{d}	7.59 ±0.07^{f}	8.00 ±0.09^{e}
ILM_2	7.65 ±0.05^{a}	7.94 ±0.03^{b}	7.55 ±0.06^{d}	7.90 ±0.05^{d}	8.16 ±0.06^{d}
ILM_3	7.65 ±0.04^{a}	7.72 ±0.06^{c}	7.88 ±0.03^{b}	8.10 ±0.03^{c}	8.33 ±0.05^{c}
ILM_4	7.65 ±0.01^{a}	8.26 ±0.03^{a}	8.62 ±0.10^{a}	8.48 ±0.06^{b}	8.52 ±0.07^{b}
ILM_5	7.65 ±0.06^{a}	7.88 ±0.07^{b}	8.40 ±0.06^{a}	8.77 ±0.07^{a}	8.86 ±0.08^{a}
ILM_6	7.65 ±0.03^{a}	7.94 ±0.06^{b}	7.84 ±0.05^{b}	7.97 ±0.07^{d}	8.32 ±0.05^{c}
ILM_7	7.65 ±0.05^{a}	7.74 ±0.05^{d}	7.90 ±0.07^{b}	7.97 ±0.03^{d}	8.09 ±0.04^{e}
ILM_8	7.65 ±0.08^{a}	7.64 ±0.06^{d}	7.72 ±0.08^{c}	7.82 ±0.04^{e}	8.16 ±0.03^{d}
ILM_9	6.49 ±0.03^{b}	7.00 ±0.08^{e}	7.73 ±0.07^{c}	7.67 ±0.05^{d}	8.03 ±0.10^{e}
LIMITS	**6.5-8.5 (WHO)**				

Note: Values are Means ±SD of triplicate determinations (n = 3); Means with different superscripts along the same column are significantly ($p \leq 0.05$) different.

The turbidity values of the sachet water samples were originally in the range of 0.37-0.57 NTU (Figure 2). There was, however, a significant ($p \leq 0.05$) difference throughout the storage period, though these were in varying degrees. Ayegbo et al. **[33]** reported 0.02-0.35 NTU values for sachet water samples sold in Sango-Otta, Ogun State of Nigeria. These are lower than the values recorded in this study. It is, however, worth noting that Ayegbo et al. **[33]** conducted their study on samples stored at 4 °C, and the age of the samples at the point of collection was not indicated. The rising turbidity recorded during the 4-month storage in this study is comparable to observations reported elsewhere by other scholars. For instance, Ajekunle et al. **[23]** noted a turbidity rise from 0.39-0.7NTU to 0.74-1.85NTU for sachet water samples after three months of storage. Regarding regulatory compliance, all the sachet water samples conformed with WHO's turbidity limit of less than 5 NTU, implying good physical quality. Turbidity is the physical property of water associated with reduced light transmission owing to absorbance and scattering by solid particles in suspension. Materials causing turbidity may include clay, silt, finely divided organic and inorganic matter, and soluble colored organic compounds. These can come from soil erosion, excess nutrients, various wastes and pollutants, and the action of bottom-feeding organisms **[34]**. Since the water samples were packaged, the increasing turbidity may be attributed to increased total dissolved solids from microbial activities. Initial turbidity in water may protect some microorganisms from disinfection during water treatment, thereby promoting microbial regrowth during storage **[35]**. This phenomenon will lead to a further rise in turbidity.

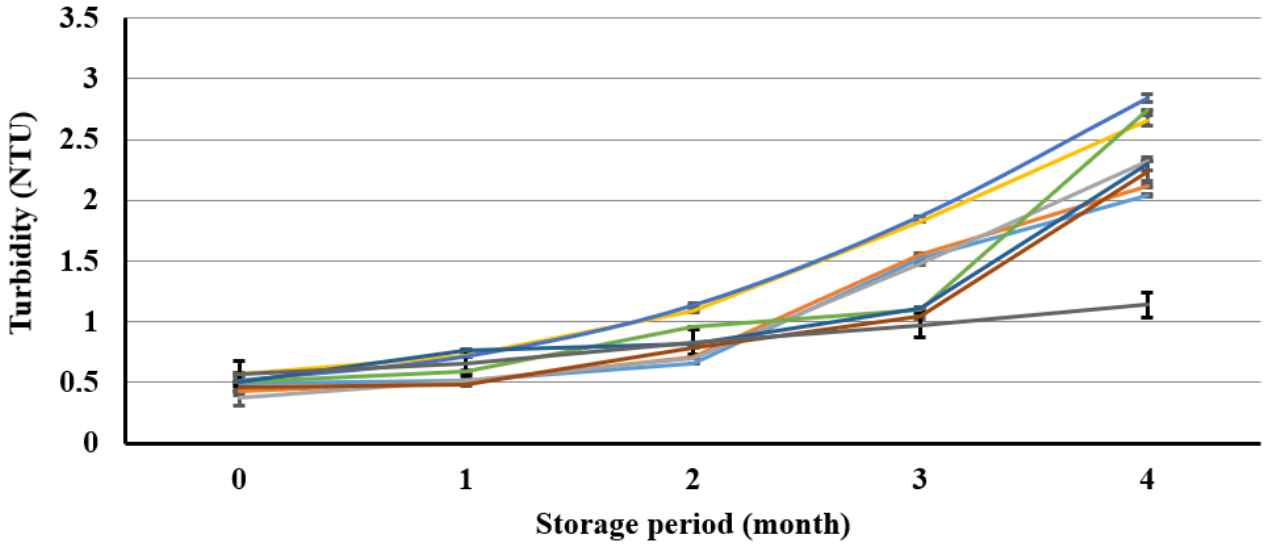


Figure 2 Turbidity of selected sachet water brands produced in the Ilorin metropolis over a 4-month storage. Note: Error bars represent standard deviations of triplicate determinations (at $p \leq 0.05$).

There was a significant ($p \leq 0.05$) difference in the total hardness of all the sachet water samples within 24 hr of production, varying from 31.33 mg/L to 90.67 mg/L (Figure 3). This is comparable with values (65.21 mg/L-90.08 mg/L) earlier reported for sachet water brands marketed in the Bauchi region of Nigeria **[1]**. The values also comply with the WHO set limit of 100 mg/L. However, total hardness increased in some samples after the second month of storage, exceeding the WHO limit. The increased total hardness may signal an increased availability of metal ions in the sachet water **[36]**. Differences in total hardness could be attributed to varying levels of magnesium and calcium in water as influenced by the geology of its source **[37]**. Water hardness can imply adverse effects such as corrosion and impaired taste **[38]**. Since it is a function of cations, hardness can also influence the electrical conductivity of water **[39]**.

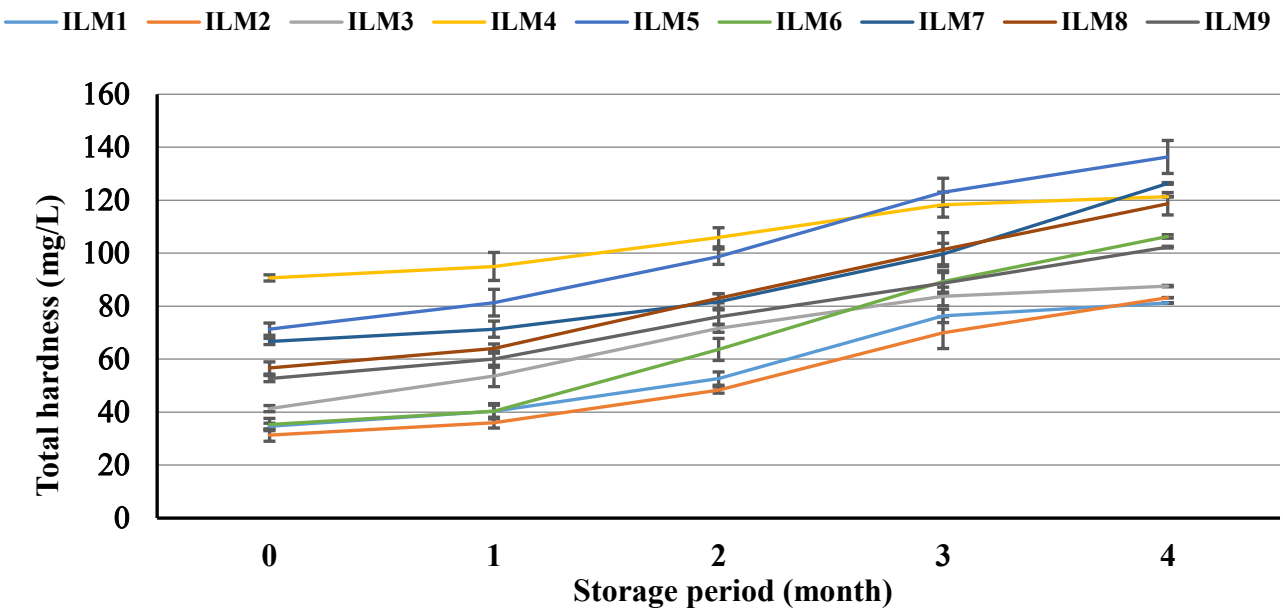


Figure 3 Total hardness of selected sachet water brands produced in the Ilorin metropolis over a 4-month storage. Note: Error bars represent standard deviations of triplicate determinations (at $p \leq 0.05$).

Figure 4 shows the electrical conductivity of all sachet water samples over 4 months of storage. Before storage, electrical conductivity values significantly ($p \leq 0.05$) varied between 110.67 µS/cm and 238.00 µS/cm. Though these were followed by some significant ($p \leq 0.05$) increase as storage progressed, the values were still below the WHO permissible limit (1000 µS/cm). The increasing electrical conductivity could be associated with rising levels of dissolved materials in the water samples during storage **[40]**. An elevated temperature of stored sachet water has also been linked to higher electrical conductivity **[28]**. Electrical conductivity denotes the ability of water to conduct electricity. Previously, Ojekunle et al. **[41]** similarly reported a significant ($p \leq 0.05$) increase in the electrical conductivity of sachet water in the Ibadan metropolis during storage. The authors recorded 60.98-117.35 µS/cm within the first week of storage, but these rose to 63.10-121.05 µS/cm after three months. An electrical conductivity higher than an acceptable level in drinking water may lead to unpleasant taste or gastrointestinal distress **[1]**.

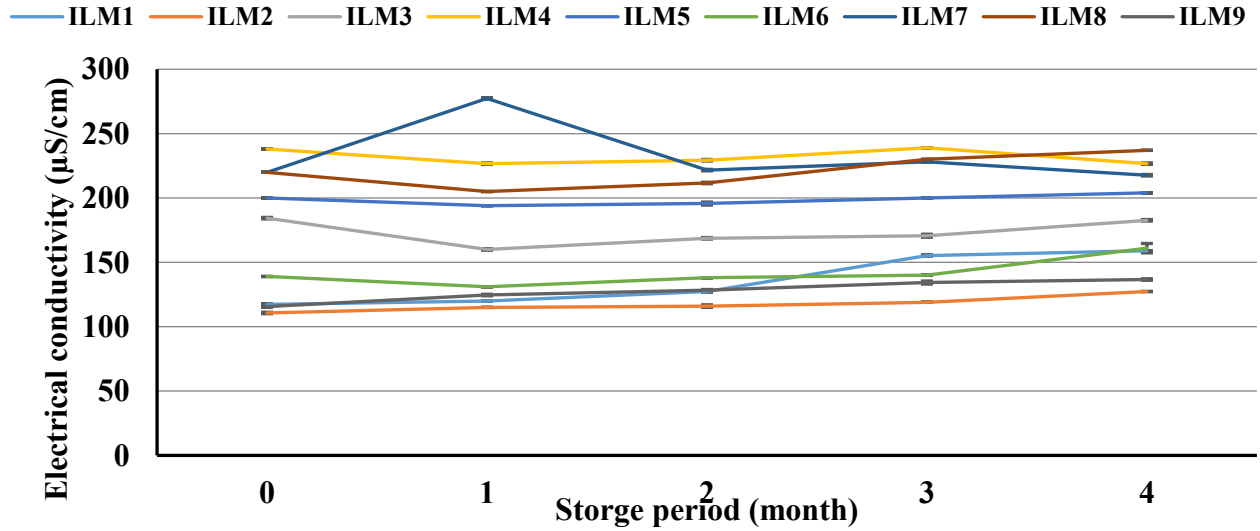


Figure 4 Electrical conductivity of selected sachet water brands produced in the Ilorin metropolis over a 4-month storage. Note: Error bars represent standard deviations of triplicate determinations (at $p \leq 0.05$).

Figure 5 shows the total dissolved solids (TDS) in the studied sachet water brands. TDS varied significantly ($p \leq 0.05$) from 55.33 mg/L to 120.00 mg/L and further rose during storage. At the end of the storage period, the

values were 70-125.7 mg/L. The variation in the levels of metabolites from growing microorganisms during storage may account for the changing TDS. Higher TDS may also be associated with possibly increasing metal availability during storage. TDS recorded in this study is higher than values (50-70 mg/L) reported for some sachet water brands marketed in a Nigerian Federal University **[14]**, probably due to geographical and environmental differences. However, the TDS of the sachet water samples remained below the WHO limits of 500 mg/L throughout the storage period. Water can dissolve many inorganic and organic minerals or salts, such as potassium, calcium, sodium, bicarbonates, chlorides, magnesium, and sulphates **[40]**. These minerals produce undesirable taste and appearance in water. While TDS in water may not directly affect consumers health, it can impair sensory attributes like taste, smell, and color **[42]**.

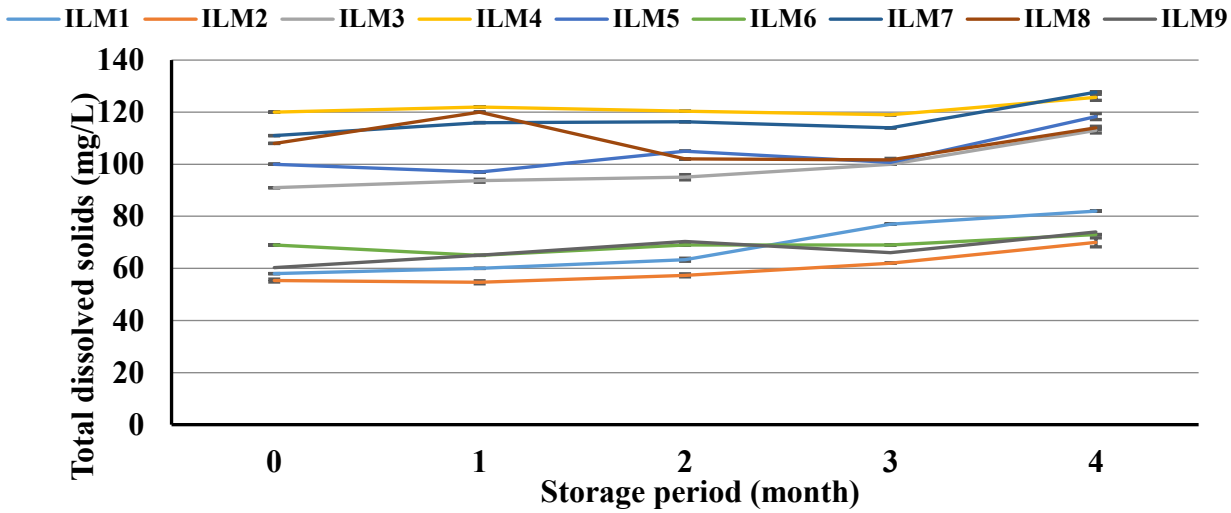


Figure 5 Total dissolved solids of selected sachet water brands produced in the Ilorin metropolis over 4-month storage. Note: Error bars represent standard deviations of triplicate determinations (at $p \leq 0.05$).

The chloride concentration of the water samples varied significantly ($p \leq 0.05$) from 2.83 mg/L to 5.21 mg/L before storage and from 4.10 mg/L to 8.57 mg/L after the fourth month (Table 3). ILM1 demonstrated the highest increasing rate in chloride concentrations throughout the storage period. However, despite the observed increase in chloride content, its level in all the sachet water samples was within WHO/NIS limits of 200-300 mg/L throughout the investigation period **[43]**. A similar case was observed by Ajekunle et al. **[23]** on the sachet water produced in Ogun State, where the chloride concentration of all the sachet water increased with the length of storage. The rise in chloride concentrations may be associated with possible leaching from chlorine-containing compounds into the packaged water during storage. For example, Farinu et al. **[36]** had earlier hypothesized the possible contribution of leached chemicals, including trichloromethane from disintegrating polyethylene packaging materials, on the increased chloride concentration of stored sachet water. Chlorides are inorganic compounds resulting from the reaction between chlorine gas and metals. Some common chlorides include sodium chloride (NaCl) and magnesium chloride ($MgCl_2$). Chlorides may get into surface water from several sources, such as rock-containing chlorides, agricultural run-off, industrial wastewater, oil well wastes, and effluent wastewater from wastewater treatment plants **[44]**. The significance of chlorides in water has been linked with heart and kidney damage, and the sensory properties of water on the other hand **[43]**.

Nitrates were not present in all the sachet water samples when analysed within 24 hr of production. However, during storage, nitrates were recorded with significantly ($p \leq 0.05$) different values: 0.25-0.59 mg/L after the first month, 0.15-0.59 mg/L after the second month, 0.22-0.57 mg/L after the third month and 0.42-0.64 mg/L after the fourth month of storage (Table 3). However, there were fluctuations, with values rising during some months while dropping in others. This observation is at variance with the report of Duru et al. **[16]**, who reported a decrease in nitrates within 24 hr of production, and this was attributed to the presence of nitrate-utilizing microorganisms. While the exact reason may account for the similarly decreasing nitrates in this study, it is likely that some contrary biochemical or microbiological processes also took place, which caused rising nitrate levels in some samples. For example, some bacteria produce nitrates from the short-lived intermediates, nitrites **[1]**. Nitrates in water may initiate the development of methemoglobinemia, otherwise known as a blue baby disease in infants **[1]**. However, concerning the sachet water samples currently under study, this condition is not of serious concern as the nitrates concentration is below the WHO permissible limits of 50 mg/L. Again, giving a sachet of water to children for drinking is not a common practice.

The values of sulphates in the various water samples differed significantly ($p \leq 0.05$) between 5.83 mg/L and 6.40 mg/L within 24 hr of production (Table 4). However, these values generally increased by the fourth month, except for some samples in which lower sulphates were observed. Notwithstanding, all sulphate levels were lower than the maximum of 250 mg/L allowed by WHO/NIS **[43]**. The sulphate content of natural waters is essential in determining their suitability for public and industrial supplies. Beyond the permissible level, sulphates in water may affect human organs, leaving vermifuge consequences on the human system **[43]**. There was no steady pattern in the amount of sulphates during storage, as some fluctuating figures were recorded. Similar fluctuating trends had been previously reported for bottled and sachet water samples stored for six weeks **[45]**. The fluctuating sulphates concentrations in the water samples might be partly due to microbial activities. For instance, Fadipe et al. **[45]** explained that some microorganisms could reduce sulphates in water, which may partly corroborate the declining sulphates noticed in some samples during storage. It has been reported that the discharge of industrial wastes and domestic sewage could increase the concentration of sulphates in water **[1]**. Sulphate may have a laxative effect that can lead to dehydration and is of particular concern for infants **[46]**.

The sodium contents of all the nine sachet water samples significantly ($p \leq 0.05$) varied from 15.17 mg/L to 18.74 mg/L during 24 hr of production (Table 4), with samples from ILM1 and ILM4 recording the highest and lowest values, respectively. Notably, sodium contents generally decreased during storage except in the second month when about two-thirds of the sachet water samples demonstrated stable sodium. A similar decreasing pattern had been earlier documented by Ajekunle et al. **[23]** while comparing the sodium contents of some sachet water samples with the WHO limit of 200 mg/L. While the general drop in sodium concentrations is yet to be precisely rationalised, the likely complexation of sodium with other elements probably reduced its detectability during analysis. In general, sodium salts are not acutely toxic because of the efficiency with which mature kidneys excrete sodium. However, acute effects and death have been reported following accidental overdoses of sodium chloride **[47]**. An excessive salt intake seriously aggravates chronic congestive heart failure, and ill effects due to high sodium levels in drinking water have been documented **[47]**. Consuming food and water containing high sodium levels can contribute to high blood pressure **[48]**.

Table 3 Chlorides and nitrates of selected sachet water brands produced in Ilorin Metropolis over a 4-month storage.

Sample	Chlorides (mg/L)				
	Storage period (month)				
	0	1	2	3	4
ILM_1	3.56 ±0.02^{cd}	3.65 ±0.37^{c}	4.00 ±0.08^{d}	4.26 ±0.26^{c}	4.10 ±0.02^{d}
ILM_2	2.83 ±0.01^{d}	3.43 ±0.33^{c}	3.80 ±0.66^{e}	4.65 ±0.36^{bc}	4.80 ±0.52^{c}
ILM_3	3.56 ±0.01^{cd}	3.72 ±0.04^{c}	4.46 ±0.47^{cd}	4.54 ±0.21^{bc}	4.54 ±0.20^{c}
ILM_4	4.27 ±0.02^{bc}	4.70 ±0.16^{b}	4.94 ±0.12^{bc}	5.20 ±0.16^{abc}	8.57 ±0.75^{a}
ILM_5	4.26 ±0.71^{be}	5.30 ±0.23^{a}	5.25 ±0.28^{b}	5.29 ±0.19^{abc}	5.29 ±0.19^{b}
ILM_6	3.08 ±0.41^{d}	3.56 ±0.35^{c}	3.87 ±0.13^{e}	4.88 ±1.18^{abc}	6.26 ±0.39^{b}
ILM_7	4.97 ±0.71^{ab}	4.97 ±0.15^{b}	5.44 ±0.13^{ab}	5.86 ±0.39^{a}	6.07 ±0.75^{b}
ILM_8	5.21 ±0.41^{a}	5.10 ±0.30^{b}	5.86 ±0.13^{a}	5.47 ±0.95^{ab}	6.22 ±0.16^{b}
ILM_9	3.56 ±0.02^{cd}	3.72 ±0.41^{c}	4.02 ±0.05^{d}	4.54 ±0.07^{bc}	4.78 ±0.07^{c}
LIMITS	**200-300 mg/L (WHO/NIS)**				
	Nitrates (mg/L)				
ILM_1	0.00^{a}	0.34 ±0.11^{bcd}	0.49 ±0.08^{ab}	0.37 ±0.02^{b}	0.54 ±0.06^{b}
ILM_2	0.00^{a}	0.30 ±0.04^{cd}	0.15 ±0.02^{d}	0.22 ±0.01^{c}	0.44 ±0.02^{c}
ILM_3	0.00^{a}	0.25 ±0.06^{c}	0.26 ±0.03^{c}	0.42 ±0.04^{ab}	0.42 ±0.09^{c}
ILM_4	0.00^{a}	0.59 ±0.11^{a}	0.59 ±0.04^{a}	0.57 ±0.13^{a}	0.64 ±0.02^{a}
ILM_5	0.00^{a}	0.52 ±0.16^{a}	0.58 ±0.07^{a}	0.47 ±0.09^{ab}	0.56 ±0.07^{b}
ILM_6	0.00^{a}	0.44 ±0.09^{abc}	0.47 ±0.07^{ab}	0.42 ±0.08^{ab}	0.47 ±0.02^{bc}
ILM_7	0.00^{a}	0.49 ±0.02^{ab}	0.46 ±0.10^{b}	0.49 ±0.09^{ab}	0.43 ±0.04^{c}
ILM_8	0.00^{a}	0.28 ±0.05^{c}	0.50 ±0.03^{ab}	0.40 ±0.03^{b}	0.54 ±0.03^{b}
ILM_9	0.01±0.02^{a}	5.21 ±0.41^{a}	0.28 ±0.02^{c}	0.29 ±0.02^{c}	0.40 ±0.41^{b}
LIMITS	**50 mg/L (WHO)**				

Note: Values are Means ± SD of triplicate determinations (n = 3); Means with different superscripts along the same column are significantly ($p \leq 0.05$) different.

Table 4 Sulphates and sodium of selected sachet water brands produced in Ilorin Metropolis over a 4-month storage.

Sample	Sulphates (mg/L)				
	Storage period (month)				
	0	1	2	3	4
ILM$_1$	5.00±0.00^{e}	4.96±0.26^{e}	5.47±1.09^{b}	6.24±0.25^{b}	6.28±0.09^{ab}
ILM$_2$	4.83±0.20^{f}	5.05±0.28^{de}	5.80±0.15^{b}	5.35±0.32^{c}	5.72±0.05^{bcd}
ILM$_3$	5.40±0.01^{c}	5.52±0.09^{cde}	5.86±0.02^{b}	6.43±0.38^{b}	6.34±0.06^{abc}
ILM$_4$	6.40±0.02^{a}	5.47±0.31^{cde}	5.53±0.32^{b}	5.89±0.13^{bc}	6.41±0.03^{ab}
ILM$_5$	6.40±0.03^{a}	6.94±0.81^{a}	6.10±0.27^{b}	6.30±0.49^{b}	5.99±0.55^{bcd}
ILM$_6$	6.03±0.01^{b}	5.77±0.50^{cd}	5.46±0.24^{b}	5.98±0.16^{b}	5.65±0.77^{cd}
ILM$_7$	6.01±0.02^{b}	6.69±0.40^{ab}	6.25±0.13^{b}	6.54±0.61^{ab}	5.48±0.25^{d}
ILM$_8$	5.26±0.03^{d}	5.98±0.22^{bc}	5.94±0.56^{b}	7.12±0.34^{a}	6.85±0.42^{a}
ILM$_9$	4.87±0.01^{e}	4.85±0.50^{f}	4.70±0.32^{a}	5.42±0.50^{c}	5.56±0.32^{cd}
LIMITS	**250 mg/L (WHO/NIS)**				
	Sodium (mg/L)				
ILM$_1$	18.26±0.07^{a}	4.96±0.26^{e}	5.47±1.09^{b}	6.24±0.25^{b}	6.28±0.09^{ab}
ILM$_2$	18.45±0.07^{a}	5.05±0.28^{de}	5.80±0.15^{b}	5.35±0.32^{c}	5.72±0.05^{bcd}
ILM$_3$	15.96±0.25^{d}	5.52±0.09^{cde}	5.86±0.02^{b}	6.43±0.38^{b}	6.34±0.06^{abc}
ILM$_4$	14.51±0.15^{f}	5.47±0.31^{cde}	5.53±0.32^{b}	5.89±0.13^{bc}	6.41±0.03^{ab}
ILM$_5$	10.40±0.20^{h}	6.94±0.81^{a}	6.10±0.27^{b}	6.30±0.49^{b}	5.99±0.55^{bcd}
ILM$_6$	17.22±0.15^{b}	5.77±0.50^{cd}	5.46±0.24^{b}	5.98±0.16^{b}	5.65±0.77^{cd}
ILM$_7$	12.32±0.22^{g}	6.69±0.40^{ab}	6.25±0.13^{b}	6.54±0.61^{ab}	5.48±0.25^{d}
ILM$_8$	16.42±0.15^{c}	5.98±0.22^{bc}	5.94±0.56^{b}	7.12±0.34^{a}	6.85±0.42^{a}
ILM$_9$	15.13±0.25^{e}	4.85±0.50^{f}	4.70±0.32^{a}	5.42±0.50^{c}	5.56±0.32^{cd}
LIMITS	**200 mg/L (WHO)**				

Note: Values are Means ± SD of triplicate determinations (n = 3); Means with different superscripts along the same column are significantly ($p \leq 0.05$) different.

The sachet water samples had no lead in them before storage. However, there was little indication of lead (0.0010-0.0020 mg/L) after one month of storage (Table 5). At the end of the second month, the lead values had increased in some samples and decreased in others, but the values were not significantly different ($p > 0.05$). The presence of lead later during storage suggests increasing detectability owing to enhanced metal availability. An increase in lead may be associated with increasing acidity and corrosiveness of water distribution systems **[32]**. The lead contents of all the samples showed a steady increase throughout the rest of the storage period but were still less than the WHO limit of 0.01 mg/L **[39]**. In a study by Ojekunle et al. **[41]**, lead was likewise detected in some sachet water samples. Considering the classification of lead as a toxic mineral, these findings call for more regulatory checks by concerned regulatory bodies in the operation of sachet water manufacturers.

Lead is present in water due to its dissolution from natural sources, primarily from household plumbing systems. This is especially common when lead-containing pipes, solder, fittings, or other service connections convey high-acidity water **[49]**. PVC pipes also contain lead compounds that can leach and produce high lead concentrations in drinking water **[44]**. Lead is a cumulative poison associated with several health hazards like anemia and reproductive effects **[50]**. According to Egbueri **[32]**, some of the possible consequences of high lead levels in drinking water also include inhibited Vitamin D metabolism, retarded mental health, as well as cancer.

There was no presence of copper in the water samples before storage. However, after one month of storage, some level was recorded, ranging from 0.0007-0.0021 mg/L. In fact, at the end of storage, copper levels were 0.030-0.060 mg/L (Table 5). This result is similar to the observation made with lead above; presumably, there was an increased solubility of copper from its compounds during storage. There was no significant ($p > 0.05$) difference in copper levels in the sachet water brands throughout the storage period, and the values remained within the WHO/NIS permissible range of 0.05-0.1 mg/L **[32]**. Consuming water whose copper level exceeds the permissible maximum can cause gastrointestinal disturbance, including nausea and vomiting, while prolonged use may cause liver or kidney damage **[51]**.

The magnesium values for the sachet water samples varied significantly ($p \leq 0.05$) from 0.42 mg/L to 1.0 mg/L before storage (Table 6). Although some fluctuations were recorded in some samples during storage, at the end of the four-month storage period, just as noted for a good number of other chemical parameters, the magnesium contents had notably increased. Storage conditions, increasing metal solubility, and microbial activities may be responsible. The results, however, appear to corroborate an earlier report suggesting that prolonged storage of sachet water may result in increased chemical properties beyond permissible limits **[16]**. Results from the present research are also similar to the increasing magnesium contents noted for some brands of sachet water evaluated by Ajekunle et al. **[23]** during a 2-month storage. However, the magnesium values of all nine samples met the WHO standard of not more than 2.0 mg/L **[30]**, except ILM5 and ILM7, which showed a tendency to exceed the limit after the fourth month. No evidence of adverse health effects attributed explicitly to magnesium in drinking water has been established; however, excess calcium and magnesium ions make water hard **[52]**.

Table 5 Lead and copper of selected sachet water brands produced in Ilorin Metropolis over a 4-month storage. Note: Values are Means ±SD of triplicate determinations (n = 3); Means with different superscripts along the same column are significantly ($p \leq 0.05$) different.

Sample	Lead (mg/L)				
	Storage period (month)				
	0	1	2	3	4
ILM_1	0.000[a]	0.0017 ±0.00[a]	0.00067 ±0.00[a]	0.001 ±0.00[a]	0.0026 ±0.00[a]
ILM_2	0.000[a]	0.0020 ±0.00[a]	0.00167 ±0.00[a]	0.0026 ±0.00[a]	0.0050 ±0.00[a]
ILM_3	0.000[a]	0.0013 ±0.00[a]	0.00200 ±0.00[a]	0.0026 ±0.00[a]	0.0033 ±0.00[a]
ILM_4	0.000[a]	0.0013 ±0.00[a]	0.00100 ±0.00[a]	0.003 ±0.00[a]	0.0040 ±0.00[a]
ILM_5	0.000[a]	0.0010 ±0.00[a]	0.0023 ±0.00[a]	0.003 ±0.00[a]	0.0040 ±0.00[a]
ILM_6	0.000[a]	0.0013 ±0.00[a]	0.0010 ±0.00[a]	0.0026 ±0.00[a]	0.0033 ±0.00[a]
ILM_7	0.000[a]	0.0013 ±0.00[a]	0.0020 ±0.00[a]	0.0026 ±0.00[a]	0.0046 ±0.00[a]
ILM_8	0.000[a]	0.0013 ±0.00[a]	0.00200 ±0.00[a]	0.0026 ±0.00[a]	0.0043 ±0.00[a]
ILM_9	0.000[a]	0.00[a]	0.00200 ±0.00[a]	0.0020 ±0.00[a]	0.0020 ±0.00[a]
LIMITS	**0.01 mg/L (WHO)**				
	Copper (mg/L)				
ILM_1	0.000[a]	0.0007 ±0.00[a]	0.0007 ±0.00[a]	0.0017 ±0.00[a]	0.030 ±0.00[a]
ILM_2	0.000[a]	0.0010 ±0.00[a]	0.0010 ±0.00[a]	0.0023 ±0.00[a]	0.023 ±0.01[a]
ILM_3	0.000[a]	0.0010 ±0.00[a]	0.0010 ±0.00[a]	0.002 ±0.00[a]	0.013 ±0.01[a]
ILM_4	0.000[a]	0.0020 ±0.00[a]	0.0020 ±0.00[a]	0.002 ±0.00[a]	0.033 ±0.01[a]
ILM_5	0.000[a]	0.0210 ±0.03[a]	0.0210 ±0.03[a]	0.003 ±0.00[a]	0.035 ±0.02[a]
ILM_6	0.000[a]	0.0013 ±0.00[a]	0.0013 ±0.00[a]	0.003 ±0.00[a]	0.040 ±0.01[a]
ILM_7	0.000[a]	0.0010 ±0.00[a]	0.0010 ±0.00[a]	0.003 ±0.00[a]	0.056 ±0.01[a]
ILM_8	0.000[a]	0.0007 ±0.00[a]	0.0007 ±0.00[a]	0.003 ±0.00[a]	0.060 ±0.01[a]
ILM_9	0.000[a]	0.000[a]	0.0010 ±0.00[a]	0.001 ±0.00[a]	0.0010 ±0.00[a]
LIMITS	**0.05-0.1 mg/L (WHO/NIS)**				

No iron was detected in the water samples before storage, but values of 0.001-0.0023 mg/L were obtained at the end of the first month (Table 6). There was no significant ($p > 0.05$) difference in the iron contents. Different trends were observed in the iron contents during the rest of the storage period. This observation contrasts the findings of Ajekunle et al. **[23]**, who reported decreasing iron contents while storing sachet water. This may be attributed to iron-utilizing species of microbes in their study, whereas more iron-releasing metabolic or biochemical processes probably occurred in the current study. All the sachet water samples contain iron levels less than the maximum (0.3 mg/L) required by WHO and NIS.

Iron in water may indicate the presence of coliforms, which can, in turn, indicate sewage contamination and could cause dysentery and typhoid **[34]**. The affected water may also demonstrate discoloration, bitter and astringent flavour, and encourage microbial growth **[32]**. Excessive consumption of iron can also result in red hot disease **[39]**.

Table 6 Magnesium and iron of selected sachet water brands produced in Ilorin Metropolis over a 4-month storage.

Sample	Magnesium (mg/L)				
	Storage period (month)				
	0	1	2	3	4
ILM$_1$	0.42 ±0.00^{g}	0.5033 ±0.04^{c}	0.6367 ±0.08^{b}	0.69 ±0.04^{b}	0.82 ±0.03^{ef}
ILM$_2$	0.43 ±0.01^{g}	0.5533 ±0.07^{c}	0.5600 ±0.03^{b}	0.59 ±0.04^{cd}	0.74 ±0.03^{fg}
ILM$_3$	0.46 ±0.01^{f}	0.5433 ±0.18^{c}	0.5700 ±0.08^{b}	0.63 ±0.06^{bcd}	0.65 ±0.07^{g}
ILM$_4$	0.77 ±0.01^{b}	0.7833 ±0.12^{b}	0.6500 ±0.09^{b}	0.67 ±0.05^{bc}	1.37 ±0.05^{c}
ILM$_5$	1.0 ±0.00^{a}	0.7667 ±0.09^{b}	1.0233 ±0.03^{a}	1.02 ±0.03^{a}	2.43 ±0.12^{a}
ILM$_6$	0.64 ±0.01^{c}	1.220 ±0.19^{a}	0.5300 ±0.10^{b}	0.69 ±0.06^{b}	1.18 ±0.09^{d}
ILM$_7$	0.49 ±0.01^{e}	0.5133 ±0.03^{c}	0.6067 ±0.04^{b}	0.55 ±0.09^{c}	2.13 ±0.03^{b}
ILM$_8$	0.52 ±0.01^{d}	0.5600 ±0.04^{c}	0.5300 ±0.08^{b}	0.73 ±0.03^{b}	0.88 ±0.04^{e}
ILM$_9$	0.53 ±0.01^{d}	0.5900 ±0.01^{c}	0.6500 ±0.03^{b}	0.74 ±0.04^{b}	0.74 ±0.00^{fg}
LIMITS	**2.0 mg/L (WHO)**				
	Iron (mg/L)				
ILM$_1$	0.00^{a}	0.0023 ±0.00^{a}	0.00200 ±0.00^{a}	0.0020 ±0.00^{b}	0.005 ±0.00^{a}
ILM$_2$	0.00^{a}	0.0010 ±0.00^{a}	0.00133 ±0.00^{a}	0.0030 ±0.00^{a}	0.003 ±0.00^{a}
ILM$_3$	0.00^{a}	0.0013 ±0.00^{a}	0.00233 ±0.00^{a}	0.0020 ±0.00^{a}	0.004 ±0.00^{a}
ILM$_4$	0.00^{a}	0.0020 ±0.00^{a}	0.00233 ±0.00^{a}	0.0026 ±0.00^{a}	0.006 ±0.00^{a}
ILM$_5$	0.00^{a}	0.0107 ±0.01^{a}	0.00167 ±0.00^{a}	0.0023 ±0.00^{a}	0.002 ±0.00^{a}
ILM$_6$	0.00^{a}	0.0017 ±0.00^{a}	0.00200 ±0.00^{a}	0.0023 ±0.00^{a}	0.004 ±0.00^{a}
ILM$_7$	0.00^{a}	0.0017 ±0.00^{a}	0.00267 ±0.00^{a}	0.0020 ±0.00^{a}	0.019 ±0.03^{a}
ILM$_8$	0.00^{a}	0.0020 ±0.00^{a}	0.00267 ±0.00^{a}	0.0026 ±0.00^{a}	0.003 ±0.00^{a}
ILM$_9$	0.00^{a}	0.0020 ±0.00^{a}	0.00233 ±0.00^{a}	0.0023 ±0.00^{a}	0.002 ±0.00^{a}
LIMITS	**0.3 mg/L (WHO)**				

Note: Values are Means ±SD of triplicate determinations (n = 3); Means with different superscripts along the same column are significantly ($p \leq 0.05$) different.

The coliform counts of the various water brands at the start of storage ranged from 2 to 26 CFU/mL (Table 7). After the first month of storage, water sample ILM1 demonstrated the highest coliform count of 280 CFU/mL, even though it had the lowest count before storage. This could be due to higher residual chlorine in other samples, which prevented coliform from multiplying. The increasing total coliform count is at variance with the loss of viability expected of coliform during storage **[23]**. However, other scholars have reported similar reports. For example, Duwiejuah et al. **[35]** reported increased coliform counts in some Ghanaian sachet water brands during refrigeration and ambient storage. Coliform counts also showed a progressive rise in different brands of sachet water during an 8-week indoor storage in Enugu, Nigeria **[53]**. While the reason for this observation is yet to be ascertained, an increase in coliform count after storage might suggest insufficient water treatment **[54]**. It is also plausible that the packaging and bagging materials were contaminated with coliform before being used, thereby serving as sources of coliform in the packaged water during storage. Accordingly, it might be necessary for the manufacturers of sachet water brands to ensure more thorough water treatment during production. In addition, more frequent cleaning and sanitation of the storage area and facilities might be required to create sanitary conditions that discourage the proliferation of coliform. A detailed risk assessment that includes inspection of packaging materials and their handlers may help improve the microbial safety of the water brands. The permeability of polyethylene materials has been featured in discussions about the possible reasons for an increasing coliform count in sachet water during storage **[23]**. The fecal contamination of drinking water has severe health implications **[24]**. Total coliforms are widely used to indicate the general sanitary quality of treated drinking water, while fecal coliforms give a much closer indication of fecal pollution **[55]**. Consequently, the WHO requires a zero count for fecal coliform in drinking water **[23]**. In a similar study by Ndamitso et al. **[51]**, a high presence of coliform was reported for some stored sachet water brands from Minna; hence, the water brands were deemed unfit for consumption. Sule et al. **[18]** also investigated bottled water brands in the Ilorin metropolis. The authors found one of the brands to contain coliform as high as 121 CFU/mL. Coupled with the findings in

the current research, this suggests that packaged water manufacturers within the Ilorin Metropolis may require more conscious efforts to protect their water sources and operational systems from fecal contamination.

At the end of storage, the total coliform count in all sachet water samples had increased markedly, especially in samples ILM1 and ILM5 (≥2400 CFU/mL each), indicating coliform growth. The recorded coliform counts in the various water samples constitute a severe health hazard, and fecal contaminations of the different production sites are strongly suspected.

Bacterial loads in the various sachet water brands at the start of storage ranged between 2 and 50 cfu/ mL (Table 7), and the values were below the permissible limit of 100 CFU/mL. However, these counts showed varied rates of increase until after the second month, when bacterial loads in a few samples declined. Samples ILM7 and ILM8 notably had bacterial counts that exceeded the permissible limit from the second month of storage.

Table 7 Total coliform and viable counts of selected sachet water brands produced in Ilorin Metropolis over 4-month storage.

Sample	Total viable counts (CFU/mL)					Total coliform counts (CFU/mL)				
	Storage period (month)					Storage period (month)				
	0	1	2	3	4	0	1	2	3	4
ILM_1	8	28	58	75	70	4	240	280	350	920
ILM_2	2	8	12	32	2	≤2	280	350	920	≥2400
ILM_3	10	30	57	70	67	2	33	43	180	220
ILM_4	10	48	100	70	80	4	34	33	49	110
ILM_5	20	68	106	100	105	2	240	220	350	1600
ILM_6	21	52	80	90	108	8	240	280	350	540
ILM_7	50	100	120	130	120	23	45	130	350	≥2400
ILM_8	38	70	111	110	105	26	34	46	110	180
ILM_9	8	26	30	38	30	≤8	110	220	220	280
LIMITS	100 CFU/mL (WHO)					0 CFU/mL (WHO)				

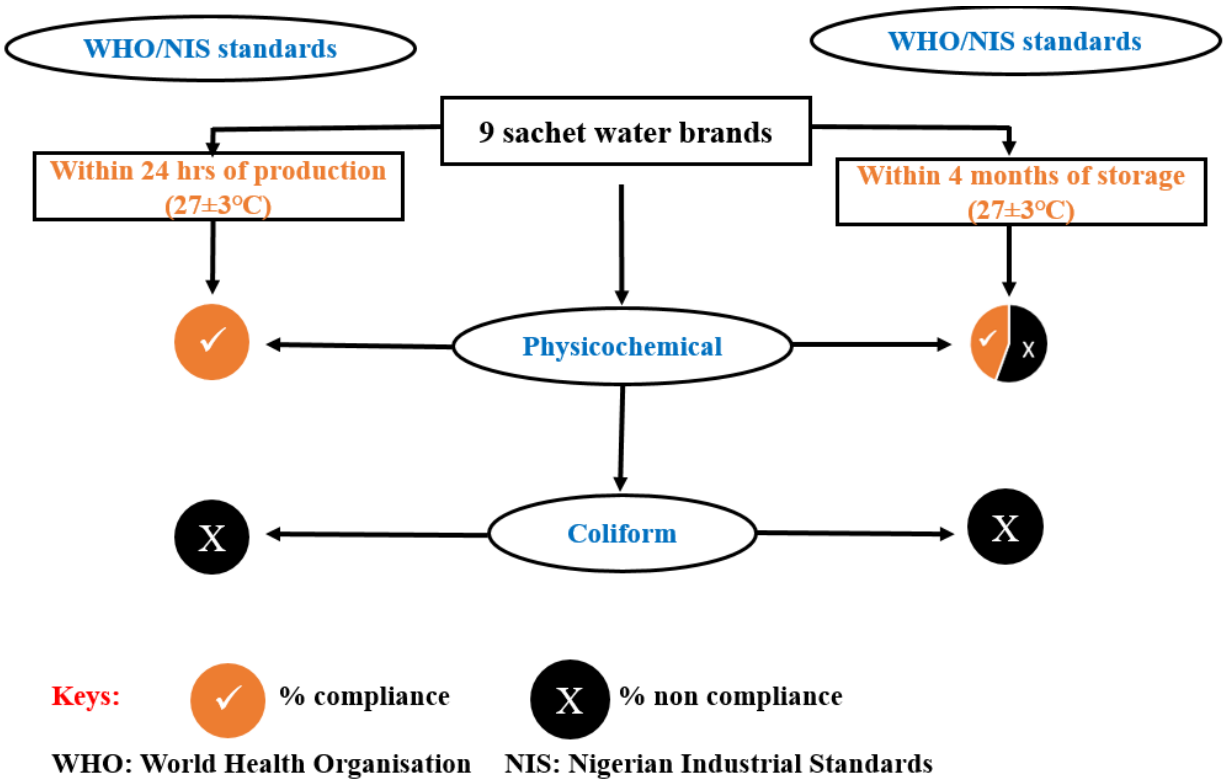


Figure 6 Graphical representation of regulatory compliance.

Based on results obtained from the current investigation, especially as it concerns microbiological safety on the one hand and physicochemical instability during storage on the other hand, concerted efforts may be required on three dimensions:

i. Addressing consumers' perceived purity of sachet water: Consumers' interpretation of sachet water as being 'pure' may represent some potential vulnerability. Nigerians typically refer to sachet water as 'pure water'. There are tendencies for many, including residents of the local government areas under study, who are not yet well-informed, to misconceive this terminology as water 'free of any probable hazard risks.' It is necessary to educate consumers that some sachet water brands may not be as safe for consumption as generally deemed. Increased awareness in this regard is helpful for consumers to be more logical by paying more attention to factory details, such as siting and operations when choosing the brands to patronize. The proposed sensitization efforts should also discourage any prolonged ambient storage of sachet water beyond two months; otherwise, physicochemical safety may not be guaranteed. Although this is in line with the two months 'Best Before Date' commonly claimed by some producers, information relating to the shelf life has been reported missing on some sachet water brands **[56]**.

ii. Manufacturers' consciousness on quality and safety: Contaminated water sources, inadequate water treatments, and unsanitary storage and packaging conditions are suspected to be responsible for the declining quality of the sachet water brands during storage. Producers are therefore encouraged to intensify surveillance on the microbiological status of raw water from the source, as well as during and after processing, extending this to the packaging materials and their handlers. In addition, storage areas and facilities should be regularly cleaned and sanitized to discourage microbial proliferation. If matched with appropriate measures to avoid fecal contamination along the line, this may check the presence of coliform in sachet water. Current and prospective sachet water producers are required to comply with standards relating to factory location and proximity to fecal sources.

iii. Importance of regulatory surveillance and penalties: Right from initial certification through to subsequent operations, relevant regulatory agencies, including NAFDAC and SON, in the country may be required to review and intensify their monitoring approaches, and focus, such that certification and presence of any sachet brand in the market can be more reliably trustable by consumers. While keeping illegal producers out of the market may be cumbersome, enacting and implementing severe sanctions on any illegal operations may be strategic.

iv. More documentation of waterborne illnesses: Some data on outbreaks of waterborne diseases are available in some different states in Nigeria, including Kwara State, where the current city under study is located. Kwara State was previously reported as the third State with the highest cholera outbreak **[57]**. This suggests that the microbial results from the current study may be strongly linked to the occurrence of certain diseases in the study area. In the same vein, leakages of storage tanks from nearby petrol filling stations have been identified as possible causes of increasing contaminants such as heavy metals found in groundwater sources in the Ilorin Metropolis **[58]**. This pollution was suggested as a possible threat to public health. However, more documentation of waterborne illnesses in the study area is recommended. This will provide for more scientific assessments of the likely roles of sachet water.

CONCLUSION

The study showed that sachet water brands from selected local government areas of the Ilorin Metropolis of Nigeria largely comply with the physicochemical requirements of WHO and NIS for drinking water. These included temperature (25.10-30.13 °C), turbidity (0.37-2.84 NTU), electrical conductivity (115.6-277.33 µS), total dissolved solids (55.33-125.70 mg/L), chlorides (2.83-8.57 mg/L), nitrates (0.15-0.64 mg/L), sulphates (4.70-7.12 mg/L), sodium (10.16-18.74 mg/L), copper (<0.01 mg/L), lead (<0.006 mg/L), and iron (<0.03 mg/L). However, two-thirds of the products exceeded the permissible level (100 mg/L) for total hardness at the end of four months of storage (102.30-136.33 µS/L), indicating their increased risk to human health when stored beyond three months. Similarly, two of the nine sachet water brands exhibited magnesium contents of 2.13-2.43 mg/L after the 4th month of storage, which was higher than the permissible level of 2.0 mg/L and may imply increased hardness during further storage. The bacterial loads of some brands demonstrated the tendency to rise (>105 CFU/mL) beyond the permissible limit of 100 CFU/mL after two months of storage. From a bacterial point of view, this may suggest that such brands are safe for consumption within the first two months of storage. However, this likelihood cannot be validated, considering that coliforms were detected (2-26 CFU/mL) in all the water brands even before the commencement of storage. As this strongly implies faecal contamination and renders the products unsafe for human consumption, it is pertinent that the sachet water producers in the study area, as well as concerned regulatory bodies, intensify efforts to check faecal contamination. It is therefore advised that the various producers put in place measures to prevent faecal contamination so as to meet the coliform zero tolerance for drinking water. Periodic inspection of sachet water factories by concerned regulatory bodies is vital to ensure conformity with set standards.

REFERENCES

1. Isikwue, M. O., & Chikezie, A. (2014). Quality assessment of various sachet water brands marketed in Bauchi metropolis of Nigeria. In International Journal of Advances in Engineering and Technology (Vol. 6, Issue 6, pp. 2489–2495). Blue Eyes Intelligence Engineering and Sciences Publication.
2. Ajala, O. J., Ighalo, J. O., Adeniyi, A. G., Ogunniyi, S., & Adeyanju, C. A. (2020). Contamination issues in sachet and bottled water in Nigeria: a mini-review. In Sustainable Water Resources Management (Vol. 6, Issue 6). Springer Science and Business Media LLC. https://doi.org/10.1007/s40899-020-00478-5
3. Stoler, J., Fink, G., Weeks, J. R., Otoo, R. A., Ampofo, J. A., & Hill, A. G. (2012). When urban taps run dry: Sachet water consumption and health effects in low income neighborhoods of Accra, Ghana. In Health & Place (Vol. 18, Issue 2, pp. 250–262). Elsevier BV. https://doi.org/10.1016/j.healthplace.2011.09.020
4. Sojobi, A., Owamah, H., & Dahunsi, S. (2014). Comparative study of household water treatment in a rural community in Kwara state, Nigeria. In Nigerian Journal of Technology (Vol. 33, Issue 1, p. 134). African Journals Online (AJOL). https://doi.org/10.4314/njt.v33i1.18
5. Dada, A. C. (2009). Sachet water phenomenon in Nigeria: Assessment of the potential health impacts. African Journal of Microbiology Research (Vol. 3, Issue 1, pp. 15–21). Academic Journals. https://doi.org/10.5942/jawwa.2017.109.0087
6. Airaodion, A. I. (2019). Assessment of Sachet and Bottled Water Quality in Ibadan, Nigeria. In Global Journal of Nutrition & Food Science (Vol. 1, Issue 4). Iris Publishers LLC. https://doi.org/10.33552/gjnfs.2019.01.000519
7. Ali, I., Singh, P., Aboul-Enein, H. Y., & Sharma, B. (2009). Chiral Analysis of Ibuprofen Residues in Water and Sediment. In Analytical Letters (Vol. 42, Issue 12, pp. 1747–1760). Informa UK Limited. https://doi.org/10.1080/00032710903060768
8. Basheer, A. A. (2017). Chemical chiral pollution: Impact on the society and science and need of the regulations in the 21st century. In Chirality (Vol. 30, Issue 4, pp. 402–406). Wiley. https://doi.org/10.1002/chir.22808
9. Basheer, A. A. (2018). New generation nano-adsorbents for the removal of emerging contaminants in water. In Journal of Molecular Liquids (Vol. 261, pp. 583–593). Elsevier BV. https://doi.org/10.1016/j.molliq.2018.04.021
10. Basheer, A. A., & Ali, I. (2018). Stereoselective uptake and degradation of (±)-o,p-DDD pesticide stereomers in water-sediment system. In Chirality (Vol. 30, Issue 9, pp. 1088–1095). Wiley. https://doi.org/10.1002/chir.22989
11. Omole, D. O., Ndambuki, J. M., & Balogun, K. (2015). Consumption of sachet water in Nigeria: quality, public health and economic perspectives. In African Journal of Science, Technology, Innovation and Development (Vol. 7, Issue 1, pp. 45–51). Informa UK Limited. https://doi.org/10.1080/20421338.2014.979654
12. Oluwaseun A Akinsola, Tolulope O Oyewumi, Adegola, B. A., & Okpe, P. O. (2021). Quality Assessment of Selected Sachet Water Brands Marketed in Owo, Akure and Ondo Areas of Ondo State, Nigeria. Unpublished. https://doi.org/10.13140/RG.2.2.22829.00481
13. Yusuf, Y. O., Jimoh, A. I., Onaolapo, E. O., & Dabo, Y. (2015). An assessment of sachet water quality in Zaria Area of Kaduna State, Nigeria. In Journal of Geography and Regional Planning (Vol. 8, Issue 7, pp. 174–180). Academic Journals. https://doi.org/10.5897/jgrp2015.0501
14. Opafola, O. T., Oladepo, K. T., Ajibade, F. O., & David, A. O. (2020). Potability assessment of packaged sachet water sold within a tertiary institution in southwestern Nigeria. In Journal of King Saud University - Science (Vol. 32, Issue 3, pp. 1999–2004). Elsevier BV. https://doi.org/10.1016/j.jksus.2020.02.004
15. Sheshe, M., & Magashi, A. (2015). Assessment of physicochemical quality of sachet water produced in selected local government areas of Kano Metropolis, Kano State - Nigeria. In Bayero Journal of Pure and Applied Sciences (Vol. 7, Issue 2, p. 31). African Journals Online (AJOL). https://doi.org/10.4314/bajopas.v7i2.7
16. Duru, C. E., Amadi, U. S., & Enyoh, C. E. (2017). Storage and its effect on chemical quality indicators in sachet water brands sold in Owerri Municipal, Imo State, Nigeria. In World News of Natural Sciences (Vol. 12, pp. 73–81). Złocieniec: Scientific Publishing House "Darwin".
17. Olayemi, A. B. (1999). Microbial potability of bottled and packaged drinking waters hawked in Ilorin metropolis. In International Journal of Environmental Health Research (Vol. 9, Issue 3, pp. 245–248). Informa UK Limited. https://doi.org/10.1080/09603129973218
18. Sule, I., Agbabiaka, T., Saliu, B., Adewumi, O., & Bello, A.B. (2017). Bacteriological and Physicochemical Analysis of Sachet Water Sold in Ilorin Metropolis, Nigeria. Al-Hikmah Journal of Pure & Applied Sciences (Vol. 30, Issue 1, pp. 2978–2986). Al-Hikmah University Central Journal.

19. Ajadi, B., Adedapo, A., & Tunde, A. M. (2011). Impact of climate on urban agriculture: case study of Ilorin City, Nigeria. In Global Journal of Human-Social Science Research (Vol. 11, Issue 1, pp. 25–29). Global Journals.
20. Idrees, M. O., Yusuf, A., Mokhtar, E. S., & Yao, K. (2022). Urban flood susceptibility mapping in Ilorin, Nigeria, using GIS and multi-criteria decision analysis. In Modeling Earth Systems and Environment (Vol. 8, Issue 4, pp. 5779–5791). Springer Science and Business Media LLC. https://doi.org/10.1007/s40808-022-01479-3
21. Ogunlela, A. O. (2012). Quantitative Analysis of Groundwater Flow in Ilorin, Nigeria. In Journal of Engineering Science and Technology Review (Vol. 5, Issue 2, pp. 57–59). International Hellenic University. https://doi.org/10.25103/jestr.052.11
22. Baird, R. B., Eaton, A. D., Rice, E. W., & Bridgewater, L. (2017). Standard methods for the examination of water and wastewater, 23rd Edition, American Public Health Association, Washington DC.
23. Ajekunle, Z., Ojekunle, V., Eruola, A., Oyebanji, F., Olatunde, K., Amujo, B., Sangowusi, O., Adekttan, A., & Taiwo, A. (2015). The Effects of Storage on Sachet Water Quality in Ogun State, Nigeria. In Journal of Applied Sciences and Environmental Management (Vol. 19, Issue 2, p. 183). African Journals Online (AJOL). https://doi.org/10.4314/jasem.v19i2.3
24. Onweluzo, J., & Akuagbazie, C. (2011). Assessment of the quality of bottled and sachet water sold in Nsukka town. In Agro-Science (Vol. 9, Issue 2). African Journals Online (AJOL). https://doi.org/10.4314/as.v9i2.64804
25. Balogun, M., Oyeyinka, A., Kolawole, F., El-Imam, A., & Oyeyinka, A. (2022). Physicochemical properties, consumer acceptability and microbial quality of saccharified sweet potato juice preserved with lemon. In The Annals of the University Dunarea de Jos of Galati. Fascicle VI - Food Technology (Vol. 46, Issue 1, pp. 9–20). Universitatea Dunarea de Jos din Galati. https://doi.org/10.35219/foodtechnology.2022.1.01
26. APHA. (1999). Standard methods for examination of water and wastewater. 20th ed, Washington DC, American Public Health Association Publication, Washington D.C.2340.
27. Sutton, S. (2010). The most probable number method and its uses in enumeration, qualification, and validation. In Journal of Validation Technology (Vol. 16, Issue 3, pp. 35–38). Institute of Validation Technology.
28. Unegbu, V. N., Ezebialu, C. U., Omeje, M. J., Nkwoemeka, N. E., Eze, E. M., & Ezennia, O. J. (2017). The Effects of storage on the physicochemical properties of the sachet water produced, sold and consumed within Owerri metropolis, Imo State. In International Journal of Research and Review (Vol. 4, Issue 8, pp. 71–83). Galore Knowledge Publication Pvt. Ltd.
29. Danso-Boateng, E., & Frimpong, I. K. (2013). Quality analysis of plastic sachet and bottled water brands produced or sold in Kumasi, Ghana. In International Journal of Development and Sustainability (Vol. 4, pp. 2222–2232). International Society for Development and Sustainability.
30. Epundu, U., Adinma, E., Ezeama, N., Emelumadu, O., & Ogbonna, B. (2017). A Review on Packaged Drinking Water, Quality Regulations and Public Health: Exploring Potability and Safety Gap Implications for Public Health in Nigeria. In International Journal of TROPICAL DISEASE & Health (Vol. 25, Issue 3, pp. 1–10). Sciencedomain International. https://doi.org/10.9734/ijtdh/2017/35846
31. Saalidong, B. M., Aram, S. A., Otu, S., & Lartey, P. O. (2022). Examining the dynamics of the relationship between water pH and other water quality parameters in ground and surface water systems. In U. Sarker (Ed.), PLOS ONE (Vol. 17, Issue 1, p. e0262117). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0262117
32. Egbueri, J. C. (2020). Heavy Metals Pollution Source Identification and Probabilistic Health Risk Assessment of Shallow Groundwater in Onitsha, Nigeria. In Analytical Letters (Vol. 53, Issue 10, pp. 1620–1638). Informa UK Limited. https://doi.org/10.1080/00032719.2020.1712606
33. Ayegbo, K. S., Ogundipe, K. E., Olimaro, G., Ajimalofin, A. D., & Akinwumi, I. I. (2021). Water Quality of Selected Sachet Water Brands Sold in Sango-Ota, Nigeria. In IOP Conference Series: Materials Science and Engineering (Vol. 1036, Issue 1, p. 012011). IOP Publishing. https://doi.org/10.1088/1757-899x/1036/1/012011
34. Bacha, A. A., Durrani, M. I., & Paracha, P. I. (2010). Physical and bacteriological characteristics of drinking water of Peshawar. In Pakistan Journal of Nutrition (Vo. 9, Issue 10, pp. 1028–1033). Asian Network for Scientific Information.
35. Duwiejuah, A. B., Cobbina, S. J., & Akrong, M. O. (2013). Effect of Storage on the Quality of Sachet-Vended Water in the Tamale Metropolis, Ghana. In Journal of Environmental Protection (Vol. 04, Issue 06, pp. 629–637). Scientific Research Publishing, Inc. https://doi.org/10.4236/jep.2013.46072

36. Adedire, O. M., Atere, A., Ogundipe, W. F., & Farinu, A. O. (2021). Effects of Direct and Indirect Sunlight on Polythene Packs, Sensory, Microbial and Chemical Properties of Sachet Water. In Journal of Advances in Biology & Biotechnology (pp. 25–34). Sciencedomain International. https://doi.org/10.9734/jabb/2021/v24i130194

37. Amuah, E. E. Y., Bekoe, E. M. O., Kazapoe, R. W., Dankwa, P., Nandomah, S., Douti, N. B., Abanyie, S. K., & Okyere, I. K. (2021). Sachet water quality and Vendors' practices in Damongo, northern Ghana during the emergence of SARS-CoV-2 using multivariate statistics, water quality and pollution indices, and panel assessment. In Environmental Challenges (Vol. 4, p. 100164). Elsevier BV. https://doi.org/10.1016/j.envc.2021.100164

38. Osmani, M., Hoxha, B., Sotir, M., Mazrreku, A., Karamelo, P., Nikolin, G., BekteshiI, L., Karasali, V., Kucaj, E., & Balla, J. (2019). Evaluation of Drinking Water Quality in Pogradec District. In The Eurasia Proceedings of Science Technology Engineering and Mathematics (Vol. 8, pp. 20–28). Konya: ISRES Publishing.

39. Onyemesili, O. O., Egbueri, J. C., & Ezugwu, C. K. (2020). Assessing the pollution status, ecological and health risks of surface waters in Nnewi urban, Nigeria: implications of poor waste disposal. In Environmental Forensics (Vol. 23, Issues 3–4, pp. 346–360). Informa UK Limited. https://doi.org/10.1080/15275922.2020.1850564

40. Meride, Y., & Ayenew, B. (2016). Drinking water quality assessment and its effects on residents health in Wondo genet campus, Ethiopia. In Environmental Systems Research (Vol. 5, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s40068-016-0053-6

41. Ojekunle, Z., & Adeleke, J. (2017). The effect of storage on Physical, Chemical and Bacteriological characteristics of Sachet and bottled water marketed in Ibadan Metropolis, Oyo State. Nigeria. In Journal of Applied Sciences and Environmental Management (Vol. 21, Issue 6, p. 1203). African Journals Online (AJOL). https://doi.org/10.4314/jasem.v21i6.35

42. Ukah, B. U., Ameh, P. D., Egbueri, J. C., Unigwe, C. O., & Ubido, O. E. (2020). Impact of effluent-derived heavy metals on the groundwater quality in Ajao industrial area, Nigeria: an assessment using entropy water quality index (EWQI). In International Journal of Energy and Water Resources (Vol. 4, Issue 3, pp. 231–244). Springer Science and Business Media LLC. https://doi.org/10.1007/s42108-020-00058-5

43. Ayejoto, D. A., Agbasi, J. C., Egbueri, J. C., & Echefu, K. I. (2022). Assessment of oral and dermal health risk exposures associated with contaminated water resources: an update in Ojoto area, southeast Nigeria. In International Journal of Environmental Analytical Chemistry (Vol. 104, Issue 3, pp. 641–661). Informa UK Limited. https://doi.org/10.1080/03067319.2021.2023515

44. Puri, A., & Kumar, M. (2012). A review of permissible limits of drinking water. In Indian Journal of Occupational and Environmental Medicine (Vol. 16, Issue 1, p. 40). Medknow. https://doi.org/10.4103/0019-5278.99696

45. Fadipe, O. O. (2020). Characteristics of Packaged Water under Different Storage Conditions Within Osogbo Metropolis. In UNIOSUN Journal of Engineering and Environmental Sciences (Vol. 2, Issue 2). Lujosh Ventures Limited. https://doi.org/10.36108/ujees/0202.20.0221

46. Jadhav, S., & Jadhav, M. (2017). Study of sulphate concentration of Pavana River at Pune: A case study. In International Journal of Chemical Studies (Vol. 5, Issue 2, pp. 198–200). AkiNik Publications.

47. Cotruvo, J. A. (2017). 2017 WHO Guidelines for Drinking Water Quality: First Addendum to the Fourth Edition. In Journal AWWA (Vol. 109, Issue 7, pp. 44–51). Wiley. https://doi.org/10.5942/jawwa.2017.109.0087

48. Bradshaw, M. H., & Powell, G. M. (2002). Sodium in drinking water. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Manhattan. Retrieved from https://www.ksre.k-state.edu/historicpublications/pubs/MF1094.pdf.

49. Egbueri, J. C. (2020). Signatures of contamination, corrosivity and scaling in natural waters from a fast-developing suburb (Nigeria): insights into their suitability for industrial purposes. In Environment, Development and Sustainability (Vol. 23, Issue 1, pp. 591–609). Springer Science and Business Media LLC. https://doi.org/10.1007/s10668-020-00597-1

50. Payne, M. (2008). Lead in drinking water. In Canadian Medical Association Journal (Vol. 179, Issue 3, pp. 253–254). CMA Joule Inc. https://doi.org/10.1503/cmaj.071483

51. Ndamitso, M., Idris, S., Likita, M., Tijani, J. O., Ajai, A., & Bala, A. (2013). Physico-chemical and Escherichia coli assessment of selected sachet water produced in some areas of Minna, Niger State, Nigeria. In International Journal of Water Resources and Environmental Engineering (Vol. 5, Issue 3, pp. 134–140). Academic Journals.

52. Oyelude, E. O., & Ahenkorah, S. (2012). Quality of sachet water and bottled water in Bolgatanga municipality of Ghana. In Research Journal of Applied Sciences, Engineering and Technology (Vol. 4, Issue 9, pp. 1094–1098). Maxwell Scientific Publications.

53. Mberekpe, P., & Eze, N. (2014). Effect of Preservation on the Quality of Sachet Water Consumed by Households in Nsukka Zone. In Civil and Environmental Research (Vol. 6, Issue 7, pp. 25–30). Sciendo.

54. Chalchisa, D., Megersa, M., & Beyene, A. (2017). Assessment of the quality of drinking water in storage tanks and its implication on the safety of urban water supply in developing countries. In Environmental Systems Research (Vol. 6, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s40068-017-0089-2

55. Ashbolt, N. J., Grabow, W. O., & Snozzi, M. (2001). Indicators of microbial water quality. In L. Fewtrell & J. Bartram (Eds.), Water quality: Guidelines, standards and health (pp. 289–316). IWA Publishing.

56. Muazu, J., Muhammad-Biu, A., & Mohammed, G. (2012). Microbial Quality of Packaged Sachet Water Marketed in Maiduguri Metropolis, North Eastern Nigeria. In British Journal of Pharmacology and Toxicology (Vol. 3, Issue 1, pp 33–38). Maxwell Scientific Publications.

57. Yusuff, A. S., John, W., & Oloruntoba, A. C. (2014). Review on Prevalence of Waterborne Diseases in Nigeria. In Journal of Advancement in Medical and Life Sciences (Vol. 2 pp. 1–3). ScienceQ Publishing Group.

58. Emmanuel Odipe, O., Olawale Sawyerr, H., & Olayinka Adewoye, S. (2020). Filling Stations and Their Effects on Groundwater Quality in Ilorin Metropolis. In International Journal of Environmental Protection and Policy (Vol. 8, Issue 1, p. 11). Science Publishing Group. https://doi.org/10.11648/j.ijepp.20200801.12

Funds:

The authors received no funds, grants nor any support from any organisation for the submitted study.

Acknowledgments:

The authors appreciate all those who assisted in the conduct of the research.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Mutiat Balogun, University of Ilorin, Department of Home Economics and Food Science, PMB 1515, Kwara State,
Tel.: +23455948515
E-mail: balogun.ma@unilorin.edu.ng
ORCID: https://orcid.org/0000-0002-4830-6573

Tawakalitu Aruna, Kwara State University, Department of Food Science and Technology, Malete, Nigeria,
Tel.: +2348099454023
E-mail: tawakalitu.aruna@kwasu.edu.ng
ORCID: https://orcid.org/0000-0002-2566-2380

***Olaide Akintayo**, University of Ilorin, Department of Home Economics and Food Science, PMB 1515, Kwara State,
Tel.: +2347037445069
E-mail: akintayo.oa@unilorin.edu.ng
ORCID: https://orcid.org/0000-0003-4705-0163

Rasheedat Imam, University of Ilorin, Department of Home Economics and Food Science, PMB 1515, Kwara State,
Tel.: +23491408145
E-mail: imamrasheedatahmad1993@gmail.com
ORCID: https://orcid.org/0009-0004-1769-0447

Olayinka Shukurat, University of Ilorin, Department of Home Economics and Food Science, PMB 1515, Kwara State,
Tel.: +2348173552728
E-mail: olayinkashukrah@gmail.com
ORCID: https://orcid.org/0009-0001-4856-3668

Oluseye Lala, University of Ilorin, Department of Home Economics and Food Science, PMB 1515, Kwara State,
Tel.: +2348088970726
E-mail: olawalelala011@gmail.com
ORCID: https://orcid.org/0009-0002-1271-7079

Fausat Kolawole, University of Ilorin, Department of Home Economics and Food Science, PMB 1515, Kwara State,
Tel.: +2348033764414
E-mail: kolawole.fl@unilorin.edu.ng
ORCID: https://orcid.org/0000-0002-6465-3057

Christianah Gbadebo, University of Ilorin, Department of Home Economics and Food Science, PMB 1515, Kwara State,
Tel.: +2348065982341
E-mail: gbadebo.ct@unilorin.edu.ng
ORCID: https://orcid.org/0000-0002-8651-5712

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 23.12.2023
Revised: 12.3.2024
Accepted: 13.3.2024
Published: 8.4.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 386-397
https://doi.org/10.5219/1946
ISSN: 1337-0960 online
www.potravinarstvo.com

Effectiveness of vitamin A supplementation among children under 5 years old in Kazakhstan

Arailym Beisbekova, Feruza Ospanova, Maikul Kainarbayeva, Elena Ushanskaya, Salima Bykybayeva, Aidar Berdygaliev, Aizhan Raushanova, Faina Linkov

ABSTRACT

Micronutrient sufficiency leads to high rates of morbidity and mortality among children in Kazakhstan. Kazakhstan does not have a program for mandatory vitamin A supplementation of children under 5 years of age. Thus, the aim of this study was to assess the effectiveness of a pilot vitamin A supplementation program among children in Kazakhstan with the ultimate goal of informing future vitamin supplementation efforts. In Akmola and Kostanay regions of Kazakhstan, 529 children (aged 6-59 months) were randomly selected in each region through the local policlinics. In the first step of the study, mothers of the children were surveyed about the health status of their children using a standard data collection tool. Children were supplemented with Vitamin A using oral at a dose of 100,000 IU for children aged 6-11 months and 200,000 IU for children aged 12-59 months. Blood serum samples were collected for determining Vitamin A status. In the second step of the study, mothers were interviewed again about the health status of their children 6 months after the intervention and blood serum samples were collected to assess the efficacy of Vitamin A supplementation program. The number of self reported diarrhea cases and other intestinal infections significantly decreased after the vitamin A supplementation. The number of children with normal level of Vitamin A signficantly increased from before to after the intervention. Data from all participants (n = 529; pre VAS and 501 post VAS) showed that mean serum retinol levels increased significantly post VAS from 30.01 ±0.5 µg/dL to 61.06 ±1.2 µg/dL (p <0.001) Likewise, a significant change was observed in the cases of reported diarrhea between pre-test and post-test assessments (30 vs. 95; p <0.01). The health status of the examined children in the Akmola and Kostanay regions had significantly improved after the vitamin A supplementation and it points to the necessity of implementing Vitamin A supplementation program on the national level. This study has important policy implications for recommending the supplementation program on the national scale.

Keywords: children, awareness, vitamin A, deficiency, prevalence, prevention, supplementation

INTRODUCTION

Deficiency of a number of micronutrients, or "hidden hunger" **[1]**, is widespread in Kazakhstan and is detrimental to the health of the population, especially for mothers and young children **[2]**. Hashizume et. Al. reported that anemia among school-aged children in rural Kazakhstan (Kzyl-Orda region) appears to be related to iron indices and vitamin A status **[3]**. In countries with a VAD prevalence rate of ≥ 20%, it is recommended to supplement children with high doses of vitamin A **[4]**. Vitamin A plays an important role in the processes of reproduction and growth, differentiation of epithelial and bone tissue, maintenance of immunological status and vision function (photoreception) **[5]**.

Vitamin A deficiency (VAD) leads to problems with vision, increased incidences of respiratory, gastrointestinal, skin and genitourinary infections, as well as a significant increase in mortality in children under

five years of age from acute respiratory infections, measles and diarrhea **[6]**. VAD reduces immunity and significantly increases morbidity and mortality in children under 5 years of age **[7]**. Vitamin A supplementation programs are useful due to their low cost and high effectiveness in preventing VAD, as well as reducing morbidity and mortality in children aged 6-59 months, and preventing other serious negative consequences of VAD **[6]**.

According to the recommendations of WHO and UNICEF, a vitamin A supplementation program for children under 5 years of age is being widely implemented throughout the world (twice a year with a vitamin A preparation in a dose of 100,000 IU for children 6-11 months and 200,000 IU for children 12-59 months) **[8]**. Consistent with these recommendations, supplementation programs are being implemented in the neighboring republics of Central Asia, including Uzbekistan, Tajikistan and Kyrgyzstan **[9]**.

The aim of this study is to determine vitamin A level before and after the supplementation program in Kazakhstani children with the ultimate goal of informing supplementation policies.

Scientific Hypothesis

The republic still lacks a system of biological monitoring of vitamin A status among children, which reduces the sustainability and reliability of ongoing preventive programs. The implementation of Vitamin A supplementation among children 6-59 month solves this problem.

MATERIAL AND METHODOLOGY

Samples

We recruited a representative sample of 529 out of 3851 potential volunteers (the total population of catchment areas of the polyclinics were working with was 27500 residents at the time of study). Participants aged 6-59 months were recruited through the network of children polyclinics (primary care centers) by reaching mothers, with mothers being surveyed pre and 6 months post the Vitamin A supplementation. For the current survey, our focus is on comparing the conditions before and six months after Vitamin A supplementation. Consequently, control groups were not included in this study. However, for future surveys, considering the potential benefits of incorporating control groups could enhance the research design. By including control groups, we can establish a more robust experimental framework, allowing for a clearer understanding of the specific effects of Vitamin A supplementation, while controlling for external factors that may influence the results. This adjustment in methodology will contribute to a more comprehensive and rigorous analysis in future research endeavors.

Survey and supplementation took place after receiving signed informed consent to participate in the study. The purpose and importance of this study were explained to the mothers. The Health Conditions Questionnaires was the standardized tool used for data collection in this study. Survey tool was translated into Kazakh and Russian languages and was conducted by trained interviewers **[10]**.

Instruments

Serum retinol levels were determined simultaneously using high-performance liquid chromatograph (HPLC) under high pressure according to the method published by Craft et al **[11]**. Used HPLC apparatus “Waters” 2487” with Breese system, USA software.

Laboratory Methods

To determine the content of vitamin A in blood serum, 5 mL of intravenous venous blood was collected by experienced medical workers. Blood was centrifuged at 3000 rpm for 5 min at room temperature. The sera and blood specimens were immediately frozen at -10 °C, kept for 1 week and then transported to Almaty in a portable ice box filled with dry ice. All specimens were kept frozen at -80 °C until analyses.

On the basis of WHO criteria **[12]**, the following serum retinol cut off levels were used **[13]**: in children aged 6-71 months >30 µg/dL (normal level) **[14] [15]**, 20-30 µg/dL (borderline level) **[16]**, and <20 µg/dL (Vitamin A deficiency) **[17]**.

Description of the Experiment

Sample preparation: First stage of the study was conducted from September to December 2012 and second stage from July to October 2013 in Akmola and Kostanay regions. Sample size was estimated taking into account the possible refusal to participate and/or potential 20% drop out rate, with the number of participants increased by 38% over the originally calculated sample size. In the two regions, children were randomly selected from 4 children’s polyclinic (2 polyclinics in each region), ensuring recruitment from at least 5 polyclinic catchment areas. Sampling plan assured representation of children from both, rural and urban areas (Table 1).

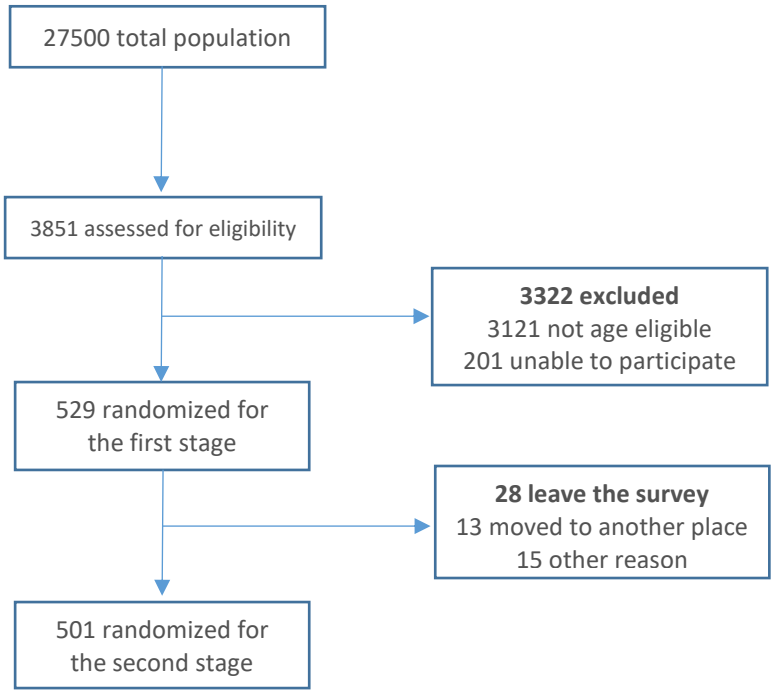


Figure 1 Random sampling of participants.

Number of samples analyzed: 529 participants
Number of repeated analyses: 501 participants
Design of the experiment: This study was carried out under the social order of the Ministry of Health of the Republic of Kazakhstan with the support of the UNICEF office in Kazakhstan. A longitudinal study was conducted in urban and rural areas of Akmola and Kostanay regions. This study was conducted in two stages. In the first stage, mothers of children aged 6-59 months were interviewed about health status of their children using a standardized data collection tool. Children's blood serum samples were collected to assess Vitamin A status. Following the initial assessment, all children were supplemented with oral drops of Vitamin A at a dose of 100,000 IU for children aged 6-11 months and 200,000 IU for children aged 12-59 months. Collection of blood samples and supplementation of children with Vitamin A were conducted by nursing staff. In the second stage, conducted 6 months after the original assessment and supplementation, mothers were interviewed again about the health status of their children. Children's blood serum samples were collected again to assess the efficacy of the Vitamin A supplementation program.

Statistical Analysis

Descriptive statistics were used to analyze the data. We looked at the mean and standard deviation for continuous variables and frequency for categorical variables. Paired t-test and McNemar tests was used to compare the outcomes of each group (pre- and post- Vitamin A supplementation) since the difference between paired samples was normally distributed. All statistical tests were two-tailed, and p values <0.05 were considered as statistically significant. Statistical calculations were performed using IBM SPSS Statistics for Windows, version 29.0.1 (IBM Corp., Armonk, N.Y., USA)

RESULTS AND DISCUSSION

General characteristic of participants

The sample comprised of a total of 529 children 6-59 month, with the majority of participants (89.8%) aged between 12-59 months and the mean age of 33.9 ±13.2 SD in the first stage of data collection. Of these children, 317 (59.9%) were Kazakh, 156 (29.5%) Russian, and 56 (16%) other nationalities/ethnicities, roughly corresponding to the ethnic composition of the Kazakhstan population. 84 (15.9%) of children were breastfed during the first assessment.

Table 1 Characteristics of study participants.

Variables	Frequency	Percentage (%)
Child age		
6-11	54	10.2
12-59	475	89.8
Maternal age		
15-24	115	21.7
25-34	278	52.6
35-49	136	25.7
Residence		
Urban	313	59.2
Rural	216	40.8
Maternal education		
Primary	180	34
Secondary	167	31.6
Tertiary	182	34.6

Maternal knowledge-related characteristics

More than half 289 (54.6%) of the mothers have not heard about medical consequences of vitamin A deficiency, and among 240 (45.4%) mothers who have heard about VAD, one third 83 (34.6%) reported that VAD may cause night blindness. More than half of the participants (55.0%) mentioned eggs and milk as sources of Vitamin A, 31.9% mentioned vegetables and fruits, and 13.0% of the respondents did not mention any food source.

Table 2 Maternal knowledge related characteristics.

Variables	Frequency	Percentage (%)
Have heard about Medical consequences of VAD		
Yes	240	45.4
No	289	54.6
Mention of Medical consequences of VAD		
Night blindness	83	34.6
Weakened immunity	23	9.6
Skin dryness	8	3.3
Growth failure	41	17.1
Other	16	6.7
Not mentioned	69	28.8
Mention of Vitamin A Food source		
Vegetable and fruits	169	31.9
Egg and milk	291	55.0
Not mentioned	69	13.0

Impact of Vitamin A supplementation on health status

The participants could be traced back successfully 6 months after the intervention and 95% response rate was obtained in the second assessment. Table 3 presents the overall differences in the prevalence of different reported health conditions among children 6-59 month pre- and post- receiving Vitamin A supplementation. Significant health status differences of children in the mother's answers were observed between pre- and post- Vitamin A supplementation. Pre- versus postsurvey data were compared by McNemar's test for paired sample. A significant change was detected. Before the Vitamin A supplementation 241 had cold last 6 months and after this number decreased to 136 (95% CI:12.178-54.214, p <0.01). Likewise, a significate percentage of change in reporting diarrhea was obtained between pre-test and post-test (30 vs. 95; p <0.01). The distribution and changes of the mothers' answer regarding acute respiratory viral infection (ARVI), cardiovascular diseases (CVD), urinary system diseases, ear, nose and throat (ENT) diseases, eye, gastrointestinal tract (GIT) diseases, food allergies, anemia, measles are presented in Table 3.

Table 3 Prevalence of health conditions among children pre and post receiving Vitamin A supplementation.

Pre \ Post	Yes	No	p-value	OR	95% CI
Cold					
Yes	241	136	<0.01*	25.695**	12.178-54.214
No	8	116			
Diarrhea					
Yes	30	95	<0.01*	59.053**	13.866-251.487
No	2	374			
ARVI					
Yes	242	140	<0.01*	32.555 **	13.955-75.945
No	6	113			
CVD					
Yes	1	24	<0.01*	0.960 ***	0.886-1.040
No	0	476			
Urinary system diseases					
Yes	1	8	0.008*	0.889***	0.706-1.120
No	0	492			
ENT					
Yes	16	187	<0.01*	12.663**	2.879-55.702
No	2	296			
Eye diseases					
Yes	5	36	<0.01*	31.806 **	5.960-169.727
No	2	458			
GIT diseases					
Yes	28	97	<0.01*	26.845 **	9.197-78.359
No	4	372			
Food allergies					
Yes	4	61	<0.01*	0.938 ***	0.882-0.999
No	0	436			
Anemia					
Yes	3	79	<0.01*	0.963 ***	0.924-1.005
No	0	419			
Measles					
Yes	0	18	<0.01*	1.004 ***	0.998-1.010
No	2	481			

Note: * McNemar test, binomial distribution; ** For cohort before Vitamin A supplementation; *** For cohort after Vitamin A supplementation.

Data from all participants (n = 529; pre VAS and 501 post VAS) showed that mean serum retinol levels increased significantly post VAS from 30.01 ±0.5 μg/dL to 61.06 ±1.2 μg/dL (p <0.001) (Table 4). The proportion of subjects with serum vitamin A levels above the upper limit of normal (normal range: ≥30 μg/dL) increased from 41.67 μg/dL to 62.66 μg/dL (p <0.001) after Vitamin A supplementation. The indicator of subjects with borderline levels of vitamin A (borderline level: 20-30 μg/dL) increased from 25.33 μg/dL to 63.61 (p <0.001). Total Vitamin A deficiency of serum level increased from 17.57 μg/dL to 27.86 μg/dL (p = 0.008). Vitamin A levels were higher than pre-Vitamin A supplementation in children 6-59 months.

Table 4 Comparison of outcomes measures pre- and post- Vitamin A supplementation.

Measure	Pre-	Post-	t-test (df)	95% CI	p-value*
Normal level of Vit A	41.67	62.66	13.34 (198)	17.88-24.09	p <0.001
Borderline level of Vit A	25.33	63.6	29.06 (187)	35.69-40.88	p <0.001
Vit A deficiency	17.57	27.86	6.27 (3)	1.64-15.51	p = 0.008
Mean of serum retinol	30.01	61.06	30.7 (500)	29.1-33.1	p <0.001

Note:*Paired samples t-test.

The survey findings indicate that vitamin A supplementation has been effective in significantly reducing VAD and leading to improved health of children in the Akmola and Kostanay regions. These findings provide a scientific basis for implementing programs to prevent VAD in children aged 6-59 months, in line with international recommendations. While several food fortifications programs have been implemented in Kazakhstan **[18]**, as of 2024 there is no active vitamin supplementation programs among children under 5 years.

Food fortification is the deliberate addition of essential vitamins, minerals, and nutrients to food during production to enhance nutritional content, addressing and preventing micronutrient deficiencies in populations. Wheat flour manufactured for retail in Kazakhstan undergoes mandatory enrichment with iron-containing vitamins, minerals, and other substances **[19]**, **[20]**. Baker's yeast, bread, bakery goods, and flour-based confectionery items are fortified with iodine preparations, B and E group vitamins, niacin, folic acid, water-soluble K-carotene preparations, and vitamin-mineral supplements **[21]**. Table salt in Kazakhstan undergoes mandatory iodization **[22]**. Fortification is also applied to milk and dairy products, fat-based items, cereal products, and pre-prepared dishes **[23]**. In preschools and other facilities serving children and adolescents, the fortification of foods with vitamin C is also taking place **[24]**. It is important to monitor existing fortification programs to see how they can be improved.

The findings on the impact of vitamin A supplementation on children's health, are consistent with the published literature on VAD prevention by Wirth et al., **[9]**. Foundational study by WHO provides a global perspective, outlining the prevalence of Vitamin A deficiency and setting the stage for interventions **[25]**. Barua et. al., and Harrison et al., explored the intricate mechanisms of Vitamin A metabolism and absorption, enhancing our understanding of its bioavailability **[26]**, **[27]**. One critical aspect of assessing the efficiency of vitamin A supplementation is its impact on child mortality. Numerous studies have demonstrated a substantial reduction in all-cause mortality among supplemented children. For instance, a randomized controlled trial by Smith et al., observed a significant decrease in mortality rates in supplemented groups in low- and middle-income countries, emphasizing the life-saving potential of vitamin A supplementation **[28]**. Amimo et al., and Yu et al., examining the role of Vitamin A in immune responses and its correlation with clinical outcomes broadens our understanding of its potential immune system impact beyond already published health impacts **[29]**, **[30]**. Vijayaraghavan et al., and Mayo-Wilson et al., focus on tackling mineral and vitamin deficiencies in rural African populations while also examining the efficacy of vitamin A supplementation in averting morbidity and mortality among children aged six months to five years. deficiency **[31]**, **[32]**. Vitamin A plays a pivotal role in bolstering the immune system, thereby influencing the incidence and severity of infectious diseases. Recent research, such as the work conducted by Abdelkader et al., suggests a notable reduction in respiratory infections among children supplemented with vitamin A **[33]**. Moreover, studies by Long et al., have explored the relationship between vitamin A supplementation and reduced occurrences of diarrheal diseases, underlining the importance of vitamin A in preventing common childhood illnesses **[34]**. Beyond its role in preventing infectious diseases, vitamin A has been implicated in cognitive development and physical growth. The study conducted by Prado et al., demonstrated positive effects on cognitive development in supplemented children. This aspect is crucial, as cognitive development during the early years lays the foundation for future learning and well-being **[35]**. Ensuring the safety and optimal dosage of vitamin A supplementation is a critical consideration. A systematic review by Soares et al., assessed potential adverse effects and recommended dosage adjustments for different age groups. Balancing the benefits and potential risks is essential for maximizing the positive impact of supplementation while minimizing any unintended consequences **[36]**.

Our results highlight a significant improvement in children's health and a reduction in VAD prevalence after vitamin A supplementation. These findings are also consistent with Villamor and Fawzi studies emphasizing the effectiveness of vitamin A supplementation in reducing VAD and associated health issues, particularly in regions where VAD is prevalent **[37]**. This reduction aligns with the goals of vitamin A supplementation programs, which aim to decrease VAD rates in populations at risk **[38]**. Considering regional perspectives and tailoring interventions to specific contexts is crucial for optimizing the efficiency of Vitamin A supplementation efforts globally **[39]**. McLean et al., and Shah et al., addressing implementation challenges **[40]** and strategies provides insights into optimizing Vitamin A supplementation programs on a global scale **[41]**.

To our knowledge, this was the first formalized effort to assess Vitamin A supplementation in Kazakhstani children. The key strength of this study is its large sample size and random selection of participants that enhances the study's statistical power and the generalizability of its findings. The study considered a diverse range of demographics, including women of various educational backgrounds and children of different ages and health conditions. This diversity allows for a more comprehensive analysis of the population's needs and the impact of vitamin A supplementation. The study compares data before and after vitamin A supplementation, allowing for a clear assessment of the intervention's effectiveness in reducing vitamin A deficiency and improving children's health. And also reports statistically significant increases in children's vitamin A levels and decreases in the

prevalence of vitamin A deficiency (VAD) after supplementation. The study follows up after six months of vitamin A supplementation, which is a reasonable duration to assess the effectiveness of the intervention.

The key weakness of this study is lack of a control group for comparison. Having a control group not receiving vitamin A supplementation would have allowed for a more robust assessment of the intervention's effectiveness by controlling for other factors that may influence the outcomes. Self-reported data by caregivers may be subject to recall bias or misreporting. Objective measures or medical records could provide more reliable data in the future investigations. The study assesses the impact of vitamin A supplementation over a relatively short period. Long-term follow-up would be valuable to understand the sustained effects of supplementation on children's health. Also, the study focuses on vitamin A but does not address the broader nutritional status of children, which could also influence health outcomes. A more holistic approach to nutritional assessment might provide deeper insights.

While the international discussion around VAS is vital, more attention should be paid to individual nations where programming choices are decided. Many nations use VAS and other initiatives to enhance VA status without having proof of the national prevalence and severity of VAD, according to our research. Moreover, we have to widely discuss the VAS programs in different countries, to evaluate those programs that really could change the statistics of VAD among their nations, and gradually implement them in other countries as well. Also taking into account socioeconomic differences between urban and rural locations, where vitamin A supply through food may be limited in relatively impoverished rural settings. In rural regions, community education on VAD awareness should be implemented so that caregivers can notice symptoms of VAD in their early stages. Localized techniques for cooking micronutrient-rich meals, as well as availability to vitamin A supplementation, are also crucial in lowering the severity of VAD in those situations. However, because frequent use of high-dose vitamin A supplements is not suggested for all children, these complete dietary methods should be targeted and applied with caution. Critical importance of it especially among infants, taking into account the role of vitamin A in maintenance of the immune system, and susceptibility of infants to different infections that is nowadays the cause of mortality and morbidity of children under 5 years, especially in cases of diarrhea or measles, as well as in visual issues that largely impact nighttime vision (i.e. xerophthalmia). Vitamin A deficiency is still one of the top causes of childhood blindness in underdeveloped nations **[42]**.

Efforts to combat VAD should be integrated into existing health programs, such as maternal and child health services, immunization campaigns, and nutrition education initiatives. This holistic approach maximizes the reach of interventions and ensures a comprehensive focus on child health Efforts to combat VAD should be integrated into existing health programs, such as maternal and child health services, immunization campaigns, and nutrition education initiatives. This holistic approach maximizes the reach of interventions and ensures a comprehensive focus on child health **[43]**.

Future research and interventions should aim to build on the current study's findings and contribute to improved child health and nutrition, particularly in regions where vitamin A deficiency is a significant public health concern. Interventions should be tailored to the specific needs of these high-risk areas, considering socio-economic factors, dietary habits, and cultural nuances. This targeted approach ensures that resources are optimally utilized and interventions are culturally sensitive **[44]**.

The study's findings have several important policy implications for public health, particularly in addressing vitamin A deficiency (VAD) among children aged 6-59 months. By implementing policy recommendations on vitamin deficiency prevention, governments and health authorities can work toward reducing the prevalence of VAD among children and improving overall child health and well-being. Addressing this nutritional deficiency is an important step in achieving better health outcomes for children in affected regions.

CONCLUSION

In conclusion, our findings indicate that vitamin A supplementation has been effective in significantly reducing VAD and leading to improved health of children in the Akmola and Kostanay regions of Kazakhstan. These findings provide a scientific basis for implementing programs to prevent VAD in children aged 6-59 months, in line with international recommendations. Improving the diet, even if it is difficult to achieve in the short term, is of paramount importance because it contributes to improving the overall nutritional status. Fortification of foods with vitamin A has proven to be an effective strategy for reducing VAD in some countries.

REFERENCES

1. Lowe, N. M. (2021). The global challenge of hidden hunger: perspectives from the field. In Proceedings of the Nutrition Society (Vol. 80, Issue 3, pp. 283–289). Cambridge University Press (CUP). https://doi.org/10.1017/s0029665121000902
2. Friesen, V. M., Jungjohann, S., Mbuya, M. N. N., Harb, J., Visram, A., Hug, J., Garrett, G. S., & Neufeld, L. M. (2019). Fortification Assessment Coverage Toolkit (FACT) Manual. Global Alliance for Improved Nutrition (Geneva) and Oxford Policy Management (Oxford). Retrieved from https://www.gainhealth.org/sites/default/files/publications/documents/fact-manual.pdf.
3. Hashizume, M., Chiba, M., Shinohara, A., Iwabuchi, S., Sasaki, S., Shimoda, T., Kunii, O., Caypil, W., Dauletbaev, D., & Alnazarova, A. (2005). Anaemia, iron deficiency and vitamin A status among school-aged children in rural Kazakhstan. In Public Health Nutrition (Vol. 8, Issue 6, pp. 564–571). Cambridge University Press (CUP). https://doi.org/10.1079/phn2004717
4. WHO. (2011). Serum retinol concentrations for determining the prevalence of vitamin A deficiency in populations. Retrieved from https://www.who.int/publications/i/item/WHO-NMH-NHD-MNM-11.3.
5. Trumbo, P., Yates, A. A., Schlicker, S., & Poos, M. (2001). Dietary Reference Intakes. In Journal of the American Dietetic Association (Vol. 101, Issue 3, pp. 294–301). Elsevier BV. https://doi.org/10.1016/s0002-8223(01)00078-5
6. Imdad, A., Mayo-Wilson, E., Haykal, M. R., Regan, A., Sidhu, J., Smith, A., & Bhutta, Z. A. (2022). Vitamin A supplementation for preventing morbidity and mortality in children from six months to five years of age. In Cochrane Database of Systematic Reviews (Vol. 2022, Issue 3). Wiley. https://doi.org/10.1002/14651858.cd008524.pub4
7. Stevens, G. A., Bennett, J. E., Hennocq, Q., Lu, Y., De-Regil, L. M., Rogers, L., Danaei, G., Li, G., White, R. A., Flaxman, S. R., Oehrle, S.-P., Finucane, M. M., Guerrero, R., Bhutta, Z. A., Then-Paulino, A., Fawzi, W., Black, R. E., & Ezzati, M. (2015). Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. In The Lancet Global Health (Vol. 3, Issue 9, pp. e528–e536). Elsevier BV. https://doi.org/10.1016/s2214-109x(15)00039-x
8. Fund, U. N. C. s. (2020). Estimates of Vitamin A Supplementation Coverage in Preschool-age Children: Methods and processes for the UNICEF global database. Retrieved from https://data.unicef.org/wp-content/uploads/2020/03/Vitamin-a-methods-paper.pdf.
9. Wirth, J., Petry, N., Tanumihardjo, S., Rogers, L., McLean, E., Greig, A., Garrett, G., Klemm, R., & Rohner, F. (2017). Vitamin A Supplementation Programs and Country-Level Evidence of Vitamin A Deficiency. In Nutrients (Vol. 9, Issue 3, p. 190). MDPI AG. https://doi.org/10.3390/nu9030190
10. Doreen Bartlett, L. C., Palisano, R., McCoy, S. W. (2012). Ed. Wong, C, Bartlett, D. J., & Chiarello, L. A. C. H., & Stoskopf, B. The Health Conditions Questionnaire. In Child Care Health and Development (Vol. 38, Issue 1, pp. 128–138). Canadian Institutes of Health Research and US Department of Education.
11. Craft, N. E., Haitema, T., Brindle, L. K., Yamini, S., Humphrey, J. H., & West, K. P., Jr. (2000). Retinol Analysis in Dried Blood Spots by HPLC. In The Journal of Nutrition (Vol. 130, Issue 4, pp. 882–885). Elsevier BV. https://doi.org/10.1093/jn/130.4.882
12. WHO. (1996). Indicators for assessing Vitamin A deficiency and their application in monitoring and evaluating intervention programmes. In WHO (66 p). Retrieved from https://www.who.int/publications/i/item/WHO-NUT-96.10.
13. de Pee, S., & Dary, O. (2002). Biochemical Indicators of Vitamin A Deficiency: Serum Retinol and Serum Retinol Binding Protein. In The Journal of Nutrition (Vol. 132, Issue 9, pp. 2895S–2901S). Elsevier BV. https://doi.org/10.1093/jn/132.9.2895s
14. Ballew, C., Bowman, B. A., Sowell, A. L., & Gillespie, C. (2001). Serum retinol distributions in residents of the United States: third National Health and Nutrition Examination Survey, 1988–1994. In The American Journal of Clinical Nutrition (Vol. 73, Issue 3, pp. 586–593). Elsevier BV. https://doi.org/10.1093/ajcn/73.3.586
15. Olmedilla, B., Granado, F., Southon, S., Wright, A. J. A., Blanco, I., Gil-Martinez, E., Berg, H. van den, Corridan, B., Roussel, A.-M., Chopra, M., & Thurnham, D. I. (2001). Serum concentrations of carotenoids and vitamins A, E, and C in control subjects from five European countries. In British Journal of Nutrition (Vol. 85, Issue 2, pp. 227–238). Cambridge University Press (CUP). https://doi.org/10.1079/bjn2000248
16. WHO. (1996). Indicators for assessing Vitamin A deficiency and their application in monitoring and evaluating intervention programmes. In WHO (77 p). Retrieved from https://www.who.int/publications/i/item/WHO-NUT-96.10.

17. WHO. Guideline: Vitamin A supplementation in infants 1–5 months of age. (2011). In WHO. Retrieved from https://iris.who.int/bitstream/handle/10665/44628/9789241501811_eng.pdf?sequence=1.
18. Jia, M., Zhen, L., & Xiao, Y. (2022). Changing Food Consumption and Nutrition Intake in Kazakhstan. In Nutrients (Vol. 14, Issue 2, p. 326). MDPI AG. https://doi.org/10.3390/nu14020326
19. Sadighi, J., Nedjat, S., & Rostami, R. (2019). Systematic review and meta-analysis of the effect of iron-fortified flour on iron status of populations worldwide. In Public Health Nutrition (Vol. 22, Issue 18, pp. 3465–3484). Cambridge University Press (CUP). https://doi.org/10.1017/s1368980019002179
20. Baizhumanova, A., Nishimura, A., Ito, K., Sakamoto, J., Karsybekova, N., Tsoi, I., & Hamajima, N. (2010). Effectiveness of communication campaign on iron deficiency anemia in Kyzyl-Orda region, Kazakhstan: a pilot study. In BMC Hematology (Vol. 10, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/1471-2326-10-2
21. Tazhibayev, S., Dolmatova, O., Ganiyeva, G., Khairov, K., Ospanova, F., Oyunchimeg, D., Suleimanova, D., & Scrimshaw, N. (2008). Evaluation of the Potential Effectiveness of Wheat Flour and Salt Fortification Programs in Five Central Asian Countries and Mongolia, 2002–2007. In Food and Nutrition Bulletin (Vol. 29, Issue 4, pp. 255–265). SAGE Publications. https://doi.org/10.1177/156482650802900402
22. Beisbekova, A., Raushanova, A., Juszkiewicz, K., Kainarbayeva, M., Chuyenbekova, A., Khassenova, G., Kozhakhmetova, A., & Kenessary, D. (2019). Medico-social effectiveness of biological monitoring of iodine deficiency status (IDS) among women of reproductive age in Kazakhstan. In Annals of Agricultural and Environmental Medicine (Vol. 26, Issue 1, pp. 73–77). Institute of Rural Health. https://doi.org/10.26444/aaem/90718
23. Adinepour, F., Pouramin, S., Rashidinejad, A., & Jafari, S. M. (2022). Fortification/enrichment of milk and dairy products by encapsulated bioactive ingredients. In Food Research International (Vol. 157, p. 111212). Elsevier BV. https://doi.org/10.1016/j.foodres.2022.111212
24. Kroupina, M. G., Toemen, L., Aidjanov, M. M., Georgieff, M., Hearst, M. O., Himes, J. H., Johnson, D. E., Miller, B. S., Syzdykova, A. M., & Sharmanov, T. S. (2014). Predictors of Developmental Status in Young Children Living in Institutional Care in Kazakhstan. In Maternal and Child Health Journal (Vol. 19, Issue 6, pp. 1408–1416). Springer Science and Business Media LLC. https://doi.org/10.1007/s10995-014-1647-0
25. WHO. (2019). Global prevalence of vitamin A deficiency in populations at risk 1995–2005: WHO global database on vitamin A deficiency.
26. Barua, A. B., & Sidell, N. (2004). Retinoyl β-Glucuronide: A Biologically Active Interesting Retinoid. In The Journal of Nutrition (Vol. 134, Issue 1, pp. 286S-289S). Elsevier BV. https://doi.org/10.1093/jn/134.1.286s
27. Harrison, E. H. (2012). Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. In Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids (Vol. 1821, Issue 1, pp. 70–77). Elsevier BV. https://doi.org/10.1016/j.bbalip.2011.06.002
28. Smith, E. R., Shankar, A. H., Wu, L. S.-F., Aboud, S., Adu-Afarwuah, S., Ali, H., Agustina, R., Arifeen, S., Ashorn, P., Bhutta, Z. A., Christian, P., Devakumar, D., Dewey, K. G., Friis, H., Gomo, E., Gupta, P., Kæstel, P., Kolsteren, P., Lanou, H., … Sudfeld, C. R. (2017). Modifiers of the effect of maternal multiple micronutrient supplementation on stillbirth, birth outcomes, and infant mortality: a meta-analysis of individual patient data from 17 randomised trials in low-income and middle-income countries. In The Lancet Global Health (Vol. 5, Issue 11, pp. e1090–e1100). Elsevier BV. https://doi.org/10.1016/s2214-109x(17)30371-6
29. Amimo, J. O., Michael, H., Chepngeno, J., Raev, S. A., Saif, L. J., & Vlasova, A. N. (2022). Immune Impairment Associated with Vitamin A Deficiency: Insights from Clinical Studies and Animal Model Research. In Nutrients (Vol. 14, Issue 23, p. 5038). MDPI AG. https://doi.org/10.3390/nu14235038
30. Yu, N., Su, X., Wang, Z., Dai, B., & Kang, J. (2015). Association of Dietary Vitamin A and β-Carotene Intake with the Risk of Lung Cancer: A Meta-Analysis of 19 Publications. In Nutrients (Vol. 7, Issue 11, pp. 9309–9324). MDPI AG. https://doi.org/10.3390/nu7115463
31. Vijayaraghavan, K. (2018). National control programme against nutritional blindness due to vitamin A deficiency: Current status & future strategy. In Indian Journal of Medical Research (Vol. 148, Issue 5, p. 496). Medknow. https://doi.org/10.4103/ijmr.ijmr_1781_18
32. Mayo-Wilson, E., Imdad, A., Herzer, K., Yakoob, M. Y., & Bhutta, Z. A. (2011). Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta-analysis. In BMJ (Vol. 343, Issue aug25 1, pp. d5094–d5094). BMJ. https://doi.org/10.1136/bmj.d5094
33. Abdelkader, A., Wahba, A. A., El-tonsy, M., Zewail, A. A., & Shams Eldin, M. (2022). Recurrent respiratory infections and vitamin A levels: a link? It is cross-sectional. In Medicine (Vol. 101, Issue 33, p. e30108). Ovid Technologies (Wolters Kluwer Health). https://doi.org/10.1097/md.0000000000030108

34. Long, K. Z., Rosado, J. L., DuPont, H. L., Hertzmark, E., & Santos, J. I. (2007). Supplementation with vitamin A reduces watery diarrhoea and respiratory infections in Mexican children. In British Journal of Nutrition (Vol. 97, Issue 2, pp. 337–343). Cambridge University Press (CUP). https://doi.org/10.1017/s0007114507257757

35. Prado, E. L., Abbeddou, S., Adu-Afarwuah, S., Arimond, M., Ashorn, P., Ashorn, U., Bendabenda, J., Brown, K. H., Hess, S. Y., Kortekangas, E., Lartey, A., Maleta, K., Oaks, B. M., Ocansey, E., Okronipa, H., Ouédraogo, J. B., Pulakka, A., Somé, J. W., Stewart, C. P., … Dewey, K. G. (2017). Predictors and pathways of language and motor development in four prospective cohorts of young children in Ghana, Malawi, and Burkina Faso. In Journal of Child Psychology and Psychiatry (Vol. 58, Issue 11, pp. 1264–1275). Wiley. https://doi.org/10.1111/jcpp.12751

36. Soares, M. M., Silva, M. A., Garcia, P. P. C., Silva, L. S. da, Costa, G. D. da, Araújo, R. M. A., & Cotta, R. M. M. (2019). Efect of vitamin A suplementation: a systematic review. In Ciência & Saúde Coletiva (Vol. 24, Issue 3, pp. 827–838). FapUNIFESP (SciELO). https://doi.org/10.1590/1413-81232018243.07112017

37. Villamor, E., & Fawzi, W. W. (2000). Vitamin A Supplementation: Implications for Morbidity and Mortality in Children. In The Journal of Infectious Diseases (Vol. 182, Issue s1, pp. S122–S133). Oxford University Press (OUP). https://doi.org/10.1086/315921

38. Ye, Y., Yang, X., Zhao, J., He, J., Xu, X., Li, J., Shi, J., & Mu, D. (2022). Early Vitamin A Supplementation for Prevention of Short-Term Morbidity and Mortality in Very-Low-Birth-Weight Infants: A Systematic Review and Meta-Analysis. In Frontiers in Pediatrics (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fped.2022.788409

39. Wirth, J. P., Woodruff, B. A., Engle-Stone, R., Namaste, S. M., Temple, V. J., Petry, N., Macdonald, B., Suchdev, P. S., Rohner, F., & Aaron, G. J. (2017). Predictors of anemia in women of reproductive age: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. The American journal of clinical nutrition, 106(Suppl 1), 416S–427S. https://doi.org/10.3945/ajcn.116.143073

40. McLean, E., Cogswell, M., Egli, I., Wojdyla, D., & de Benoist, B. (2008). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. In Public Health Nutrition (Vol. 12, Issue 04, p. 444). Cambridge University Press (CUP). https://doi.org/10.1017/s1368980008002401

41. Shah, D., & Sachdev, H. P. S. (2001). Effect of gestational zinc deficiency on pregnancy outcomes: summary of observation studies and zinc supplementation trials. In British Journal of Nutrition (Vol. 85, Issue S2, pp. S101–S108). Cambridge University Press (CUP). https://doi.org/10.1079/bjn2000301

42. Lyellu, H. Y., Hussein, T. H., Wandel, M., Stray-Pedersen, B., Mgongo, M., & Msuya, S. E. (2020). Prevalence and factors associated with early initiation of breastfeeding among women in Moshi municipal, northern Tanzania. In BMC Pregnancy and Childbirth (Vol. 20, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12884-020-02966-0

43. WHO. (2017). Global accelerated action for the health of adolescents (AA-HA!): guidance to support country implementation. Retrieved from https://www.who.int/publications/i/item/9789240081765.

44. Stevens, G. A., Bennett, J. E., Hennocq, Q., Lu, Y., De-Regil, L. M., Rogers, L., Danaei, G., Li, G., White, R. A., Flaxman, S. R., Oehrle, S.-P., Finucane, M. M., Guerrero, R., Bhutta, Z. A., Then-Paulino, A., Fawzi, W., Black, R. E., & Ezzati, M. (2015). Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. In The Lancet Global Health (Vol. 3, Issue 9, pp. e528–e536). Elsevier BV. https://doi.org/10.1016/s2214-109x(15)00039-x

Funds:

This study was carried out under the social order №271 from 13.04. 2012 of the Ministry of Health of the Republic of Kazakhstan with the support of the UNICEF office in Kazakhstan.

Acknowledgments:

We thank the National Center for Healthy Nutrition for their contribution to this data set. We are also grateful the study participants for their willingness involve in the study. The authors wish to thank Dr. Ariel Shensa and Dr. Ahmad Khanijahani for their support.

Conflict of Interest:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Statement:

The study design and questionnaires were preliminarily tested and approved by the Local Ethical Commission of Kazakh Academy of Nutrition (Protocol No. 04, 01.04.2012). The participants provided their written informed consent to participate in this study.

Contact Address:

***Arailym Beisbekova**, Kazakh National Medical University named after S. D. Asfendiyarov, Department of Nutrition, 66 Klochkov st, 050008, Almaty, Republic of Kazakhstan,
Tel.: +77072037823
E-mail: abeisbekova@gmail.com
ORCID: https://orcid.org/0000-0001-6105-7068

Feruza Ospanova, Kazakh Academy of Nutrition, 66 Klochkov st, 050008, Almaty, Republic of Kazakhstan,
Tel.: +77273762313
E-mail: fospan@mail.ru
ORCID: https://orcid.org/0000-0003-2499-4989

Maikul Kainarbayeva, Kazakh National Medical University named after S. D. Asfendiyarov, Department of Nutrition, 66 Klochkov st, 050008, Almaty, Republic of Kazakhstan,
Tel.: +77273762313
E-mail: kainarbayeva.m@mail.ru
ORCID: https://orcid.org/0000-0002-5356-9546

Elena Ushanskaya, Kazakh National Medical University named after S. D. Asfendiyarov, Department of Nutrition, 66 Klochkov st, 050008, Almaty, Republic of Kazakhstan,
Tel.: +77273762313
E-mail: lenaushanski@mail.ru
ORCID: https://orcid.org/0009-0007-6612-5948

Salima Bykybayeva, Kazakh National Medical University named after S. D. Asfendiyarov, Department of Nutrition, 66 Klochkov st, 050008, Almaty, Republic of Kazakhstan,
Tel.: +77273762313
E-mail: salima_7@mail.ru
ORCID: https://orcid.org/0000-0001-9549-3538

Aidar Berdygaliev, Kazakh National Medical University named after S. D. Asfendiyarov, Department of Nutrition, 66 Klochkov st, 050008, Almaty, Republic of Kazakhstan,
Tel.: +77273762313
E-mail: berdaidar@mail.ru
ORCID: https://orcid.org/0000-0002-3163-5135

Aizhan Raushanova, al-Farabi Kazakh National University, Department of Epidemiology, Biostatistics and Evidence-Based Medicine, 71 al-Farabi Ave., 050040, Almaty, Republic of Kazakhstan,
Tel.: +77273762313
E-mail: ram7202699@mail.ru
ORCID: https://orcid.org/0000-0003-4584-885X

Faina Linkov, Duquesne University, Department of Health Administration and Public Health, John G. Rangos Sr. School of Health Sciences, 600 Forbes Avenue, 15282, Pittsburgh, USA,
Tel.: +77273762313
E-mail: linkovf@duq.edu
ORCID: https://orcid.org/0000-0002-3707-0104

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 14.2.2024
Revised: 24.4.2024
Accepted: 30.4.2024
Published: 9.5.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 398-407
https://doi.org/10.5219/1983
ISSN: 1337-0960 online
www.potravinarstvo.com

Effective creation of ESG reporting using data from financial accounting in the food industry company

Petra Súlovská, Anna Látečková

ABSTRACT

Environmental, social, and governance (ESG) reports in the synergy of the accounting system of the company. In building a system for ESG reporting, it is necessary to comply with valid transnational and national levels and at the same time create a system that will be able to fulfil its tasks in the future. This study presents an analysis possibility of using the company's accounting system for the efficient creation of ESG reporting. The paper presents the focused on the investigation of factors that influence the tendency to using the financial data for effective ESG reporting. Next step of the conducted analysis was the estimation of individual model to investigate specific factors and to identify differences between selected companies of food industry in region of western Slovakia. We used Binary logit model for estimated, based on the data coming from questionary survey in selection company. In this study strongly significant variable DocFA with a positive effect on the dependent variable referred to the agreement of respondents to use documentation from financial accounting for effective ESG reporting. For instance, respondents from the selected western region of Slovakia in the field of food industry have an 82% higher chance of using this documentation for effective ESG reporting. Significant variable AccountS is strongly significant (p-value less than 0.01) in selected companies in western region of Slovakia in field of food industry, which has positive impact to use financial data for effective ESG reporting.

Keywords: ESG reporting, financial accounting, accounting system, binary logit model, ESG preferences

INTRODUCTION

Research on the impact of environmental reporting on financial performance tends to lead to inconsistent findings, as concepts such as managerial attitudes, regulation and governance, reporting quality and stakeholder perceptions, audit activities, and government policies are important areas to investigate more in the future **[1]**. While research on voluntary reporting is extensive, mandatory reporting, particularly under IAS/IFRS, is limited, so there are opportunities for further investigation **[2]**. There are some research opportunities in integrated reporting, such as what information stakeholders find relevant, whether this might influence their decisions, and the impact on capital costs and assurance judgments **[3]**.

The obligation to report data related to ESG is transformed at the national level of the Slovak Republic in the Accounting Act and other related regulations.

Act no. 431/2002 Coll. on Accounting as amended, in § 20 Annual report an accounting unit must have its financial statements verified by an auditor following § 19, except for the accounting unit listed in § 17a par. 1 letter b) and a branch of a foreign securities dealer, is required to prepare an annual report. The annual report contains the financial statements for the accounting period for which the annual report is drawn up and the auditor's report on these financial statements, unless a special regulation provides otherwise and in particular information on the development of the accounting unit, the state in which it is located, and the significant risks and uncertainties to which the accounting unit is exposed; the information is provided in the form of a balanced

and comprehensive analysis of the state and development forecast and contains important financial and non-financial indicators, including information on the impact of the accounting unit's activities on the environment and employment, concerning the relevant data presented in the financial statements **[4]**.

Specifically, the obligations for subjects of public interest are set out in paragraphs 9-12 **[4]**:
An entity of public interest, whose average calculated number of employees for the accounting period exceeded 500 employees, also includes in the annual report non-financial information on the development, proceedings, position, and the impact of the activity of the accounting unit on the environmental, social and employment areas, information on compliance with human rights and on the fight against corruption and bribery (hereinafter referred to as the "area of social responsibility, if as of the date on which the financial statements are drawn up and for the immediately preceding financial period, also meets one of the entities of public interest according to paragraph 9 shall state in the annual report in particular.

Description of the main risks of the accounting entity's impact on the area of social responsibility arising from the accounting entity's activities, which could have adverse consequences, and, if appropriate, also a description of its business relationships, products, or services that related to these impact risks, and a description of how the entity manages these risks. significant non-financial information about the activity of the accounting unit by individual activities. Specifically, it is about a reference to information on the amounts reported in the financial statements and an explanation of these amounts in terms of impacts on the area of social responsibility, if appropriate **[4]**.

An entity of public interest may also use the framework of the European Union or another international framework that governs non-financial information when providing information according to paragraphs §9 and §10 if it specifies exactly which framework it followed **[4]**.

From another regulation, Measure of the Ministry of Finance of the Slovak Republic of 16 December 2002 No. 23054/2002-92 on stipulation of details of accounting procedures and framework chart of accounts for entrepreneurs keeping double-entry accounting, the issue concerns the following paragraphs § 19, § 30 a § 35 **[5]**:

- § 19 Principles of Creation and Use Provision—Provisions are related to liabilities following general legislation, executed contracts, or voluntary decisions of the accounting unit relating to third-party liabilities. For example, if based on past conduct of the accounting unit, publication of rules, or notification of recognition of liability of the accounting unit, the third-party expects the accounting unit to meet the liability. Provisions shall be created for costs related to the elimination of environmental pollution.
- § 30 Accounting Procedures Related to Construction Contract—also includes Provision of services directly related to the creation of the asset pursuant, services of the project manager or architect(s), and Reconstruction, liquidation of the asset pursuant, or rehabilitation of the environment after liquidation of the asset pursuant.
- § 35 Accounting for procurement of long-term tangible assets:

On account 042 – Acquisition of long-term tangible assets, acquired long-term tangible assets and their technical valorisation until the time the assets are put into use including acquisition-related costs shall be accounted for, especially the cost of Reimbursement for forced limitation of real estate use and reimbursement for pecuniary injury provided to the real estate owner according to specific regulations and payments for ecologic injury related to construction **[5]**.

The required data result (Figure 1) from the company's activities, from which it is possible to obtain an overview of the impact on the state and development of ESG **[6]**:

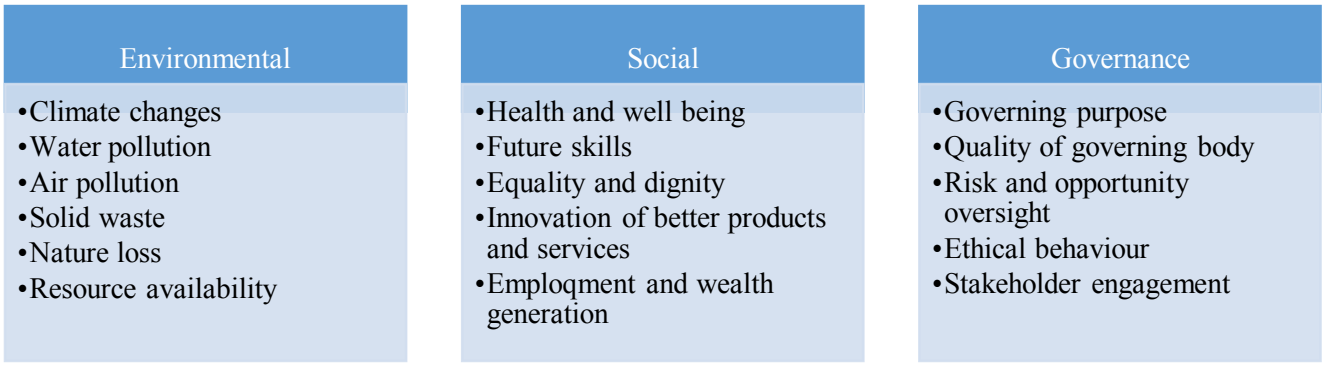


Figure 1 The company's activities necessary for the ESG report.

Scientific Hypothesis

The paper presents focuses on the investigation of factors that influence the tendency to use financial data for effective ESG reporting. According to the results of the model estimated from data collected in 68 selected companies in the region of western Slovakia, the most important factors are individual support for environmental protection awareness, agreement with the statement that companies should also use the financial data from financial accounting for effective ESG reporting, which would have an impact on environmental protection. The next step of the conducted analysis was the estimation of individual models of companies to investigate specific factors and to identify differences between the selected western regions of Slovakia in the field of the food industry.

MATERIAL AND METHODOLOGY

The survey database includes information about 690 managers, financial directors, and accountants who responded to the questionnaire survey. During the analyzed period, 68 companies in the Slovak Republic's food industry were included in the survey.

Samples

Our sample consisted of respondents from selected companies in western Slovakia. The structure of the respondents was:

- Total number of respondents: 690
- Man: 65%
- Woman: 35%
- The age categories:
- 18 – 30: 14%
- 31 – 40: 29%
- 41 – 50: 33%
- 51 – 64: 20%
- 65 and >: 4%

Education:

- Basic (primary school): 0%
- Secondary (high school): 36%
- Higher education (university): 64%

Questionnaire preparation: In creating the questionnaire, we relied on the investigation of factors that influence the tendency to use financial data for effective ESG reporting. Respondents were allowed to answer the questions yes or no. All questions were mandatory. Number of questions analysed: 8

1. Is it possible to use the company's accounting system for the efficient creation of ESG reporting? (yes, no)
2. Do you agree with increasing awareness about ESG to protect the environment? (yes, no)
3. Do you think accounting documents are a sufficient source for ESG reporting? (yes, no)
4. Do you think the accounting system in your company is efficient in ESG reporting? (yes, no)
5. Should the government decrease environmental pollution without decreasing the company´s income? (yes, no)
6. Do you have children? (yes, no)
7. Do you trust your government? (yes, no)
8. Do you trust Environmental Organizations? (yes, no)

Conducting a questionnaire survey: The questionnaire survey was conducted via selected companies in the field of the food industry of the Slovak Republic. The questionnaire was prepared by Google Form and consequently shared with managers in selected companies. Only one person from the research team had access to the collected raw data. We prevented any manipulation of these data.

We complied with the requirements of the GDPR legislation of the European Union. Data collection was anonymous. Each participant could complete the questionnaire only once. The survey took place in the period: 10th of January 2024 to 31st January 2024.

Number of answers: The total number of processed answers was 5 520.

Creating a dataset: We processed the raw data. The database was corrected from discrepancies and outliers The structure of the dataset was adapted to further statistical processing.

Statistical Analysis

For econometrics analyses was used statistical system Gretl. If a binary response variable Y is equal to 1 when the attribute is present and 0 if it is not present in observation. If x=(x1,x2,x3….xk) is a set of explanatory variables which can be discrete, continuous or a combination. The binary dependent variable was preferFA (1 if the

respondent agreed that it is possible to use the company's accounting system for the efficient creation of ESG reporting; otherwise, 0). Other factors described above were considered explanatory variables.

The logistic regression model presents conditional probabilities through the linear function of the predictors expressed as:

$$\ln\left(\frac{P(y_i=1)}{P(y_i=0)}\right) = \beta_0 + x_i^T\beta = I_i \quad (1)$$

Where:

$\beta = (\beta_1, \beta_2, \ldots . \beta_k)^T$ is the estimated vector of k predictor coefficients. The vector of parameters β^ is estimated using the maximum likelihood method. The following likelihood function is maximized:

$$\ln[L(\beta)] = \sum_{i=1}^{n}\left\{y_i \ln\left[\frac{exp(I_i)}{1+exp(I_i)}\right] + (1-y_i)\ln\left[\frac{1}{1+\exp(I_i)}\right]\right\} = \sum_{i=1}^{n}\langle y_i I_i - \ln[1+\exp(I_i]\rangle \quad (2)$$

Then predicted probability can be expressed as follows:

$$F_l(I_i) = P(y_i = 1) = \frac{\exp(I_i)}{1+\exp(I_i)} \quad (3)$$

It is difficult to relate estimated parameter values only to the outcome. A better way to explain the influence of explanatory variables on the outcome is to interpret the odds ratio. It is better than the estimated parameters of logistic regression. The odds ratio is the Euler number raised to the value of the estimated coefficient of logistic regression.

$$Odds\ Ratio_j = e^{B_j} \quad (4)$$

If the odds ratio of the explanatory variable is higher than 1, it means that increasing the value of the explanatory variable will also increase the odds in favour of a positive outcome. On the other hand, if the odds ratio is smaller than 1, increasing the value of an explanatory variable will decrease the chance of a positive outcome.

In logistic regression, it is no longer necessary to hold the assumptions of a classical linear econometric model based on ordinary least squares. Linear relationships between dependent and independent variables, explained variables, and error terms do not need to be normally distributed. Logistic regression also does not need variances to be homoscedastic and can handle nominal or ordinal data as explanatory variables.

RESULTS AND DISCUSSION

The binary logit model was estimated based on data from a questionnaire survey in the selection company´s region. McFadden R-Squared and the number of correct predictions were the basic indicators of model quality. Due to the nature of the dependent variables, was McFadden pseudo-R-square value 0.24 which means excellent model fit (The interpretation of McFadden R-square is different from the classical R-square known from OLS; in this case, lower values are expected due to the nature of the dependent variable).

The model's accuracy measured by correct predictions was 95.1%, which also suggests the model's good prediction ability. The model was evaluated as significant and appropriate to describe the suggested relationship among variables. That means that most of the estimated model parameters are significantly different from zero (p-value 0.0000). Table 1 shows the factors that significantly affect respondents' tendency to financial accounting for the efficient creation of ESG reporting.

From all the explanatory variables in Table 1, the following factors were evaluated as significant the following: InfESG, DocFA, AccountS, and Trust in EU. It means that people who agree with increasing awareness about ESG and also support financial accounting system for the efficient creation of ESG reporting. The strength of their conviction correlates with their tendency to use financial accounting, which is necessary for ESG reporting. This variable was evaluated as the most significant, it means that respondents who agree with ESG would probably use more data from the financial accounting for ESG reporting.

Table 1 Estimated logit model.

Variable	Estimation of logit model			
	p-value	slope at mean	odds ratio	significance
Intercept	<0.0001		1.46	***
InfESG	<0.0001	0.38	1.35	***
DocFA	<0.0001	0.22	0.82	***
AccountS	0.03	0.09	0.88	***
Government	0.31	-0.08	1.64	
Children	0.82	-0.11	1.32	
Age	0.33	0.10	1.34	*
Trust Gov	0.45	0.05	0.58	
Trust EU	0.04	0.02	0.36	**

Significant factor DocFA represents the impact of accounting documents as a sufficient source for ESG reporting. Respondents prefer using documentation from financial accounting, and the favour of using this documentation for efficient ESG reporting will increase by 82%. Another significant factor is AccountS; for efficient ESG reporting, it is necessary to map the data from the accounting system, and the favour of using this system will increase by 88%.

The last significant factor was Trust in the EU. Respondents who trust in the EU will be more likely to use financial data from accounting for ESG reporting. If the level of trust in the EU increases by one odd in favour of using data from financial accounting, it will increase by 36%. All the other indicators included in the model were evaluated as insignificant.

The results interpreted above come from an econometric model estimated using the data from the questionnaire survey. It provides general information about factors that influence the ESG preferences of respondents in the analyzed companies.

The estimation of individual models for each analysed country

Analogous models were estimated for the selected company´s region of western Slovakia, which uses the same explained and explanatory variables as in the case of the pooled model. All individual models were significant with the joint significance of the estimated coefficients was less than 0.05. The significance of individual variables was different. This was mainly influenced by specific socio-economic and cultural conditions in each analysed company. Odds indicators offer better information than estimated coefficients, because of direct connection with the modeled phenomenon. An odds ratio greater than 1 indicates that the variable supports respondents to use financial data for effective ESG reporting, and an odds ratio less than 1 indicates a factor that reduces the chance that respondents use this data for effective ESG reporting, which would lead to protecting the environment.

In all the company´s studied, InfESG was the most significant variable, which is consistent with the general model. The reason of this is the strong correlation of this question with the dependent variable.

Table 2 Estimated odd ratios in individual models for each selected company´s region.

Variable	Estimated odd ratios of each selected company´s region			
	Bratislava	Nitra	Trnava	Trencin
Intercept	0.924***	1.286**	1.743***	1.876**
InfESG	1.668***	0.938***	1.248***	1.562***
DocFA	0.882**	0.741**	0.853**	0.821**
AccountS	0.623***	1.104***	0.721***	1.086***
Government	1.737	1.458	1.893	1.489
Children	1.369	1.239	1.225	1.433
Age	1.535	1.552**	0.438	1.834
Trust Gov	0.685	0.633	0.423	0.566
Trust EU	0.358**	0.365**	0.495***	0.232**

The variable InfESG referred to agreement with increasing awareness about ESG to protect environment, was evaluated as significant in the general model with a positive effect on the dependent variable. In the case of individual models for all selected regions of companies, the parameter of this variable was strongly significant (*p*-value less than 0.01).

The strongly significant variable DocFA with a positive effect on the dependent variable referred to the agreement of respondents to use documentation from financial accounting for effective ESG reporting. Strongly significant (*p*-value less than 0.01) was in the selected western regions of Slovakia, which are Bratislava, Nitra, Trnava, and Trenčín in the field of food industry. For instance, respondents from region Bratislava have an 88% higher chance of using this documentation for effective ESG reporting. Nitra only has a 74% higher chance of using this documentation for effective reporting of ESG.

The significant variable AccountS is strongly significant (p-value less than 0.01) in all of the selected regions in the food industry. Region Trnava has a 72% higher chance of preferring using an accounting system that has effective ESG reporting, which has a positive impact on using financial data for effective ESG reporting.
Age was a factor specific only for region Nitra. According to the estimated model, respondents who were 31-40 years old had 55% higher odds than others to support using the data from financial accounts for effective ESG reporting.

The last factor included in the estimated models is the variable Trust EU. This variable was significant (*p*-value less than 0.05) in all regions without Trnava. In region Trnava was a strongly significant variable Trust EU and respondents who trust the European Union have a 55% higher chance to use financial data from accounting systems for effective ESG reporting. Bratislava region has 36%, Nitra region has 37% and Trenčín has a 23% chance to do effective ESG reporting using data from financial accounting.

Growing interest in social and environmental issues has resulted in an increase in academic publications, which have adopted different perspectives. Most of the research focuses on analyzing social and environmental reports (SER) and searching for the theoretical statements that justify these practices **[7]**.

Concern for taking care of the environment is increasing as is interest in accounting for the environment **[8]**. The response from the business community has been to gather and report more information about environmental activities to stakeholders **[9]**.

In recent years, the company´s sustainability disclosure has attracted the attention of investors, researchers and policymakers **[10]**. Environmental, social and governance (ESG) disclosure levels have increased significantly over the years **[11]**. A more transparent ESG disclosure could provide relevant information for effective portfolio management and sustainable financial policy proposals. Therefore, level of company´s ESG disclosure is expected to be closely related to a country's involvement in sustainable development **[12]**.

Voluntary disclosure theory proposes that sharing private information with investors can improve the efficiency of resource allocation and reduce information asymmetry **[13]**. The existing literature theoretically suggests and empirically finds that analysts can estimate earnings more accurately if they have better quality information through voluntary disclosure **[14]**. Financial analysts are intermediaries between companies and investors who collect, process and analyze financial information. When analysts' forecasts are more accurate, investors can allocate their capital more efficiently, which benefits the capital market. The utility of voluntary disclosure for financial analysts can be assessed by examining the relationships between the choice of reporting framework and the accuracy of financial analysts' forecasts **[15]**. The findings raise questions about how we evaluate environmental accounting interventions and about the role of research in helping environmental movement adherents on the inside of organizations to stay engaged **[16]**. The growing importance of sustainable behavior in the economy has paved the way for increased corporate reporting of information related to ESG companies in the last decade **[17]**. Based on disclosure guidelines from market regulators such as the Securities and Exchange Commission (SEC) and authorities and information demand from market participants, most companies worldwide pay special attention to reporting on life policies and activities. environment, social affairs, and governance areas **[18]**.

For investment management purposes, the framework implemented in this study could serve as a management tool for companies to optimize their communication with investors regarding ESG information reporting **[19]**, thus improving the delivery of the intended meaning of messages to recipients through qualitative information disclosure. In addition, determining the ESG words with the most negative impact on share prices could help companies identify critical aspects of sustainability for investors and adjust their business strategy accordingly **[20]**. Identifying the most important ESG topics could improve the work of regulatory agencies such as the SEC in developing new ESG disclosure regulations for companies, as in the case of the upcoming climate disclosure rules, which cover both material risks and direct and indirect emissions from company activities **[21]**.

Among the financial trends that have emerged after COVID-19, green and sustainable finance is most likely the strongest, following the first ESG-related regulation after years of a complete lack of standardization and laws

for environmental and social disclosures **[22]**. Policymakers identify finance as a key driver of economic transformation **[23]**, while ESG integration is further supported by corporate and retail demand **[24]**. Many global public and corporate entities use automated financial tools and services in their sustainable transition **[25]. T**echnology is key to creating a green and inclusive framework to address ESG market challenges.

Sustainability accounting and related terms are more often used in academic conferences and business practice. This raises the question of the relationship between accounting and sustainability, the role of accounting for sustainability, and what might be understood by sustainability accounting **[26]**.

Social accounting scholars have suggested social and environmental disclosure practices as a mechanism by which accountability obligations can be met because they can inform a wide range of stakeholders about companies' social and environmental impacts **[27]**. However, to be held accountable, these disclosures must demonstrate the company's acceptance of corporate social and environmental responsibility **[28]**. This acceptance can be demonstrated by a clear statement of values with relevant objectives and quantified targets with expected achievement dates against which the company must report its progress **[29]**. "Sustainability accounting" and related terms are more often used in academic conferences and in business practice. This raises the question of the relationship between accounting and sustainability and the role of accounting for sustainability, as well as what might be understood by sustainability accounting. The paper examines the literature on sustainability accounting from an information management perspective and distinguishes between different interpretations of sustainability accounting. Based on existing accounting tools, environmental management accounting (EMA) has emerged as an important approach to transforming firms' environmental strategies into firm performance **[30]**. The information provided by EMA is of two types: financial information related to costs, revenues, etc., and physical resource and impact information. Such information can help control environmental costs, support the realization of environmental returns and profits, and generally improve business performance **[31]**.

CONCLUSION

Companies think about ESG in the context of economic profitability and ecology. It is, therefore, a logical consequence that the return on invested funds is decisive when creating the concept of ESG monitoring and reporting. In building a system for ESG reporting, it is necessary to comply with valid transnational and national levels and at the same time create a system that will be able to fulfil its tasks in the future. We can discuss the need to create an ESG strategy in the food industry. We see the need to create ESG strategies in individual companies. Therefore, the paper focuses on investigating factors that influence the tendency to use financial data for effective ESG reporting. According to the results of the model estimated from data collected in 68 companies in the food industry, the most important factors are individual support for environmental protection awareness, agreement with the statement that companies should also use the financial data from financial accounting for effective ESG reporting, which would have an impact on environmental protection. Based on the analysis of preferences, we consider it necessary to use data from financial accounting for ESG reporting. We found a positive influence in the monitored variables on the usability of data from financial accounting. The next step of the conducted analysis was the estimation of individual models for each analyzed western region of selected companies to investigate specific factors and to identify differences between these western regions. We found trust in the European Union to be a significant variable, which also has the impact of supporting environmental factors. Despite expectations, variable Education, Children and Trust in Government were insignificant in any selected region. The results in most of the monitored regions were similar, it concerns the variables InfESG, DocFA, AccountS, and Trust EU. Only one variable, Age, was significant in the Nitra region, which means that respondents aged 31-40 years old have 55% higher odds than others to support using the data from financial accounts for effective ESG reporting.

REFERENCES

1. Mata, C., Fialho, A., & Eugénio, T. (2018). A decade of environmental accounting reporting: What we know? In Journal of Cleaner Production (Vol. 198, pp. 1198–1209). Elsevier BV. https://doi.org/10.1016/j.jclepro.2018.07.087
2. Ball, A. (2007). Environmental accounting as workplace activism. In Critical Perspectives on Accounting (Vol. 18, Issue 7, pp. 759–778). Elsevier BV. https://doi.org/10.1016/j.cpa.2006.04.005
3. Baldassarre, B., Keskin, D., Diehl, J. C., Bocken, N., & Calabretta, G. (2020). Implementing sustainable design theory in business practice: A call to action. In Journal of Cleaner Production (Vol. 273, p. 123113). Elsevier BV. https://doi.org/10.1016/j.jclepro.2020.123113
4. Statutory Audit and on Amendments and Supplements to Act No. 431/2002 Coll. Retrieved from https://www.mfsr.sk/files/en/taxes-customs-accounting/accounting/laws/A ct_423_20 15_EN.pdf.

5. Measure Of the Ministry of Finance of the Slovak Republic of 16 December 2002 No. 23054/2002-92. Retrieved from https://www.mfsr.sk/files/archiv/priloha-stranky/20204/29/Measure_No_2305 4_as_amended.pdf.
6. Dorauz, G., Bartenschlager-Igel, S., Taschner, M., Batliner, A., Koumbarakis, A., & Ledebur-Wicheln, O. (2018). Sustainability – ESG. PwC. Retrieved from https://www.pwc.ch/en/publications/2018/Sustainability_EN_ web.pdf.
7. Burnett, R. D., & Hansen, D. R. (2008). Ecoefficiency: Defining a role for environmental cost management. In Accounting, Organizations and Society (Vol. 33, Issue 6, pp. 551–581). Elsevier BV. https://doi.org/10.1016/j.aos.2007.06.002
8. Su, S., Baird, K., & Phan, T. (2023). The association between ethical leadership and environmental activity management: The mediating role of employee environmental empowerment. In Advances in Accounting (p. 100682). Elsevier BV. https://doi.org/10.1016/j.adiac.2023.100682
9. Dimitropoulos, P. E. (2024). Sustainability Accounting. In Reference Module in Social Sciences. Elsevier. https://doi.org/10.1016/b978-0-443-13701-3.00008-6
10. Li, Y., Gong, M., Zhang, X.-Y., & Koh, L. (2018). The impact of environmental, social, and governance disclosure on firm value: The role of CEO power. In The British Accounting Review (Vol. 50, Issue 1, pp. 60–75). Elsevier BV. https://doi.org/10.1016/j.bar.2017.09.007
11. Giakoumelou, A., Salvi, A., Bekiros, S., & Onorato, G. (2024). ESG and FinTech funding in the EU. In Research in International Business and Finance (Vol. 69, p. 102233). Elsevier BV. https://doi.org/10.1016/j.ribaf.2024.102233
12. Bueno-García, M., Delgado-Márquez, B., Georgallis, P., & Aragón-Correa, J. A. (2022). How do shareholders influence international firms' environmental strategies? The differential impact of strategic and financial investors. In Long Range Planning (Vol. 55, Issue 6, p. 102183). Elsevier BV. https://doi.org/10.1016/j.lrp.2022.102183
13. Gerged, A. M., Zahoor, N., & Cowton, C. J. (2024). Understanding the relationship between environmental management accounting and firm performance: The role of environmental innovation and stakeholder integration – Evidence from a developing country. In Management Accounting Research (Vol. 62, p. 100865). Elsevier BV. https://doi.org/10.1016/j.mar.2023.100865
14. Pizzi, S., Corbo, L., & Caputo, A. (2021). Fintech and SMEs sustainable business models: Reflections and considerations for a circular economy. In Journal of Cleaner Production (Vol. 281, p. 125217). Elsevier BV. https://doi.org/10.1016/j.jclepro.2020.125217
15. Rossi, P., & Candio, P. (2023). The independent and moderating role of choice of non-financial reporting format on forecast accuracy and ESG disclosure. In Journal of Environmental Management (Vol. 345, p. 118891). Elsevier BV. https://doi.org/10.1016/j.jenvman.2023.118891
16. Johnstone, L. (2020). A systematic analysis of environmental management systems in SMEs: Possible research directions from a management accounting and control stance. In Journal of Cleaner Production (Vol. 244, p. 118802). Elsevier BV. https://doi.org/10.1016/j.jclepro.2019.118802
17. Ignatov, K. (2023). When ESG talks: ESG tone of 10-K reports and its significance to stock markets. In International Review of Financial Analysis (Vol. 89, p. 102745). Elsevier BV. https://doi.org/10.1016/j.irfa.2023.102745
18. Danso, A., Adomako, S., Lartey, T., Amankwah-Amoah, J., & Owusu-Yirenkyi, D. (2020). Stakeholder integration, environmental sustainability orientation and financial performance. In Journal of Business Research (Vol. 119, pp. 652–662). Elsevier BV. https://doi.org/10.1016/j.jbusres.2019.02.038
19. Hoang, T. H. V., Pham, L., & Nguyen, T. T. P. (2023). Does country sustainability improve firm ESG reporting transparency? The moderating role of firm industry and CSR engagement. In Economic Modelling (Vol. 125, p. 106351). Elsevier BV. https://doi.org/10.1016/j.econmod.2023.106351
20. Christ, K. L., & Burritt, R. L. (2013). Environmental management accounting: the significance of contingent variables for adoption. In Journal of Cleaner Production (Vol. 41, pp. 163–173). Elsevier BV. https://doi.org/10.1016/j.jclepro.2012.10.007
21. Jasch, C. (2003). The use of Environmental Management Accounting (EMA) for identifying environmental costs. In Journal of Cleaner Production (Vol. 11, Issue 6, pp. 667–676). Elsevier BV. https://doi.org/10.1016/s0959-6526(02)00107-5
22. de Beer, P., & Friend, F. (2006). Environmental accounting: A management tool for enhancing corporate environmental and economic performance. In Ecological Economics (Vol. 58, Issue 3, pp. 548–560). Elsevier BV. https://doi.org/10.1016/j.ecolecon.2005.07.026

23. Gale, R. (2006). Environmental management accounting as a reflexive modernization strategy in cleaner production. In Journal of Cleaner Production (Vol. 14, Issue 14, pp. 1228–1236). Elsevier BV. https://doi.org/10.1016/j.jclepro.2005.08.008
24. Bernardi, C., & Stark, A. W. (2018). Environmental, social and governance disclosure, integrated reporting, and the accuracy of analyst forecasts. In The British Accounting Review (Vol. 50, Issue 1, pp. 16–31). Elsevier BV. https://doi.org/10.1016/j.bar.2016.10.001
25. Bouten, L., Everaert, P., Van Liedekerke, L., De Moor, L., & Christiaens, J. (2011). Corporate social responsibility reporting: A comprehensive picture? In Accounting Forum (Vol. 35, Issue 3, pp. 187–204). Informa UK Limited. https://doi.org/10.1016/j.accfor.2011.06.007
26. Schaltegger, S., & Burritt, R. L. (2010). Sustainability accounting for companies: Catchphrase or decision support for business leaders? In Journal of World Business (Vol. 45, Issue 4, pp. 375–384). Elsevier BV. https://doi.org/10.1016/j.jwb.2009.08.002
27. Alonso-Paulí, E., & André, F. J. (2015). Standardized environmental management systems as an internal management tool. In Resource and Energy Economics (Vol. 40, pp. 85–106). Elsevier BV. https://doi.org/10.1016/j.reseneeco.2015.02.001
28. Phan, T. N., Baird, K., & Su, S. (2017). The use and effectiveness of environmental management accounting. In Australasian Journal of Environmental Management (Vol. 24, Issue 4, pp. 355–374). Informa UK Limited. https://doi.org/10.1080/14486563.2017.1354235
29. Sari, R. N., Pratadina, A., Anugerah, R., Kamaliah, K., & Sanusi, Z. M. (2020). Effect of environmental management accounting practices on organizational performance: role of process innovation as a mediating variable. In Business Process Management Journal (Vol. 27, Issue 4, pp. 1296–1314). Emerald. https://doi.org/10.1108/bpmj-06-2020-0264
30. Endenich, C., & Trapp, R. (2018). Ethical Implications of Management Accounting and Control: A Systematic Review of the Contributions from the Journal of Business Ethics. In Journal of Business Ethics (Vol. 163, Issue 2, pp. 309–328). Springer Science and Business Media LLC. https://doi.org/10.1007/s10551-018-4034-8
31. Gunarathne, A. D. N., Lee, K., & Hitigala Kaluarachchilage, P. K. (2020). Institutional pressures, environmental management strategy, and organizational performance: The role of environmental management accounting. In Business Strategy and the Environment (Vol. 30, Issue 2, pp. 825–839). Wiley. https://doi.org/10.1002/bse.2656

Funds:

This article received no external funds.

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Petra Súlovská**, Slovak University of Agricultural in Nitra, Faculty of Economics and Management, Institute of Accounting and Informatics, Tr. Andreja Hlinku 2, 949 76 Nitra, Slovakia,
Tel.: 0907 772 555
E-mail: xdurkacovap@is.uniag.sk
ORCID: https://orcid.org/0009-0005-6301-6661

Anna Látečková, Slovak University of Agricultural in Nitra, Faculty of Economics and Management, Institute of Accounting and Informatics, Tr. Andreja Hlinku 2, 949 76 Nitra, Slovakia,
Tel.: +421 37 641 4149
E-mail: anna.lateckova@uniag.sk
ORCID: https://orcid.org/0000-0002-0682-5025

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 18.11.2023
Revised: 16.4.2024
Accepted: 14.5.2024
Published: 20.5.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 408-424
https://doi.org/10.5219/1939
ISSN: 1337-0960 online
www.potravinarstvo.com

Application of a new brine of sprouted grains for delicatessen products from horse meat, beef, and pork

Gulmira Kenenbay, Tamara Tultabayeva, Urishbay Chomanov, Gulzhan Zhumalieyeva, Aruzhan Shoman

ABSTRACT

The main task of the meat processing industry is to produce meat products as the primary source of animal protein that ensures the vital activity of the human body in the necessary volumes, high quality, and a diverse assortment. Providing the population with high-quality food products that are biologically complete, balanced in the composition of the primary nutrients, and enriched with target physiologically active components is one of the most priority scientific and technical problems to be solved. In this regard, a recipe for a new brine from sprouted grains for delicatessen products from horse meat, beef, and pork was developed. The composition of the new brine includes flavoring and aromatic ingredients, juice of sprouted grains, and juices of raw vegetable materials. The viscosity of horse meat, beef, and pork during massaging was studied. Thermodynamic parameters such as water activity and moisture binding energy of horse meat, beef, and pork using a new brine were studied. The data analysis shows that the values of the "aw" indicator and the moisture binding energy in the experimental samples of meat products are higher than in the control samples. Studies have found that with an increase in the activity of water and the moisture binding energy, the tenderness of finished delicatessen meat products with a new brine increases. As a result, it was found that the maximum amount of brine in horse meat is retained at 160 minutes of continuous massaging, in beef – at 130 minutes, in pork – at 120 minutes of mechanical processing.

Keywords: recipe, functional products, delicatessen products, brine, vegetable additives

INTRODUCTION

Currently, the most popular and priority products for consumers are functional products that help improve metabolism and increase the body's immune properties by correcting the protein, fat, and carbohydrate composition. The domestic food industry should respond to the growing concern of the population about maintaining health, which, on the one hand, is associated with an increase in consumer awareness of the relationship between nutrition and health and, on the other hand, with the deterioration of statistics on nutrition-related diseases **[1]**.

In this regard, research on the development of products with directed correction of the composition with the use of biologically active additives that contribute to strengthening all physiological functions of the human body is becoming relevant. The most promising essential product for functional nutrition is meat products, since the meat of slaughtered animals is a source of high-grade protein, has a high biological value, and is similar in terms of amino acid score to the standard recommended by FAO/WHO. This makes it a valuable raw material for the production of functional products **[2]**.

The healing properties of sprouted seeds have been known for a very long time. Even 3000 years B.C., the Chinese used bean sprouts for food. In Rus, our ancestors fed weak, sick children with sprouted grains, and the

children quickly recovered and gained weight. Since the second half of the 20th century, sprouted seeds have been widely used in America and Western Europe. Sprouted seeds of various crops are increasingly being introduced into medical practice due to their amazing properties. Sprouts are not a health supplement artificially enriched with a particular substance. This is a very special, healing food, the only case when a complete living organism is used as food in a state of maximum activity. The healing effect of seedlings on the human body is determined by the changes that occur in the seeds during their germination **[3]**.

Regular consumption of sprouts stimulates metabolism and hematopoiesis, increases immunity, compensates for vitamin and mineral deficiencies, normalizes the acid-base balance, helps to cleanse the body of toxins and effective digestion, increases potency, and slows down the aging process. In addition to the general positive effect on the human body, the seedlings of each crop, having a certain set of useful substances, vitamins, and microelements in their composition, have a specific healing effect **[4]**.

Use in cooking: Cumin, ginger, and coriander are spicy seasonings used to flavor spicy dishes, such as meat, sausage products, and vegetables. They are also part of various spicy curry mixes. Application in medicine: Infusion of coriander, cumin, and ginger improves digestion and helps with flatulence. Aroma and taste: Plants have a very strong, peculiar smell **[5]**.

Delicacies occupy a very special place among all sausage products. No matter how unusual the sausage is, it will always remain a familiar product for everyone in the well-established form of a loaf. Delicacies are quite another matter. There are several time-tested traditional varieties of delicacies - boiled pork, carbonade, smoked and boiled pork, or beef fillet. In French, "delicacy" is translated as "dainty dishes". Indeed, few people can resist the spicy smell of pale pink pork belly. Delicacies are made according to traditional recipes that have survived to this day, with the addition of only natural spices **[3]**.

Using vegetable raw materials in the technology of meat products is one of the most promising directions in creating products with a given chemical composition **[6]**. Combining ingredients of animal and vegetable origin **[7]** in prescription compositions leads to mutual addition and enrichment of the missing biologically active substances **[8]**.

The above was the basis for conducting research at the Kazakh Research Institute of Processing and Food Industry; the subject is the development of meat delicacies balanced in amino acid composition from horse meat, beef, and pork with a new brine from sprouted grains.

Sprouted wheat grain and brine from such grain have enormous practical value for the food industry since they allow us to produce products with highly valuable substances. The benefit of sprouted grains of various crops for the food industry has been repeatedly noted in the specialized literature since any sprouted grain is rich in enzymes necessary for the digestion and assimilation of food, such as easily digestible monosaccharides. At the same time, it provides the human body with active energy in the form of ATP (adenosine triphosphate) since it is most actively synthesized and used during the germination period.

Scientific hypothesis

Theoretically, we hypothesize that as water activity and moisture binding energy increase, the tenderness of finished deli meats using the new brine increases. This paper presents the results of a study of delicacy products from horse meat, beef, and pork prepared from a new brine from sprouted grains, which was our goal.

MATERIAL AND METHODOLOGY

Samples

The study's objects were horse meat, beef, and pork, which were purchased from the Sozak et al. Company.

Chemicals

All reagents were of analytical grade and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

Animals, Plants and Biological Materials

The study used beef, horse meat, and pork for analysis.

Instruments

We used AquaLab 4TE water activity analyzer (Decagon Devices Inc, USA), automatic amino acid analyzer AAA-881 (Mikrotechna, Czech Republic), and TMS-PRO Structure Analyzer (Food Technology Corporation, USA).

Laboratory Methods

All analyses were conducted in an accredited laboratory of the Almaty Technological University and Kazakh Research Institute of Processing and Food Industry LLP. Laboratory studies of raw materials were carried out based on Almaty Technological University JSC (Almaty, Kazakhstan). The general chemical composition of the

original grain and sprouted grain was determined: moisture, protein, carbohydrates, amino acid composition, and others. The pH, moisture, water activity, moisture binding capacity, and organoleptic properties of beef, horse meat, and pork delicacies were also studied.

The grain moisture content was determined according to GOST 13586.5-2015 **[9]**. The essence of the method is to dehydrate a sample of crushed grain in a drying cabinet (installation) with fixed parameters: temperature, drying time, and calculation of humidity as a percentage based on the change in its mass by weighing the sample before and after drying.

The grain protein content was determined according to GOST 10846-91 **[10]**. The essence of the method is the mineralization of organic matter with sulfuric acid in the presence of a catalyst, which forms ammonium sulfate; the destruction of ammonium sulfate with alkali, which releases ammonia; and the distillation of ammonia with water vapor into a solution of sulfuric or boric acid, followed by titration.

The grain carbohydrate content was determined by determining the mass fraction of soluble carbohydrates according to Bertrand according to GOST 26176-2019 **[11]**. The essence of the method lies in the ability of reducing sugars to reduce divalent copper in an alkaline medium into copper oxide (I), which is oxidized with ferric ammonium alum. This is followed by titration of the reduced divalent iron with a solution of potassium permanganate.

The grain amino acid content was determined according to GOST 32195-2013 **[12]** using an automatic amino acid analyzer AAA-881. An amino acid analyzer is a special compact liquid chromatograph designed for the analysis of amino acids on an ion exchanger column with a post-column derivatisation by means of ninhydrin and for the determination of biogenic amines.

The hydrogen ions (pH) concentrations of meat and meat products were determined according to GOST R 51478-99 **[13]**. The electrical potential differences between a glass electrode and a reference electrode placed in a sample of meat or meat products are measured. Calibrate the pH meter using a buffer solution with a known pH value close to the pH value of the analyzed solution at the measurement temperature. Insert the electrodes into the sample and set the temperature regulator of the pH meter to the sample temperature. pH measurements are carried out depending on the design of the pH meter. After the instrument readings have reached a steady value, read the pH value directly from the device scale with an accuracy of ±0.05 pH units. The arithmetic mean of three individual measurements is taken as the final result if the requirements for convergence of results are met.

The mass fraction of moisture in meat and meat products was determined according to GOST 33319-2015 **[14]**. The method is based on drying the analyzed sample with sand to constant weight at a temperature of 103 ±2 °C.

The water activity of meat and meat products was determined according to GOST ISO 21807-2015 **[15]** using an AquaLab 4TE analyzer. The Aqualab 4TE meter can measure water activity in 5 minutes or less (average reading time: 2.5 minutes) with an accuracy of ±0.003 aw. Internal temperature control allows you to set the temperature measurement from 15 to 50 °C and use the device anywhere – even outside the laboratory. Water activity can be used to predict the growth of microorganisms and determine the microbiological stability of food products and is also an important, quantitative criterion for assessing the length of time for which food products can be stored.

To determine the moisture-binding capacity of meat and meat products, the pressing method developed by the German researcher Hoffman is of particular interest, in which the moisture-binding capacity is judged by the volume of product moisture absorbed when the gypsum working fluid comes into contact with the object. However, the most widely used methods for practice are pressing on filter paper and centrifugation methods. The method for determining the moisture binding capacity of raw meat (according to Grau-Hamm as modified by Volovinskaya-Kelman) is based on the release of moisture from the test sample when it is lightly pressed, the sorption of the released water by filter paper and the determination of the amount of released moisture by the size of the spot area left by it on the filter paper. paper. The reliability of the results can be ensured by repeating the determinations three times or more **[16]**.

Organoleptic characteristics of meat and meat products were determined according to GOST 9959-2015 **[17]**. The following were determined: appearance, color, taste, smell (aroma), consistency and others through the senses. Organoleptic evaluation was carried out by specialist tasters with experience in assessing the quality of meat products. The tasting commission was created based on the selection of tasters, taking into account their sensitivity and ability to establish specific differences in color, taste, smell (aroma) and consistency of samples of meat products by GOST ISO 8586 (the competence of the tasters is confirmed by relevant documents).

All natural food products contain water in their composition in various quantities and states; their technological properties and shelf life largely depend on this. The “water activity” indicator is an important tool in developing technological processes and producing public catering products, as well as ensuring high quality and increasing the shelf life of food products. Considering the importance and greater information availability of the water

activity indicator aw in the countries of the United Europe, its determination, along with the indicators "humidity" and "concentration of hydrogen ions" (pH), is mandatory when examining a number of products. In other foreign countries, the determination is included in the control algorithm for food quality **[18]**. Moisture activity was examined by AquaLab (USA), and the equipment was developed by academics Rogov and Chomanov **[19]** method of determining the moisture activity at the Rogov-Chomanov equipment is as follows: in sample No. 2 placed examined product, sample No. 6 is filled with distilled moisture. After fixing the samples at the equipment with opened valves No. 4, Air is pumped by vacuum pump No. 8. The duration of pumping is 5 minutes. Then, valves are closed, and after 6-8 min, fluid movement stops in the manometer. The manometer indication ΔP is the difference between the equilibrium vapor pressure of moisture over distilled moisture P_0 and the equilibrium pressure of moisture vapor over the product P_{pr}, i.e.,

$$\Delta P = P_0 - P_{pr} \quad (1)$$

The equation calculates the moisture activity value:

$$a_w = \frac{P_0 - \Delta P}{P_0} \quad (2)$$

Some of meat's most important sensory qualities are appearance, juiciness, taste, texture, and consistency. Textural characteristics are assessed by various indicators, which vary depending on the purpose of the study (for example, hardness, strength, elasticity, viscousness, juiciness, and stickiness **[20]**, **[21]**.

Cooked meat texture is determined by connective tissue, myofibrillar proteins, and related components, the contributions of which vary according to concentration, quaternary structure, and strength of intermolecular bonds. The texture of meat is one of the important culinary properties that determines its tenderness, juiciness, and pleasantness when eating. It can be different: from soft and tender to dense and elastic. Proper meat processing before cooking, such as marinating, braising, or frying, is essential for optimal texture. When instrumentally assessing the texture of meat, texturemeters are often used – devices that analyze the resistance of tissues both during shear and compression of the sample **[22]**, **[23]**.

Texture analysis on the analyzer TMS-PRO (Figure 1) is carried out as follows: The texture analyser moves in either an up or down direction to squash or stretch a food sample. The traveling beam is fitted with a load cell measuring the food's response as a force. The load cell acts like an upside-down laboratory balance in a compression test. It is "triggered" when the sample surface is detected, giving a constant start point to the texture test. The texture analyser then travels to either a "target" distance or load, recording the force response of the sample to the deformation imposed. Time is the common variable in all texture tests, e.g. the faster we travel the less time a sample has to respond.

The traveling beam then returns, and the sample adjusts to the conditions created. Probes and fixtures manipulate forces, recreating conditions exposed to the food during handling or consumption.

The energy put into the sample has to be absorbed, stored, or returned. We measure this response in graphical form and can assign numbers to sensory characteristics. Instrumental results are correlated to sensory panels, and sensory characteristics such as hardness, cohesiveness, stickiness, etc., can be calculated **[24]**.

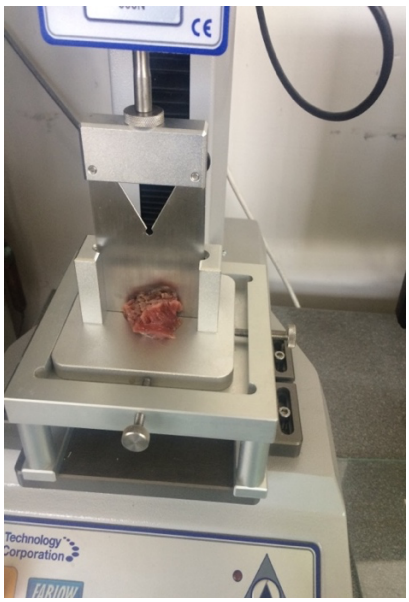


Figure 1 TMS-PRO Structure Analyzer.

The samples were thawed in air at 15 °C to a core temperature of 0-4 °C and measured at 10-12 °C. The meat was deboned and cut into cubes measuring 60 × 60 × 30 mm in width, length, and height, respectively.

The meat texture was examined as follows: A TMS-PRO Structure Analyzer with a force sensor of 1000 N and a movement speed of 500 mm/min was used for the study. A meat sample of about 300 g is placed on a cutting table between the cutting knife and the force plate to carry out measurements. The texture analyzer cross moves downward, compressing the meat sample. The moving beam is equipped with a strain gauge to measure the reaction of the product force to deformation. The load element acts like an inverted laboratory balance in a compression test. Data collection can be "triggered" and begin when a sample surface is detected, providing a constant starting point for repeated testing. The texture analyzer crossbar then moves either a programmed distance or a programmed load, recording the force response of the meat sample to the applied deformation. The traverse is then returned, and the meat sample reacts to the conditions created. The forces are manipulated using sensors and devices that recreate the conditions to which food is exposed during chewing or processing. The energy put into the sample is absorbed, stored, or returned. Next, the results were obtained in graphical form. Instrumental results almost always correlate with sensory panel scores, and sensory characteristics such as hardness, cohesiveness, and tack can be calculated.

Description of the Experiment

Sample preparation: For the experiment, boneless muscles were used and removed from beef, horse meat, and pork carcasses two days after slaughter. Each muscle was cut into six samples weighing about 300 g. To prepare the delicacy, a new brine was used (1 litre each), which contained the following ingredients: Geleon 209 M, sodium chloride (table salt), sodium nitrate, cumin, black pepper, ginger, cinnamon, onion juice, carrot juice, sprouted wheat juice, garlic juice and kiwi juice. A control sample of brine, also 1 litre, was prepared from the following ingredients: Geleon 209M, sodium chloride (table salt), and sodium nitrite. To prepare 1 kg of delicacy meat, 400 ml of brine was needed. The prepared brine was cooled to a temperature of 0 to 4 °C, and then the prepared samples of beef, horse meat, and pork were weighed and filled with the appropriate brines.

Number of samples analyzed: We analyzed three samples.

Number of repeated analyses: All measurements of instrument readings were performed two times.

Number of experiment replications: The number of repetitions of each experiment to determine one value was two times.

Design of the experiment: To experiment in the laboratory, brine for beef, horse, and pork was obtained. To get 1 kg of meat delicacy, 400 ml of brine is needed. The composition of the control brine includes galleon 209M, salt, and sodium nitrite. The experimental brine added flavoring and spicy aromatic ingredients: cumin, black pepper, ginger, coriander, onion juice, carrots, sprouted grains, garlic, and kiwi. The prepared brine was cooled to a temperature of 0 to 4 °C. The raw meat prepared for salting was weighed and then injected with brine, giving the product the necessary organoleptic characteristics **[25]**, **[26]**.

Based on the results obtained, a recipe for a new brine was developed from flavoring aromatic ingredients, sprouted grains juice, and raw vegetable juices (Table 1).

Table 1 Brine for delicatessen meat products (percentage of substances in ml).

Name of the raw material	Control brine	Experienced brine
Geleon 209 M	20	20
Salt	20	20
Sodium nitrite	0.2	0.2
Cumin	-	1.5
Black pepper	2	2
Ginger	-	0.5
Cinnamon	-	1
Onion juice	-	50
Carrot juice	-	50
Sprouted grains juice	-	50
Garlic juice	5	10
Kiwi juice	-	2

As a result of the research, a recipe for meat delicatessen products with the addition of brine of vegetable raw materials was developed (Tables: 2, 3 and 4).

Table 2 Recipe of delicatessen products from beef with the addition of brine of vegetable raw materials.

Raw materials, in kg per 100 kg	Meat delicatessen products
Beef of the category I	75.00
Brine with vegetable raw materials	25.00
Total	100.00

Table 3 Recipe of meat delicatessen products from horse meat with the addition of brine of vegetable raw materials.

Raw materials, in kg per 100 kg	Meat delicatessen products
Horse meat of category I	70.00
Brine with vegetable raw materials	30.00
Total	100.00

Table 4 Recipe of meat delicatessen products from pork with the addition of brine of vegetable raw materials.

Raw materials, in kg per 100 kg	Meat delicatessen products
Pork of category I	70.00
Brine with vegetable raw materials	30.00
Total	100.00

The appearance of deli meats is shown in Figure 2, 3 and 4.

Figure 2 Beef delicacy.

Figure 3 Pork delicacy.

Figure 4 Horse meat delicacy.

Statistical Analysis

The experiments were carried out in triplicate. Standard deviation values are given for all measurements. Differences in the measurements of the experimental and control groups were calculated using analysis of variance (one-way ANOVA) using Tukey's test. The measurement value p <0.05 was considered significant. The STATISTICA Microsoft Excel editor processed experimental data using mathematical statistics methods. Statistical processing was performed in Microsoft Excel 2010 in combination with XLSTAT.

RESULTS AND DISCUSSION

The nutritional value of brines based on sprouted grains and the valuable properties of such a brine make it urgent to create new delicatessen products from horse meat, beef, and pork with the addition of such a brine **[27]**. During the germination of grain **[28]**, the content of individual biologically active substances **[29]** increases significantly since many of them are necessary for the development and formation of a new plant, **[30]**. Let's consider some features of sprouted wheat grain, the solution from which is planned to be introduced into meat products. The characteristics of wheat grain during germination are shown in Figure 5.

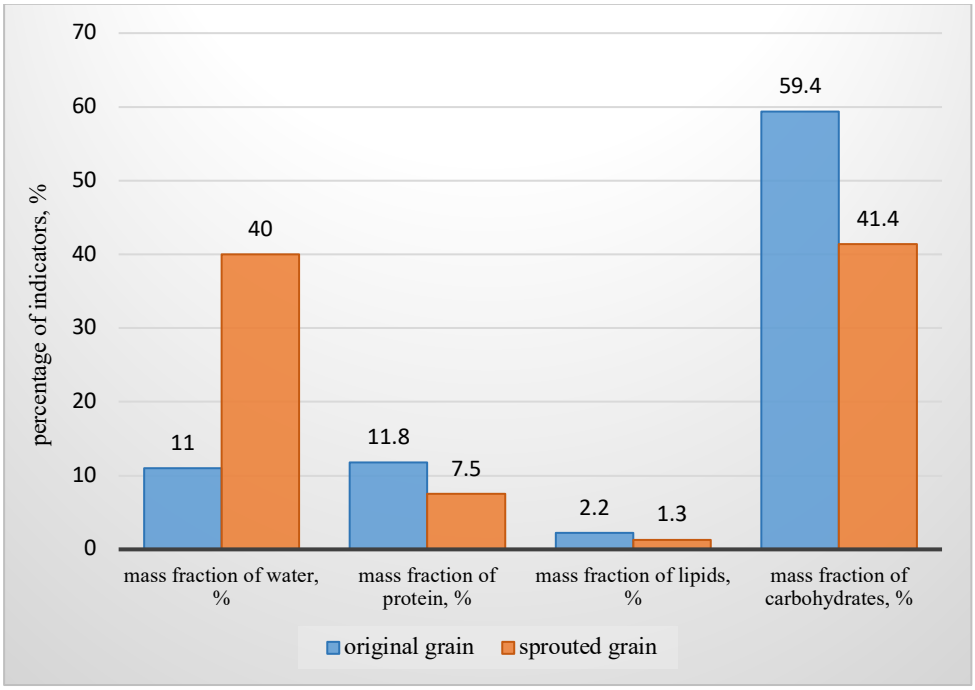


Figure 5 Comparative indicators of the original and sprouted grain (%).

As can be seen from the data presented in Figure 1, the sprouted grain has a more significant mass fraction of water **[31]** than the original non-sprouted grain but slightly lower indicators **[32]** in terms of the mass fraction of protein, carbohydrates, and lipids compared to the original grain **[33]**. This property of sprouted grain is because

these substances are spent on the processes associated with the formation and development of the plant embryo **[34]**. At the same time, there is a large amount of various vitamins in sprouted grains, often exceeding the amount of vitamins in non-sprouted grains (Figure 6).

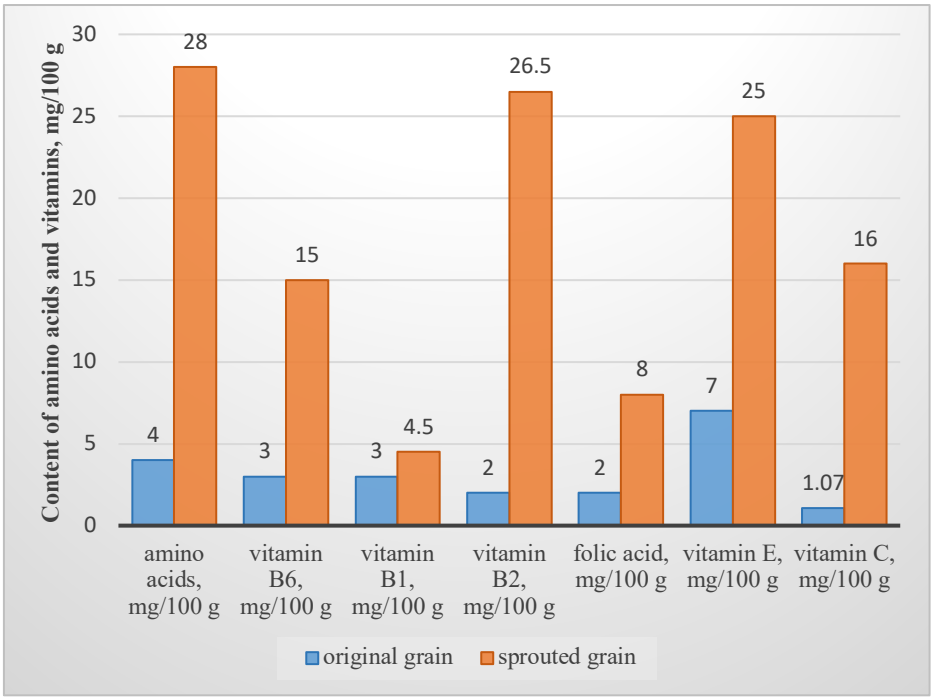


Figure 6 Comparative indicators on the content of amino acids and vitamins of the original grain and sprouted grain (mg/100 g).

The data presented in Figure 2 show that sprouted grain contains much more valuable acids and vitamins than ordinary wheat grain.

A number of research studies are devoted to the study of the benefits of using sprouted grain in the food industry **[35]**. Thus, several researchers prove that the use of sprouted grains gives products a specific taste range **[36]**, while the share of valuable substances in the finished product increases by a certain percentage (Figure 7) **[37]**.

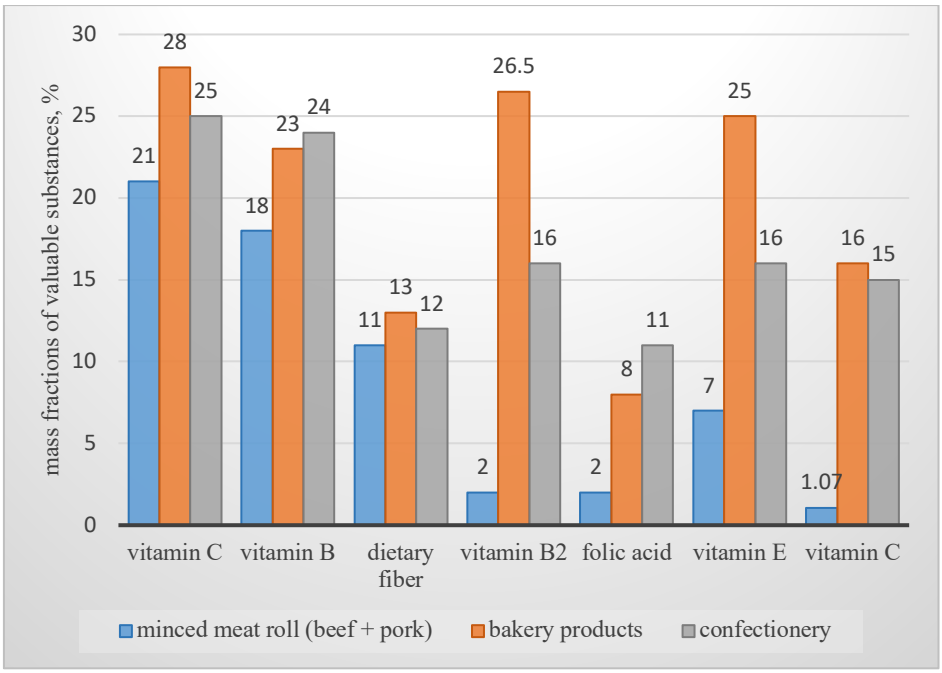


Figure 7 Comparative indicators for increasing the share of valuable substances in ready-made food products with the addition of sprouted grains (%).

The above data allow us to conclude the benefits and practical significance of sprouted grain in the food industry.

During the salting process, the meat matures under the action of tissue enzymes and enzymes of microorganisms **[38]**, which gives it the necessary functional and technological properties - plasticity, stickiness, and high moisture-binding ability **[39]**. When salted, the muscle tissue swells, increasing in volume, its moisture-binding ability increases, the concentration of hydrogen ions changes in the acidic direction **[40]**, and the meat acquires several new properties, including organoleptic ones **[41]**. Subsequently, the salting ingredients provide the desired color and aroma of the product and have an antioxidant and preservative effect **[42]**. So, products from pork, beef, and horse meat, after salting using a new brine based on sprouted grains **[43]**, have a moderately salty taste, a specific (ham) aroma, a stable pinkish-red color, become more tender, tasty and are better absorbed by the body **[44]**.

To calculate the optimal required amount of introduced brine, a study of the technological characteristics of meat delicacies with a level of syringing of 10%, 20%, and 30% of brines from vegetable raw materials was conducted.

The study's objects were experimental industrial samples of delicatessen products with different levels of syringing. The control for determining the relative biological value was meat delicacies prepared from the appropriate meat products with the addition of the following components (according to Table 1): Geleon 209 M, salt, sodium nitrite, black pepper, and garlic juice.

The results of studies of meat delicacies containing 10%, 20%, and 30% brine are shown in Table 2-4. The physical and chemical parameters of meat delicacies were studied (Tables: 5, 6 and 7).

Table 5 Study of physical and chemical indicators of delicacies from beef meat.

The amount of brine introduced, %	Physical and chemical indicators	
	pH	moisture, %
10	6.1 ±0.13 [a]	46.3 ±0.43 [a]
20	6.14 ±0.09 [a]	47.8 ±0.32 [b]
30	6.21 ±0.14 [a]	50.7 ±0.48 [b]
Control	6.30 ±0.13 [b]	50.3 ±0.37 [b]

Note: Indicated values: ± – standard deviation calculated from three parallel measurements. [a-b] values with different letters inside the graph mean a significant difference between different batches (p <0.05).

Table 6 Study of physical and chemical indicators of delicacies from horse meat.

The amount of brine introduced, %	Physical and chemical indicators	
	pH	moisture, %
10	6.0 ±0.16 [a]	45.9 ±0.24 [a]
20	6.12 ±0.19 [b]	48.5 ±0.29 [b]
30	6.23 ±0.07 [c]	50.5 ±0.36 [b]
Control	6.33 ±0.13 [d]	50.8 ±0.44 [b]

Note: Indicated values: ± – standard deviation calculated from three parallel measurements. [a-d] values with different letters inside the graph mean a significant difference between different batches (p <0.05).

Table 7 Study of physical and chemical indicators of delicacies from pork meat

The amount of brine introduced, %	Physical and chemical indicators	
	pH	moisture, %
10	6.13 ±0.06 [a]	45.8 ±0.73 [a]
20	6.16 ±0.19 [a]	47.7 ±0.58 [b]
30	6.24 ±0.10 [b]	50.6 ±0.41 [c]
Control	6.37 ±0.18 [c]	50.7 ±0.62 [c]

Note: Indicated values: ± – standard deviation calculated from three parallel measurements. [a-c] values with different letters inside the graph mean a significant difference between different batches (p <0.05).

From the data in tables 5, 6 and 7, it is clear that the pH and moisture content of delicacies made from beef, horse meat, and pork are closer to the content of control samples. This proves that brine from sprouted grains can be expediently used when introduced in an amount of 30% by weight of the products since the use of 30% brine

allows you to maintain not only the cost-effectiveness of using the main raw materials but also the technological indicators of the raw materials at a fairly high level. Next, deli meats with 30% brine were studied.

Now, we focus on why the method of syringing was chosen out of all the methods available in the food industry for salting meat. According to the classical theory of cooking meat delicacies, the salting method is chosen based on what kind of finished product you need **[45]**. So, for example, for raw-dried delicatessen products, the dry method will be the optimal acceptable salting method. For such delicacies as carbonate, brisket, balyk, loin, and other similar products, it is necessary to choose a wet or salting method associated with syringing **[46]**.

In essence, syringing is also a wet method of salting since a solution of substances is used, but the method of bringing the solution to the meat product is different. If during the wet method of salting, the product is dipped in brine, then when syringing into lump meat, namely deep into the muscle tissue, a specially prepared brine is injected with a part of the production raw materials, salt and spices, after which it is subjected to heat treatment. This method of making meat delicacies improves the structure, consistency, and nutritional value of delicatessen products **[47]**. At the same time, as noted in the specialized literature in the food industry **[48]**, with this method of salting meat products, the consumption of brine, compared with the wet method of salting, is reduced by 70%, and in some cases, it can reach up to 80-90%.

Special importance is given to the viscosity of minced meat in manufacturing meat delicacies and studying their nutritional properties. The viscosity of minced meat is one of the most important indicators that characterize the quality and determine the readiness of minced meat **[49]**. Continuous viscosity monitoring allows for obtaining constant information about the course of the massaging process **[50]**, adjusting the amount of water introduced depending on the thermodynamic parameters of the raw material, and automating the technological process **[51]**.

Mechanical processing- massaging of salted raw materials – is chosen to speed up the salting process. Massaging was performed on an installation developed in the laboratory at 15-20 rpm at a temperature of 0-4 °C and 90% vacuum **[52]**. That is, from a technological point of view, a mechanical method-massaging was added to the chemical method of improving the properties of meat (syringing with a solution) **[53]**.

The viscosity of horse meat, beef, and pork was studied during massaging on the TMS-PRO structure analyzer (USA) (Figure 4). During the study, 6 variants of meat delicacies were obtained from horse, pork, and beef with the addition of experimental and control brines. 1 experience – horse meat with experimental brine, 2 experience – horse meat with control brine, 3 experience – beef meat with experimental brine, 4 experience – beef meat with control brine, 5 experience – pork meat with experimental brine, and 6 experience – pork meat with control brine (Figure 8).

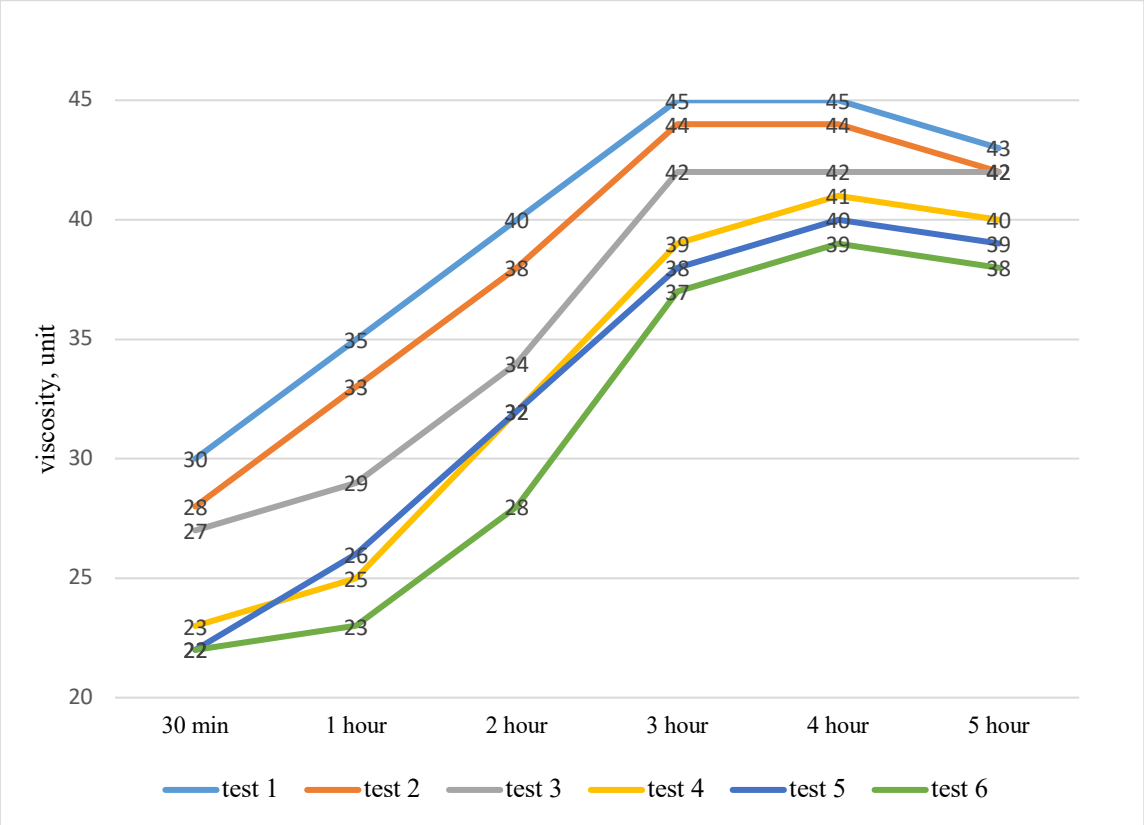


Figure 8 The dependence of the viscosity of meat on the duration of grinding.

Research found that with the duration of massaging 2-3 hours, the viscosity of meat products was 40.0-45.0 units, and further processing leads to a decrease in viscosity.

Tables 8, 9 and 10 show the control organoleptic indicators of meat products (by aroma, consistency, and taste) after the solution was introduced.

Table 8 Control organoleptic indicators of ready-made beef meat delicacies.

Name of the indicator	Name of the raw material for the product	
	Control sample	**With the addition of a solution**
Appearance	The surface is clean, dry, without shades	
Surface color	Dark red, light pink on the cut	Dark red, light pink on the cut with a slight shade of yellow
Smell	Pronounced meat, with a smoked aroma, with a hint of spices	Pronounced meat, with a smoked aroma, with a hint of spices and a fresh smell
Consistency	Elastic, without foreign inclusions	
Taste	Pleasant meat, slightly spicy, moderately salty, without an extraneous taste characteristic of this type of product	Pleasant meat, slightly spicy, moderately salty with a characteristic pleasant organic taste

The organoleptic characteristics of beef delicacies showed that the appearance of the control and test samples was clean, dry, and without shades. The surface color of the control sample is dark red, and light pink on the cut surface, the surface color of the test sample is dark red, and light pink on the cut surface with a slight tint of yellow. The smell of both samples: pronounced meaty, with a smoked aroma, with notes of spices; the sample under study has a fresh smell. The consistency of both samples is the same: elastic, without foreign inclusions. The sample's taste differs from a characteristic pleasant organic taste.

Table 9 Control organoleptic indicators of ready-made pork meat delicacies.

Name of the indicator	Name of the raw material for the product	
	Control sample	**With the addition of a solution**
Appearance	The surface is clean, dry, without shades	
Surface color	Pink, light pink on the cut	Pink, light pink on the cut with a slight shade of yellowness
Smell	Pronounced meat, with a smoked aroma, with a hint of spices	Pronounced meat, with a smoked aroma, with a hint of spices and a fresh smell
Consistency	Elastic, without foreign inclusions	
Taste	Pleasant meat, slightly spicy, moderately salty, without an extraneous taste characteristic of this type of product	Pleasant meat, slightly spicy, moderately salty with a characteristic pleasant organic taste

Organoleptic characteristics of pork delicacies showed that the appearance of the control and test samples was clean, dry, and without shades. The surface color of the test sample differs from the control sample with a slight tint of yellowness. The smell of both samples: pronounced meaty, with a smoked aroma, with notes of spices; the sample under study has a fresh smell. The consistency of both samples is the same: elastic, without foreign inclusions. The taste of the test sample differs from the control sample with a characteristic pleasant organic taste.

Organoleptic indicators of horse meat delicacies showed that the appearance of both samples was clean, dry, and without shades. The surface color of the control sample is dark brown, with dark red on the cut; the surface color of the test sample is dark brown, with dark red on the cut with a slight tint of yellow. The smell of both samples: pronounced meaty, with a smoked aroma, with notes of spices; the sample under study has a fresh smell. The consistency of both samples is the same: elastic, without foreign inclusions. The control sample has a pleasant taste, slightly spicy, moderately salty, without any foreign aftertaste characteristic of this type of product; the test sample has a pleasant taste, slightly spicy, moderately salty, with a pleasant organic taste.

Table 10 Control organoleptic indicators of ready-made meat delicacies from horse meat.

Name of the indicator	Name of the raw material for the product	
	Control sample	With the addition of a solution
Appearance	The surface is clean, dry, without shades	
Surface color	Dark brown, dark red on the cut	Dark brown, dark red on the cut with a slight shade of yellow
Smell	Pronounced meat, with a smoked aroma, with a hint of spices	Pronounced meat, with a smoked aroma, with a hint of spices and a fresh smell
Consistency	Elastic, without foreign inclusions	
Taste	Pleasant meat, slightly spicy, moderately salty, without an extraneous taste characteristic of this type of product	Pleasant meat, slightly spicy, moderately salty with a characteristic pleasant organic taste

The organoleptic indicators of the finished product using brine based on sprouted grain practically do not differ from similar indicators of finished meat products in which brine was not used.

Also, in the study to clarify the nature of the production process and the feasibility of using ready-made brine a number of thermodynamic indicators was analyzed: water activity, the moisture binding energy of horse meat, beef and pork for production of meat delicatessen products on the Testo 650 (Germany) and on the installation developed by academicians Rogov and Chomanov (Figure 9 and 10).

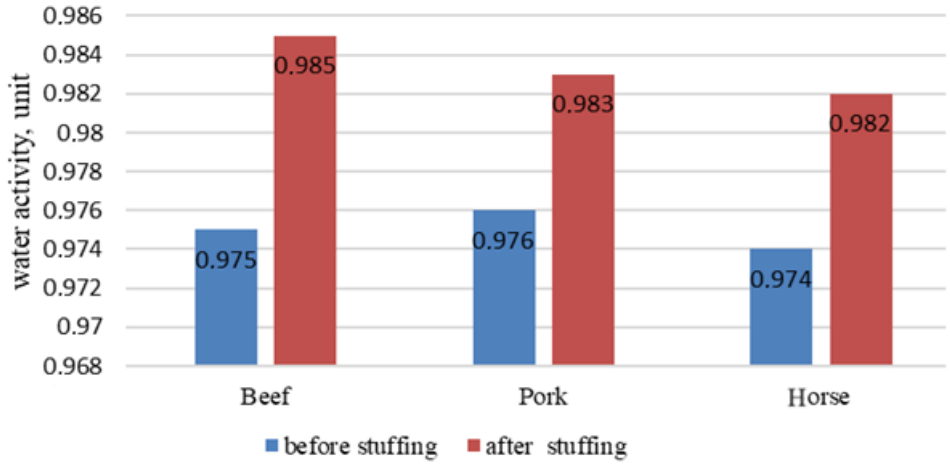


Figure 9 Water activity of horse meat, beef and pork.

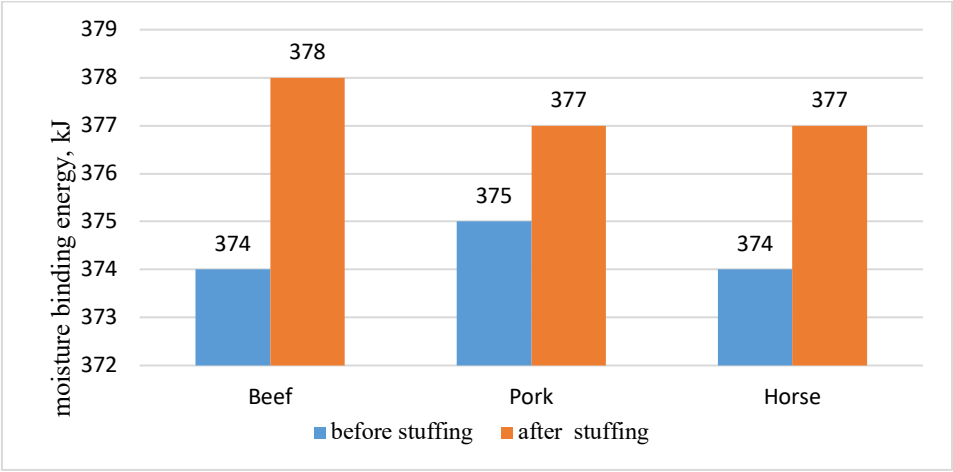


Figure 10 Moisture binding energy of horse meat, beef and pork.

The need for water control in production processes is due to the fact that controlling the water activity in beef, horse meat, and pork allows for maintaining the optimal structure, texture, stability of the products, density, and hydration properties. Studies have found that after syringing, the activity of water and energy value in various meat products increase (Table 11).

Table 11 Dynamics of the increase in energy value indicators in meat products after syringing with a special brine based on sprouted grains.

Name of the indicator	Name of the meat product		
	beef	pork	horse meat
Water activity (unit fraction)	0.975 to 0.985	0.976 to 0.983	0.973 to 0.981
Energy value (kJ)	374 -378	375-377	374-377

The increase in meat tenderness and water-binding ability is due to the fact that when brine is introduced into meat by syringing, muscle fibers break.

The use of brine from sprouted grains will not affect the taste preferences of delicacies consumers but will make their diet more useful in terms of obtaining additional nutrients. It is also assumed that the new products will interest adherents of a healthy diet.

During germination, the calorie content of the grain decreases, and the nutrients become easier for humans to absorb. In sprouted grains, starch is modified into dextrins and maltose, protein into easily digestible amino acids, and vitamins C, B6, B2, B6, E, and carotene are formed. Mineral substances and dietary fiber (fiber, hemicellulose, pectin, lignin) are preserved, concentrated mainly in the fruit and seed shells of the grain, which practically do not undergo qualitative changes during germination **[54]**. Sprouted grains are high in protein, vitamins and minerals, making them valuable to the product's nutritional value. The use of sprouted grains for delicacy meat products allows you to increase the economic performance of production by reducing the cost of raw materials and increasing the profitability of production: make the most efficient use of raw meat, reduce weight loss of finished products after technological processing, increase production volume and expand the range of high-quality food products. Therefore, using brine from sprouted grains reduces the cost of production and improves the organoleptic and physicochemical characteristics of deli meats.

CONCLUSION

The viscosity of horse meat, beef, and pork during massaging was determined, and the following thermodynamic parameters were also studied: water activity and moisture binding energy of horse meat, beef, and pork using a new brine. Studies have found that the tenderness of finished products increases with increased water activity and the binding energy of moisture with the substance. After massaging, the indicators of thermodynamic characteristics increased by 8-10% compared to the control. The data obtained shows that the maximum amount of brine in horse meat is kept at 160 minutes of continuous massaging, beef at 130 minutes, and pork at 120 minutes of mechanical processing. The results showed that the “aw” index and moisture binding energy in the experimental samples of meat products were higher than in the control samples. As a result, it was found that with increased water activity and moisture binding energy, the tenderness of finished gourmet meat products using the new brine increases. The developed recipe and technology for the production of May delicacies from horse meat, pork, and beef using brine from sprouted wheat grains is new and relevant for the social nutrition of various consumer groups, as it allows the production of food products with specified functional properties for the healthy nutrition of the population.

REFERENCES

1. Zhukova, S. B., & Stepanenko, T. S. (2010). Review of development of promising directions for creating functional products for herodietic nutrition of people suffering from diseases of the musculoskeletal system. In Processes and Apparatuses of Food Production (Vol. 1, p. 23). ITMO University.
2. Potipayeva, N. N., Gurinovich, G. V., & Patrakova, I. S. (2008). Food additives and protein preparations for the meat industry. In Technological Institute of Food Industry (168 p.). Kemerovo Technological Institute of Food Industry.
3. Nunes, J. L., Piquerez, M., Pujadas, L., Armstrong, E., Fernández, A., & Lecumberry, F. (2015). Beef quality parameters estimation using ultrasound and color images. In BMC Bioinformatics (Vol. 16, Issue S4). Springer Science and Business Media LLC. https://doi.org/10.1186/1471-2105-16-s4-s6
4. Bazarnova, Y. G. (2010). Improving the nutritional value of meat products. In Meat Industry (Vol. 3, p. 17). Meat Industry’ Journal, LLC.

5. Kuzmicheva, M. B. (2010). The Russian market of non-traditional meats. In Meat Industry (Vol. 6, p. 21). Meat Industry' Journal, LLC.
6. Caraveo, O., Alarcon-Rojo, A. D., Renteria, A., Santellano, E., & Paniwnyk, L. (2014). Physicochemical and microbiological characteristics of beef treated with high-intensity ultrasound and stored at 4 °C. In Journal of the Science of Food and Agriculture (Vol. 95, Issue 12, pp. 2487–2493). Wiley. https://doi.org/10.1002/jsfa.6979
7. Ježek, F., Kameník, J., Macharáčková, B., Bogdanovičová, K., & Bednář, J. (2019). Cooking of meat: effect on texture, cooking loss and microbiological quality – a review. In Acta Veterinaria Brno (Vol. 88, Issue 4, pp. 487–496). University of Veterinary and Pharmaceutical Sciences. https://doi.org/10.2754/avb201988040487
8. Chomanov, U., Kenenbay, G., Tursynov, A., Zhumalieva, T., & Tultabayev, N. (2023). The Influence of Various Combinations of Aging and Freezing/Defrosting Modes on the Quality Indicators of Saanen Goat Meat. In OnLine Journal of Biological Sciences (Vol. 23, Issue 4, pp. 451–457). Science Publications. https://doi.org/10.3844/ojbsci.2023.451.457
9. GOST 13586.5-2015. Grain. Method of moisture content determination. (2019). Moscow, Standardinform. (p. 16).
10. GOST 10846-91. Grain and products of its processing. Method for determination of protein. (2009). Moscow, Standardinform.
11. GOST 26176-2019 Fodders, mixed feeds. Methods for determination of soluble and hydrolysable carbohydrates. (2019). Moscow, Standardinform.
12. GOST 32195-2013 (ISO 13903:2005). Feed, compound feed. Method for determining amino acid content. (2020). Moscow, Standardinform.
13. GOST R 51478-99. Meat and meat products. Reference method for measurement of pH. (2014). Moscow, Standardinform.
14. GOST 33319-2015. Meat and meat products. Method for determination of moisture content. (2019). Moscow, Standardinform.
15. GOST ISO 21807-2015. Microbiology of food products and feed. Determination of water activity. (2016). Moscow, Standardinform.
16. Fatyanov, E. V. (2016). Biotechnology in the production of food of animal origin: guidelines for performing laboratory work for the direction of preparation. In Food products of animal origin (70 p.). Federal State Educational Institution of Higher Education Saratov State Agrarian University Retrieved from https://www.vavilovsar.ru/files/pages/27220/14722088195.pdf?ysclid=lvgmqbjoq0300279928.
17. GOST 9959-2015. Meat and meat products. General conditions of organoleptical assessment. (2016). Moscow, Standardinform.
18. Rogov, I. A., Tokaev, E. S., Kovalev, Y. I., & Tolstoguzov, V. B. (1992). Collagen and its rational content in meat products: Part 1. Analytical studies. In Meat Science (Vol. 31, Issue 1, pp. 35–42). Elsevier BV. https://doi.org/10.1016/0309-1740(92)90070-k
19. Kenenbai, G. S., Chomanov, U. C., Omirzhanova, B. B., Tatieva, A. N., Kassymbek, R., & Zhumaliyeva, G. (2022). Processing of beef rumen with ultrasonic waves. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 810–823). HACCP Consulting. https://doi.org/10.5219/1794
20. Nasrollahzadeh, F., Alexi, N., Skov, K. B., Roman, L., Sfyra, K., & Martinez, M. M. (2024). Texture profiling of muscle meat benchmarks and plant-based analogues: An instrumental and sensory design approach with focus on correlations. In Food Hydrocolloids (Vol. 151, p. 109829). Elsevier BV. https://doi.org/10.1016/j.foodhyd.2024.109829
21. Kiswanto, Hadiyanto, & Sediyono, E. (2024). Modification of the Haar Wavelet Algorithm for Texture Identification of Types of Meat Using Machine Learning. In Proceedings of Data Analytics and Management (pp. 225–239). Springer Nature Singapore. https://doi.org/10.1007/978-981-99-6547-2_18
22. Bruce, H. L., & Aalhus, J. L. (2022). Advances in the understanding and measurement of meat texture. In New Aspects of Meat Quality (pp. 163–194). Elsevier. https://doi.org/10.1016/b978-0-323-85879-3.00015-5
23. Schreuders, F. K. G., Schlangen, M., Kyriakopoulou, K., Boom, R. M., & van der Goot, A. J. (2021). Texture methods for evaluating meat and meat analogue structures: A review. In Food Control (Vol. 127, p. 108103). Elsevier BV. https://doi.org/10.1016/j.foodcont.2021.108103
24. Food Technology Corporation. (2020). Food Texture Analyser Brochure. Retrieved from https://www.si-instruments.com.au/products/test-stands/food-texture-analyser.html.

25. Honsor, O., & Oberyshyn, R. (2023). A Computerised System for Monitoring Water Activity in Food Products Using Wireless Technologies. In Advances in Artificial Systems for Logistics Engineering III (pp. 393–403). Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-36115-9_36
26. Conceição, S., Queiroga, M. C., & Laranjo, M. (2023). Antimicrobial Resistance in Bacteria from Meat and Meat Products: A One Health Perspective. In Microorganisms (Vol. 11, Issue 10, p. 2581). MDPI AG. https://doi.org/10.3390/microorganisms11102581
27. Kumar, Y., Bashir, A. A., & Choudhary, P. (2023). Processed Meat Products. In Fat Mimetics for Food Applications (pp. 313–342). Wiley. https://doi.org/10.1002/9781119780045.ch15
28. Miyahira, R. F., Lopes, J. de O., & Antunes, A. E. C. (2021). The Use of Sprouts to Improve the Nutritional Value of Food Products: A Brief Review. In Plant Foods for Human Nutrition (Vol. 76, Issue 2, pp. 143–152). Springer Science and Business Media LLC. https://doi.org/10.1007/s11130-021-00888-6
29. Dubtsov, G. G., Berezhnaya, O.V., & Voino L. I. (2013). Improving the microbiological safety of sprouted wheat grain. In Food industry (Vol. 6, pp. 28–29). Publishing House «Food Industry».
30. Iztayev, A., Yakiyayeva, M., Kulazhanov, T., Kizatova, M., Maemerov, M., Stankevych, G., Toxanbayeva, B., & Chakanova, Z. (2018). Efficient mathematical models of ion-ozon cavitation treatment for long-term storage of grain legume crops. In Acta Technica CSAV (Vol. 63, Issue 1, pp. 1–10). Academy of Sciences of the Czech Republic.
31. Hwang, I. H., & Thompson, J. M. (2001). The effect of time and type of electrical stimulation on the calpain system and meat tenderness in beef longissimus dorsi muscle. In Meat Science (Vol. 58, Issue 2, pp. 135–144). Elsevier BV. https://doi.org/10.1016/s0309-1740(00)00141-8
32. May, S. G., Mies, W. L., Edwards, J. W., Harris, J. J., Morgan, J. B., Garrett, R. P., Williams, F. L., Wise, J. W., Cross, H. R., & Savell, J. W. (2000). Using live estimates and ultrasound measurements to predict beef carcass cutability. In Journal of Animal Science (Vol. 78, Issue 5, p. 1255). Oxford University Press (OUP). https://doi.org/10.2527/2000.7851255x
33. Tursunbayeva, S., Iztayev, A., Mynbayeva, A., Alimardanova, M., Iztayev, B., & Yakiyayeva, M. (2021). Development of a highly efficient ion-ozone cavitation technology for accelerated bread production. In Scientific Reports (Vol. 11, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-021-98341-w
34. Iztayev, A., Baibatyrov, T., Mukasheva, T., Muldabekova, B., & Yakiyayeva, M. (2020). Experimental studies of the baisheshek barley grain processed by the ion-ozone mixture. Periodico Tche Quimica, (Vol. 17, Issue 35, pp. 239–258). https://doi.org/10.52571/PTQ.v17.n35.2020.22_IZTAYEV_pgs_239_258.pdf
35. Xu, S., & Falsafi, S. R. (2023). Juiciness of Meat, Meat Products, and Meat Analogues: Definition, Evaluation Methods, and Influencing Factors. In Food Reviews International (pp. 1–34). Informa UK Limited. https://doi.org/10.1080/87559129.2023.2273923
36. Simonova, I., Drachuk, U., Halukh, B., Basarab, I., Koval, H., & Kinash, S. (2023). Innovative meat products from non-traditional sources. In Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies (Vol. 25, Issue 100, pp. 26–34). Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. https://doi.org/10.32718/nvlvet-f10005
37. Kumar, Y., Bashir, A. A., & Choudhary, P. (2023). Processed Meat Products. In Fat Mimetics for Food Applications (pp. 313–342). Wiley. https://doi.org/10.1002/9781119780045.ch15
38. Biryukova, T. V., Enkina, C. V., Vorozheykina, T. M., Ashmarina, T. I., & Yagudaeva, N. A. (2023). The approach to assessing the characteristics significant for consumers when buying meat and meat products of organic origin. In V. Trukhachev, I. Jafarov, A. Shitikova, R. Migunov, & V. Kukhar (Eds.), BIO Web of Conferences (Vol. 66, p. 14015). EDP Sciences. https://doi.org/10.1051/bioconf/20236614015
39. Biryukova, T., & Ashmarina, T. (2023). Approach to assessing consumers' purchasing choices for premium meat and meat products. In M. Pushkarev (Ed.), E3S Web of Conferences (Vol. 460, p. 11015). EDP Sciences. https://doi.org/10.1051/e3sconf/202346011015
40. Munekata, P. E. S., Domínguez, R., Pateiro, M., Andrés, S. C., Santos, E. M., Fraqueza, M. J., Campagnol, P. C. B., & Lorenzo, J. M. (2023). Meat and meat products: animal species, products, processing, quality, and shelf life. In Meat and Meat Replacements (pp. 45–76). Elsevier. https://doi.org/10.1016/b978-0-323-85838-0.00001-8
41. Simonova, I., Drachuk, U., Halukh, B., Basarab, I., Koval, H., & Kinash, S. (2023). Innovative meat products from non-traditional sources. In Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies (Vol. 25, Issue 100, pp. 26–34). Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. https://doi.org/10.32718/nvlvet-f10005
42. Zahorulko, A., Cherevko, O., Zagorulko, A., Yancheva, M., Budnyk, N., Nakonechna, Y., Oliynyk, N., & Novgorodska, N. (2021). Design of an apparatus for low-temperature processing of meat delicacies. In

Eastern-European Journal of Enterprise Technologies (Vol. 5, Issue 11 (113), pp. 6–12). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2021.240675

43. Nemkov, G. G., Belousov, V. I., & Bazarbayev, S. B. (2020). Properties of dynamics of microbiological seeding of equipment for serving slicing of meat delicacies from beef meat. In Problems of Veterinary Sanitation, Hygiene and Ecology (Vol. 1, Issue 2, pp. 236–239). The publishing house - Scientific library. https://doi.org/10.36871/vet.san.hyg.ecol.202002018
44. Shakin, A. P. (2021). Qualimetric forecasting of quality requirements for meat delicacies. In Russian State Agrarian University-Moscow State Agricultural Academy named after K. A. Timiryazev. Agrobiotechnology-2021. Publishing house RGAU-MSHA. https://doi.org/10.26897/978-5-9675-1855-3-2021-256
45. Krasnikova, E. S., Morgunova, N. L., Krasnikov, A. V., Ivanova, Z. I., Shorina, A. V., & Sukharev, M. I. (2021). Development of technology for cooked smoked lamb products using ultrasonic treatment. In IOP Conference Series: Earth and Environmental Science (Vol. 845, Issue 1, p. 012085). IOP Publishing. https://doi.org/10.1088/1755-1315/845/1/012085
46. Mohan, A., & Long, J. M. (2021). Valorization of wastes and by-products from the meat industry. In Valorization of Agri-Food Wastes and By-Products (pp. 457–474). Elsevier. https://doi.org/10.1016/b978-0-12-824044-1.00010-6
47. Gheorghe-Irimia, R.-A., Tapaloaga, D., Petcu, C.-D., Ghimpeteanu, O. M., & Militaru, M. (2023). Current trends in the application of vaccinium myrtillus and aronia melanocarpa as natural preservatives in meat and meat products: a review. In Scientific Papers Journal Veterinary series (Vol. 66, Issue 1, pp. 48–52). https://doi.org/10.61900/spjvs.2023.01.10
48. Echegaray, N., Rosmini, M., Fernandez, J., Munekata, P. E. S., Frizzo, L., Campagnol, P. C. B., & Lorenzo, J. M. (2023). Natural Additives in Meat Products as Antioxidants and Antimicrobials. In Functional Meat Products (pp. 111–124). Springer US. https://doi.org/10.1007/978-1-0716-3573-5_9
49. St. Pierre, S. R., Rajasekharan, D., Darwin, E. C., Linka, K., Levenston, M. E., & Kuhl, E. (2023). Discovering the mechanics of artificial and real meat. In Computer Methods in Applied Mechanics and Engineering (Vol. 415, p. 116236). Elsevier BV. https://doi.org/10.1016/j.cma.2023.116236
50. Siegel, D. G., Theno, D. M., & Schmidt, G. R. (1978). Meat massaging: the effects of salt, phosphate and massaging on the presence of specific skeletal muscle proteins in the exudate of a sectioned and formed ham. In Journal of Food Science (Vol. 43, Issue 2, pp. 327–330). Wiley. https://doi.org/10.1111/j.1365-2621.1978.tb02297.x
51. Palamarchuk, I., Shtonda, O., Semeniuk, K., Topchii, O., & Petryna, A. (2023). Physical and mathematical modelling of the massing process of marinated pork and beef preparation technology. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 929–944). HACCP Consulting. https://doi.org/10.5219/1926
52. Sharedeh, D., Mirade, P.-S., Venien, A., & Daudin, J.-D. (2015). Analysis of salt penetration enhancement in meat tissue by mechanical treatment using a tumbling simulator. In Journal of Food Engineering (Vol. 166, pp. 377–383). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2015.06.023
53. Gombozhapova, N. I., Bazhenova, B. A., Leskova, S. Yu., Badmaeva, T. M., & Danilov, A. M. (2017). Influence of the new multicomponent brine on the quality characteristics of the boiled horse meat product. In Foods and Raw materials (Vol. 5, Issue 1, pp. 11–19). Kemerovo State University. https://doi.org/10.21179/2308-4057-2017-1-11-19
54. Studref. (2024). Ways to increase the nutritional value of culinary meat products. Justification of the technology of minced semi-finished meat products and ready culinary products using sprouted wheat grain. Retrieved from: https://studref.com/547670/tovarovedenie/obosnovanie_tehnologii_myasnyh_rublenyh_polufabrikatov_gotovyh_kulinarnyh_izdeliy_ispolzovaniem_pror#aftercont.

Funds:

The presented studies were carried out within the framework of the program-targeted financing for 2021-2023 "Development of high-tech technologies for deep processing of agricultural raw materials in order to expand the range and output of finished products with units of raw materials, as well as reducing the share of waste in the production of products" BR10764970, funded by the Ministry of Agriculture of the Republic of Kazakhstan.

Acknowledgments:

The authors express their gratitude to the administration of the Kazakh Research Institute of Processing.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Gulimira Kenenbay, Kazakh Research Institute of Processing and Food Industry LLP, Laboratory of Technology for Processing and Storage of Animal Products, 238 Gagarin Ave., 050060, Almaty, Republic of Kazakhstan,
Tel.:+ 77023205856
E-mail: g.kenenbay@bk.ru
ORCID: https://orcid.org/0000-0002-8332-8102

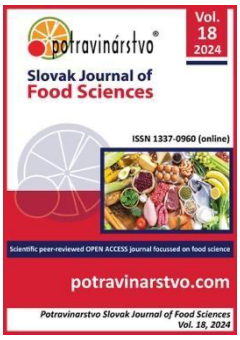
Tamara Tultabayeva, Kazakh Research Institute of Processing and Food Industry LLP, Laboratory of Technology for Processing and Storage of Animal Products, 238 Gagarin Ave., 050060, Almaty, Republic of Kazakhstan,
Tel.:+ 77089728228
E-mail: g.kenenbay@bk.ru
ORCID: https://orcid.org/0000-0003-2483-7406

Urishbay Chomanov, Kazakh Research Institute of Processing and Food Industry LLP, Laboratory of Technology for Processing and Storage of Animal Products, 238 Gagarin Ave., 050060, Almaty, Republic of Kazakhstan,
Tel.:+ 77017884556
E-mail: u.chomanov@bk.ru
ORCID: https://orcid.org/0000-0002-5594-8216

Gulzhan Zhumalieyeva, Kazakh Research Institute of Processing and Food Industry LLP, Laboratory of Technology for Processing and Storage of Animal Products, 238 Gagarin Ave., 050060, Almaty, Republic of Kazakhstan,
Tel.:+77787930493
E-mail: gulzhan_71711@mail.ru
ORCID: https://orcid.org/0000-0003-4010-6064

***Aruzhan Shoman**, Kazakh Research Institute of Processing and Food Industry LLP, Laboratory of Technology for Processing and Storage of Animal Products, 238 Gagarin Ave., 050060, Almaty, Republic of Kazakhstan,
Tel.:+77787930493
E-mail: shoman.aruzhan@mail.ru
ORCID: https://orcid.org/0000-0002-7844-8601

Corresponding author: *



potravinárstvo®

OPEN ACCESS

Received: 11.1.2024
Revised: 3.4.2024
Accepted: 16.4.2024
Published: 20.4.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 425-438
https://doi.org/10.5219/1952
ISSN: 1337-0960 online
www.potravinarstvo.com

Regional features of camel milk composition and properties in the Republic of Kazakhstan

Aidana Yessenova, Fatima Dikhanbayeva, Elmira Assembayeva, Dinara Nurmukhanbetova, Elmira Zhaxybayeva

ABSTRACT

Camel milk, renowned for its distinctive nutritional qualities, has captured the interest of scientific researchers due to its potential health benefits. This study aims to compare the biochemical composition of camel milk sourced from two distinct regions of Kazakhstan: Jetisu and Mangystou. Analytical methods were employed to achieve this objective, including gas chromatography for fatty acid analysis, chemical methods for physicochemical parameter determination, and assessment of amino acid, fatty acid, mineral, and vitamin content. In samples from the Jetisu region, protein content ranged from 3.61% to 3.70%, fat from 3.85% to 4.64%, and lactose from 4.80% to 4.85%. In comparison, samples from the Mangystou region exhibited protein content ranging from 3.65% to 3.81%, fat from 4.72% to 5.75%, and lactose from 4.21% to 4.28%. Regarding amino acid composition, Mangystou region samples contained more essential amino acids per 100 g of protein than Jetisu region samples: 41.29 g versus 38.20 g, respectively. Additionally, the Jetisu region sample contained 64.291% saturated fatty acids, while the Mangystou region sample had 62.135%, indicating differences in fatty acid composition based on geographical origin. In terms of mineral composition, camel milk from the Mangystou region exhibited higher calcium and zinc content compared to Jetisu region samples, with calcium and zinc content measured at 124.50 mg/100 g and 490.15 µg/100 g, respectively, for Mangystou samples, and 112.50 mg/100 g and 321.24 µg/100 g, respectively, for Jetisu samples. Overall, the study underscores regional variances in camel milk's biochemical composition, which can impact its nutritional and biological value. These findings provide dairy product producers in Kazakhstan with enhanced opportunities to create healthy, high-quality dairy products.

Keywords: amino acids, camel milk, chemical composition, fatty acids, mineral

INTRODUCTION

Camel breeding has been a longstanding practice in the Republic of Kazakhstan, dating back to ancient times. This livestock farming sector stands out as one of the most lucrative, particularly in desert climates, as it provides the population with milk, meat, wool, and leather.

According to the Bureau of National Statistics **[1]**, Kazakhstan's camel population is currently experiencing a consistent rise, as illustrated in Figure 1.

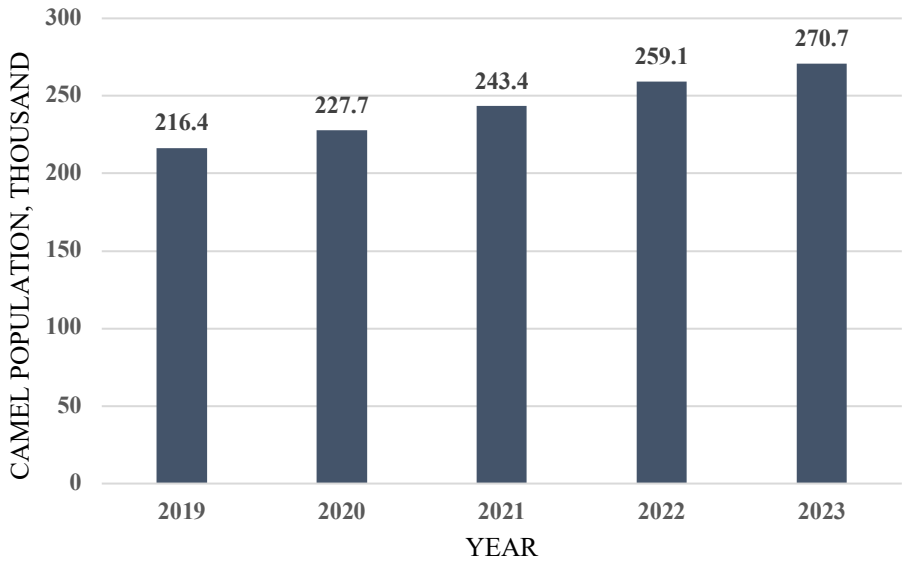


Figure 1 Number of Camels in the Republic of Kazakhstan.

Naturally, Kazakhstan's substantial population of camels (270 thousand heads in 2023) presents an opportunity to utilize camel milk productivity. This entails allocating additional resources from the agro-industrial complex for the dairy industry of the Republic of Kazakhstan. It also underscores the need to process camel milk into high-quality, safe dairy products, expanding and diversifying their range.

Camel milk products such as shubat, saumal, kurt, and balkaimak are integral to Kazakh culture and culinary heritage. These products offer delightful taste and health benefits and carry significant traditional value, reflecting Kazakhstan's rich cultural stories and traditions.

There is a growing interest in camel milk globally, making it increasingly popular in various cuisines and cultures. Producing camel milk-based cheeses, yogurts, and dairy drinks presents a unique approach to diversifying dairy products in the global market.

While the use of camel milk in the global food industry is not yet as extensive as that of milk from other animals, there is a clear trend toward leveraging this product's potential. Camel milk's distinctive nutritional and medicinal properties attract the attention of producers and consumers alike, offering new prospects for innovation in the food industry.

Categorised as an albumin-type product, camel milk resembles female breast milk composition **[2]**. Noteworthy for its optimal protein content and easily digestible fats, camel milk distinguishes itself by the absence of β-lactoglobulin, rendering it suitable for consumption without eliciting allergic reactions **[3]**.

The diminutive size of camel milk fat globules accelerates their hydrolysis, facilitating superior absorption by the human body **[4]**. The quality of camel milk fat surpasses that of fat derived from other types of farm animal milk. Furthermore, the relatively elevated presence of unsaturated fatty acids positions camel milk as a product with notable dietary properties **[5]**.

Camel milk is a valuable source of vitamins essential for ensuring the normal progression of biochemical and physiological processes within the human body. It contains 3-5 times more vitamin C than cow's milk **[4]**.

Furthermore, camel milk is enriched with macro- and microelements crucial for sustaining the body's normal development. A review article by Konuspayeva et al. noted that camel milk is relatively rich in potassium (K), sodium (Na), chloride (Cl), iron (Fe), and zinc (Zn) **[6]**.

Studies indicate that various factors, including geographical area, feeding conditions, season, and stage of lactation influence camel milk's composition. Therefore, understanding the composition of camel milk in different regions of Kazakhstan is crucial for optimising the production and utilisation of this valuable product in the food industry.

This study aims to comprehensively analyse the physicochemical and biochemical indicators that characterise the nutritional and biological value of camel milk in the western and southern regions of Kazakhstan, with a specific focus on the Mangystou and Jetisu regions. These areas have traditionally considered camel milk a key element of their food culture. Analysing these data will enhance our understanding of camel milk's potential to promote the health and well-being of these regions' populations. Additionally, it will help justify practical measures to improve the quality and efficiency of camel milk production and utilisation.

Scientific hypothesis

This study's hypothesis suggests that camel milk produced in the Mangystou and Jetisu regions of western and southern Kazakhstan has unique physicochemical and biochemical characteristics that affect its nutritional and biological value. This hypothesis is based on the assumption that local camel housing conditions, nutritional habits, and climate influence the composition and properties of the milk.

MATERIAL AND METHODOLOGY

Samples

Samples of whole fresh milk from dromedary camels were obtained from farms in the Jetisu region (n = 12) and Mangystou region (n = 12).

The milking of camels took place during two seasons, the summer (July) and winter (December) of 2022, in the early morning. The samples were collected from the milk that was obtained. The camels are under constant veterinary and zootechnical supervision.

The regions from which the camel milk samples were collected have diverse climates (see Figure 2).

The Mangystou region is situated southwest of Kazakhstan. The climate is desert-like and arid, with extremely hot summers. The average temperature in January is -1 °C, rising to +26.4 °C in July. During the summer, temperatures can reach as high as +44 °C. Strong winds and storms are common, with minimal precipitation, with an average annual amount not exceeding 100-150 mm.

The Jetisu region is located in the southeast of Kazakhstan. The climate there is continental, with an average annual precipitation of 600-650 mm. The primary maximum rainfall occurs in April-May, with a secondary maximum in October-November.

Chemicals

All reagents used were of U.S.P. purity or higher. All solvents, including water, were used with the LC/MS label.

Instruments

pH meter: pH-150 MI (Measuring Technologies LLC, Russia).

Gas chromatograph: Shimadzu GC-2010 (Shimadzu Corporation, Japan).

Distiller for steam distillation: Velp Scientifical UDK 129 (Himlaborreaktiv LLC, Italy).

Mass spectrometer: Agilent 7900 (Agilent Technologies, Japan).

High-performance liquid chromatograph: LC-20 Prominence (Shimadzu Corporation, Japan).

Capillary electrophoresis system “KAPEL-205” (Lumex-Marketing LLC, Russia)

Laboratory Methods

The laboratory of Nutritest LLP in Almaty conducted analyses on camel milk samples. The following parameters were assessed: protein content **[7]**, fat content **[8]**, lactose **[9]**, titratable acidity **[10]**, active acidity **[11]**, amino acid composition **[12]**, fatty acid composition **[13]**, vitamins **[14]**, **[15]**, **[16]**, and minerals **[17]**. The vitamin content was determined partially, excluding some vitamin types.

To determine the fatty acid composition, camel milk samples were prepared according to SST 32915-2014: each milk sample was divided into two centrifuge tubes (50 cm^3 each), and then centrifuged at 10,000 rpm for 15 ±1 minutes. After centrifugation, the upper-fat fraction was collected and transferred to a 250 cm^3 glass container. Next, 150 cm^3 of hexane was added to the fat fraction, gently mixed, and homogenized for 3-5 minutes. The hexane layer containing the dissolved fat was separated and transferred to a 250 cm^3 round-bottomed flask. The flask was connected to a rotary evaporator and the solvent was completely distilled off at a temperature of 70 ±2 °C. Methyl ether was added to the resulting fatty fraction, and 1 µl of a solution of fatty acid methyl esters was injected into a Shimadzu GC-2010 Plus gas chromatograph with a flame ionization detector. An Agilent HP-88 capillary column with dimensions of 100 m × 0.250 mm × 0.20 µm was used. The detector was supplied with gas from a gas flow regulator with the following gases: nitrogen, hydrogen, and air; the maximum detector temperature was set to 260 °C. The temperature parameters were as follows: 100 °C for 5 minutes, increased to 210 °C for 8 minutes at a speed of 4 °C/min, and further increased to 240 °C for 25 minutes at a speed of 10 °C/min. The sample flow division was 1/40, and the total analysis time was 48.25 minutes.

The amino acid composition was determined by capillary electrophoresis (CEP), which involves preliminary acid and alkaline (only for tryptophan) hydrolysis to convert amino acids into free forms, obtaining phenylisothiocarbamyl derivatives and their subsequent separation and quantitative determination by capillary electrophoresis. Tryptophan was an exception, as its determination utilized direct detection on Kapel-205 equipment in the UV spectral region at a wavelength of 254 nm, using a quartz capillary with an internal diameter of 50 µm and a total length of 75 cm, with a sample volume of 2.5 cm^3.

Description of the Experiment

Sample preparation: Milk samples are collected from dromedary camels by the sample preparation process at farms in designated regions. This collection process typically involves using specialized containers to minimize potential contamination and maintain the sample's integrity. Subsequently, the milk samples must be transported to the laboratory carefully for further analysis.

Number of samples analyzed: we analyzed 24 samples.

Number of repeated analyses: 3

Number of experiment replication: 3

Design of the experiment: During the initial phase of the experiment, samples of fresh whole camel milk were collected from dromedary camels at farms in the Jetisu region (six samples each from summer and winter) and the Mangystou region (six samples each from summer and winter). Following the milking process, the temperature and pH of the camel milk were promptly measured using an electronic thermometer and a pH meter. Each camel milk sample (approximately 500 ml) was carefully collected into clean, sterilised bottles. The samples were refrigerated at 4 °C (<24 h) and transported for further laboratory analysis.

For the analysis, the physicochemical parameters, amino acid and fatty acid composition, and vitamin and mineral content were taken and investigated at the local accredited laboratory (Nutritest LLP).

In the final stage, we analyzed the obtained results, conducted statistical analysis, and verified the validity of our hypotheses.

Figure 2 Map of Kazakhstan, indicates the locations of sampled Jetisu (Zhetysu) and Mangystau regions.

Statistical Analysis

The results of the experimental studies were processed using mathematical statistics. The experimental data was analysed using the Data Analysis in Microsoft Excel and Statistica. Each experiment was performed with a minimum of three to seven repetitions. The acquired results were subjected to standard processing methods and are presented as average values and standard errors of the mean (± SEM). Statistical results were assessed using the Student's t-test, with differences considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Camel milk is a historically renowned source of nutrition, offering essential nutrients and possessing adaptive properties that enhance survival in challenging climatic conditions. This study aimed to identify the difference in several parameters of dromedary camel milk during the summer and winter seasons, which helps determine that the local camel housing conditions, nutritional habits, and local climate influence the composition and properties of the milk. The received results are illustrated in Table 1.

Table 1 The physicochemical composition of camel milk in the Jetisu and Mangystou regions during the summer and winter seasons.

Seasons	Protein, %	Fat, %	Lactose, %	Dry matter, %	Titratable acidity, °T	pH
Jetisu region						
summer	3.61 ±0.02	3.85 ±0.03	4.85 ±0.04	13.06 ±0.03	16.50 ±0.5	6.56 ±0.05
winter	3.70 ±0.03	4.64 ±0.05[a]	4.80 ±0.03	13.92 ±0.05[b]	16.30 ±0.3	6.47 ±0.02
Mangystou region						
summer	3.65 ±0.03	4.72 ±0.05	4.21 ±0.05	13.98 ±0.03	17.00 ±0.5[d]	6.30 ±0.05[e]
winter	3.81 ±0.05[a]	5.75 ±0.03[b]	4.28 ±0.03	14.66 ±0.04[c]	16.60 ±0.2	5.86 ±0.03

Note: [a, b, c, d, e] – means significantly differ from other season samples of the indicated region (p <0.05). All values are expressed as the mean of ±SD (Standard Deviation).

The above-presented results revealed the variability in the chemical composition of milk based on the region and time of year. These findings are consistent with previous studies Ishill et al. **[18]** and Dikhanbayeva et al. **[19]** on Kazakh camel milk, but surpass the data from a study on Egyptian dromedary camel milk **[20]**.

The fat and protein content increased from summer to winter. During winter, camel milk from the Mangystou region exhibited a higher protein content (3.81%) compared to the Jetisu region (3.70%). The same scenario was with the protein content, where Mangystou region (3.65%) was higher by 3.68% than Jetisu region (3.61%). The fat content, particularly in winter, was also higher in camel milk from the Mangystou region, reaching a maximum of 5.75%.

The fat content in camel milk from the Mangystou region rose from 4.72% in summer to 5.75% in winter, with protein content increasing from 3.65% to 3.81%. A similar trend was observed in camel milk from the Jetisu region.

In the Jetisu region, the lactose content in camel milk was approximately 4.85% in summer and 4.80% in winter, while in the Mangystou region, it was about 4.21% in summer and 4.28% in winter. Thus, the lactose content of camel milk demonstrates relative stability under different conditions, which may be crucial for understanding its nutritional value and relevance to consumers.

Our study confirms the variability in protein content of camel milk, as noted in the works of other authors. For example, the average protein content in camel milk from the Jetisu region ranged from 3.61% to 3.70% in different seasons, consistent with the findings **[21]**. However, it's worth noting that some studies, such as **[22]**, indicate a wider range of protein content, from 2.04% to 3.05%.

In one line, our study indicates camel milk's relatively low lactose content. Our data shows that the average lactose content ranges from 4.21% to 4.85% in different seasons and regions, comparable to other studies. For instance, **[23]** reported similar lactose content in Egyptian camel milk (4.86 g/100 g), and **[24]** and **[25]** also demonstrated low lactose levels in camel milk. Thus, the consistency of this parameter in camel milk across different geographical regions is confirmed, emphasizing its uniqueness among other types of milk.

A comparative analysis of dry substance content in camel milk during summer and winter in various regions of Kazakhstan revealed an increase in dry substance content in winter compared to summer. In the Jetisu region, the average dry matter content was 13.06% in summer and 13.92% in winter, while in the Mangystou region, these values were 13.98% and 14.66%, respectively. This indicates a more concentrated nature of camel milk in winter, likely due to the increased fat content.

During hot months, camels require more fluid due to high temperatures and intense evaporation. This can lead to milk production with higher water content, reducing the overall solids content.

The analysis of camel milk's chemical composition from different regions and seasons is valuable for understanding the variability in its quality characteristics based on the animals' environmental and living conditions. Our results show that camel milk produced in the Mangystou region generally has higher protein and fat content than milk from the Jetisu region.

Milk protein and fat content variations can be attributed to differences in housing conditions and animal diets across regions. The relatively high-fat content in camel milk from the Mangystou region, particularly in winter, may be due to the physiological characteristics of camels in this region. It is possible that they actively store fat reserves in winter to survive harsh climatic conditions.

Additionally, changes in lactose content between seasons may be associated with variations in the camels' diet and the composition of vegetation in their environment. Importantly, in the current investigation, camel milk samples from the Jetisu region exhibited higher lactose content than those from the Mangystou region. This difference may be attributed to the predominant desert conditions in the Mangystou region, where camels

primarily graze on halophytic plants. These plants, consumed by camels in desert environments, fulfil their physiological salt requirements, reducing lactose content in camel milk **[26]**.

In both regions, milk's acidity and pH levels are consistently within the normal range. Samples of camel milk from the Jetisu and Mangystou regions consistently exhibited pH values ranging from 6.36 to 6.56, irrespective of the season. The low pH of camel milk could be attributed to its high Vitamin C content **[2]**. Additionally, milk pH may change depending on the animals' water availability and fodder quality **[27]**.

Titrated acidity (°T) in camel milk samples from these regions ranged from 16.30 to 17.00 °T, consistent with previous findings. These results align with the scientific work Cherifa et al. **[28]**.

According to **[22]**, camel milk's low pH value and high titratable acidity may be associated with its microbial flora, particularly lactic acid bacteria producing lactic acid under milking conditions at ambient temperatures.

The results underscore the significance of considering regional characteristics when analyzing and utilizing camel milk within the food industry. These findings confirm the importance of such considerations and offer potential insights for developing strategies to enhance milk quality through camel feeding and management.

Amino Acid Composition: Proteins, as high-molecular compounds composed of amino acids, play a crucial role in the body's functional activity. Amino acids serve both substrate and regulatory functions in protein biosynthesis, actively participate in energy processes, act as a source of physiologically active amines, and contribute to forming nucleic acids, lipids, and hormones **[29]**.

As highlighted by **[23]**, camel milk is notably rich in essential and non-essential amino acids, except for lysine, glycine, threonine, and valine.

Research indicates that camel milk contains higher levels of methionine, valine, phenylalanine, arginine, and leucine than cow's milk **[30]**.

The amino acid composition of camel milk from the regions above was examined to assess the biological value. The results are detailed in Table 2.

Table 2 Comparative analysis of the amino acid composition of camel milk.

Amino acids, g/100 g	Jetisu region	Mangystou region
Essential amino acids		
Valine	5.46 ±0.04	6.03 ±0.05[a]
Methionine	2.07 ±0.003	3.4 ±0.004[a]
Phenylalanine	4.28 ±0.05	4.64 ±0.05[a]
Isoleucine	4.96 ±0.02[b]	4.53 ±0.03
Leucine	8.17 ±0.06	9.00 ±0.05[a]
Lysine	7.19 ±0.05	7.56 ±0.06 [a]
Threonine	4.77 ±0.04	4.67 ±0.04
Tryptophan	1.3 ±0.003	1.46 ±0.003
Nonessential amino acids		
Aspartic acid	7.01 ±0.06[b]	6.01 ±0.06
Glutamic acid	20.25 ±0.09 [b]	19.2 ±0.06
Histidine	2.65 ±0.003	2.75 ±0.005
Arginine	4.55 ±0.03	5.08 ±0.04 [a]
Serine	4.66 ±0.05 [b]	2.82 ±0.02
Glycine	1.65 ±0.002	1.15 ±0.003
Alanine	3.02 ±0.03	3.23 ±0.05
Tyrosine	4.45 ±0.05	4.25 ±0.05
Cysteine	1.56 ±0.003	1.58 ±0.002
Proline	11.95 ±0.05	12.63 ±0.06 [a]

Note: [a, b] – means significantly differ from other season samples of the indicated region (p <0.05). All values are expressed as the mean of ±SD (Standard Deviation).

The results of a comparative analysis of the amino acid composition (Table 2) indicate that both samples of camel milk contain all eight essential amino acids. In 100 g of camel milk protein from the Jetisu region, 38.20 g of essential and 61.74 g of non-essential amino acids were detected. Meanwhile, 41.29 g of essential and 58.70 g of non-essential amino acids were found in 100 g of camel milk protein from the Mangystau region.

The largest amounts of essential amino acids in both samples were leucine (8.17-9.00 g/100 g), lysine (7.19-7.56 g/100 g), and valine (5.46-6.03 g/100 g), while the tryptophan content was lower (1.30-1.46 g/100 g). The total amount of essential amino acids is higher in milk from the Mangystou region.

Regarding non-essential amino acids, both camel milk samples contain the largest amounts of aspartic acid (6.01-7.01 g/100 g), glutamic acid (19.20-20.25 g/100 g), and proline (11.95-12.63 g/100 g), while cysteine (1.56-1.58 g/100 g) and glycine (1.15-1.56 g/100 g) are present in smaller quantities. Previous studies on the amino acid composition of camel milk **[23]** also confirm that glutamic acid, proline, and aspartic acid are major components. At the same time, methionine and glycine are present in lesser quantities.

It is noted that camel milk from the Jetisu region has a higher total level of essential amino acids than camel milk from the Mangystou region.

The obtained data on the amino acid composition of camel milk from both regions (Table 2) are consistent with the authors' previous works. Thus, the study revealed similar general characteristics of the biological value of camel milk in both regions, with minor differences in the content of individual amino acids. These results underscore the importance of camel milk as a food product with high biological value, providing essential amino acids to support a healthy diet and supporting the potential for further research in the food industry.

Fatty Acid Composition: Camel milk, renowned for its high nutritional value, is a subject of interest for research in the food industry. The fatty acid composition of camel milk plays a crucial role in its biological value. It can vary depending on several factors, including the animals' location and feeding conditions. This study compared camel milk's fatty acid composition from two Kazakhstan regions: Jetisu and Mangystou.

Table 3 presents the quantitative composition of fatty acids in camel milk from both regions.

Table 3 Comparative Analysis of the Fatty Acid Composition in Camel Milk.

FA name	Fatty acid code	Fatty acid, %	
		Jetisu region	Mangystou region
Saturated FA			
Butyric acid	C4:0	-	-
Caproic acid	C6:0	0.145 ±0.003[a]	0.097 ±0.002
Caprylic acid	C8:0	0.228 ±0.002 [a]	0.068 ±0.001
Capric acid	C10:0	0.259 ±0.004 [a]	0.101 ±0.003
Lauric acid	C12:0	1.103 ±0.005 [a]	0.820 ±0.002
Myristic acid	C14:0	9.484 ±0.006 [a]	8.027 ±0.002
Pentadecylic acid	C15:0	1.009 ±0.002	1.678 ±0.004 [b]
Palmitic acid	C16:0	32.461 ±0.004 [a]	29.321 ±0.005
Margaric acid	C17:0	1.571 ±0.002	1.704 ±0.002[b]
Stearic acid	C18:0	16.540 ±0.005	18.866 ±0.003[b]
Arachidic acid	C20:0	0.452 ±0.002	0.540 ±0.001[b]
Behenic acid	C22:0	0.551 ±0.006 [a]	0.494 ±0.003
Lignoceric acid	C24:0	0.488 ±0.002 [a]	0.419 ±0.002
Monounsaturated FA			
Myristoleic acid	C14:1	0.484 ±0.003 [a]	0.442 ±0.004
Pentadecylic acid	C15:1	0.200 ±0.002	0.380 ±0.005[b]
Palmitoleic acid	C16:1	6.836 ±0.004	6.606 ±0.003
Heptadecanoic acid	C17:1	0.453 ±0.003	0.774 ±0.006 [b]
Oleic acid	C18:1 (ω-9)	18.558 ±0.005	23.684 ±0.005[b]
Polyunsaturated FA			
Linolenic acid	C18:2n6t	0.528 ±0.004	1.070 ±0.008 [b]
Linoleic acid	C18:2n6c	4.357 ±0.005[a]	2.905 ±0.005
γ -Linolenic acid	C18:3n6	3.223 ±0.002[a]	1.194 ±0.006
Eicosadienoic acid	C20:2	0.422 ±0.005	0.414 ±0.002
Arachidonic acid	C20:4n6	0.313 ±0.002[a]	-
Eicosapentaenoic acid	C20:5n3	0.337 ±0.001	0.366 ±0.004
Saturated FA		64.291 ±0.043	62.135 ±0.032
Monounsaturated FA		26.531 ±0.017	31.886 ±0.023
Polyunsaturated FA		9.18 ±0.019	5.494 ±0.025

Note: [a, b] – means significantly differ from other season samples of the indicated region (p <0.05). All values are expressed as the mean of ±SD (Standard Deviation).

The data obtained revealed a significant disparity in saturated and unsaturated fatty acid concentrations between two distinct regions of Kazakhstan.

In the fat extracted from camel milk samples in the Jetisu region, 23 fatty acids were identified. In comparison, 22 types were discerned in the camel milk sample from the Mangystou region. Generally, the Jetisu region sample exhibited higher levels of saturated and polyunsaturated fatty acids, whereas the Mangystou camel milk sample displayed elevated monounsaturated fatty acids. Predominantly, saturated fatty acids constituted the major proportion in both samples, accounting for 62.135% to 64.291% of the total fatty acids. These data are consistent with the results of the researchers' work Konuspayeva et al. **[31]** and Teng et al. **[32]**.

Specifically, the concentration of saturated fatty acids in the Jetisu region's camel milk sample was 64.291%, while in the Mangystou region, it amounted to 62.135% of the overall fatty acid composition.

In both samples, the predominant saturated fatty acids were myristic (C14:0), palmitic (C16:0), and stearic (C18:0). The obtained data are similar to previous data for Turkish camel milk **[33]**.

The camel milk samples from the Jetisu region exhibited higher concentrations of capron ($C_{6:0}$), caprylic ($C_{8:0}$), caprine ($C_{10:0}$), lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic ($C_{16:0}$), lignoceric ($C_{20:0}$), and behenic acids ($C_{22:0}$) compared to the samples from the Mangystou region. Conversely, camel milk samples from the Mangystou region demonstrated elevated levels of arachidic, stearic, margaric, and pentadecanoic acids.

Notably, neither of the samples contained butyric acid ($C_{4:0}$). These data are similar to those from previous scientists Dreiucker and Vetter **[34]**.

The most significant intergroup difference was observed in the concentrations of specific saturated fatty acids. For instance, the palmitic acid content ($C_{16:0}$) in the camel milk sample from the Jetisu region was 3.14% higher than that in the Mangystou region. In contrast, the stearic acid content ($C_{18:0}$) in the Jetisu camel milk sample was 2.33% lower than in the Mangystou region's camel milk sample.

As per Table 2, the mean content of monounsaturated fatty acids in both samples was 26.531% to 31.886%. Notably, camel milk from the Mangystou region stands out for its richness in monounsaturated fatty acids, constituting 31.886% of the total sum of all fatty acids. Oleic acid (C18:1) emerged as the predominant acid, comprising 23.684%.

In contemporary perspectives, considerable emphasis is placed not only on the quantity but also on the chemical composition of fats, with a particular focus on the content of polyunsaturated acids. This attention stems from the fact that the human body cannot synthesize linoleic and linolenic acids, and the biosynthesis of arachidonic acid is limited, categorizing them as essential or irreplaceable.

It is noteworthy that polyunsaturated fatty acids play a crucial role in eliminating excess cholesterol from the body, impeding its deposition on the walls of blood vessels and safeguarding the body against the development of atherosclerosis.

The findings of our study revealed distinct lipid profiles in camel milk between the Mangystou and Jetisu regions, with a notable difference in polyunsaturated fatty acid content. Specifically, the lipids in camel milk from the Mangystou region exhibited a concentration of -5.494%, while in the Jetisu region, the content was 9.18%. Among the Jetisu region's camel milk, the highest concentration of polyunsaturated fatty acids was observed at 9.18%, prominently featuring linoleic (4.36%) and γ-linolenic (3.22%) acids. Additionally, arachidonic acid (0.33%) was identified in camel milk samples from the Jetisu region, aligning with the findings in the prior work of Teng et al. **[32]**.

In conclusion, the analysis of the fatty acid composition of camel milk from both the Jetisu and Mangystou regions indicated the prevalence of specific fatty acids in both samples. $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{16:1}$, and $C_{18:1n9c}$ were identified as the most abundant fatty acids in both samples. These findings are consistent with Kazakh scientists studying Bactrian camel milk **[35]**.

Mineral Substances: According to a review of publications on camel milk, the overall mineral content, as measured by total ash content, varied between 0.60% and 1.30%, with an average value of 0.80% **[36]**.

Figure 1 illustrates the outcomes of a comparative analysis of mineral content in camel milk samples collected from the Jetisu and Mangystou regions.

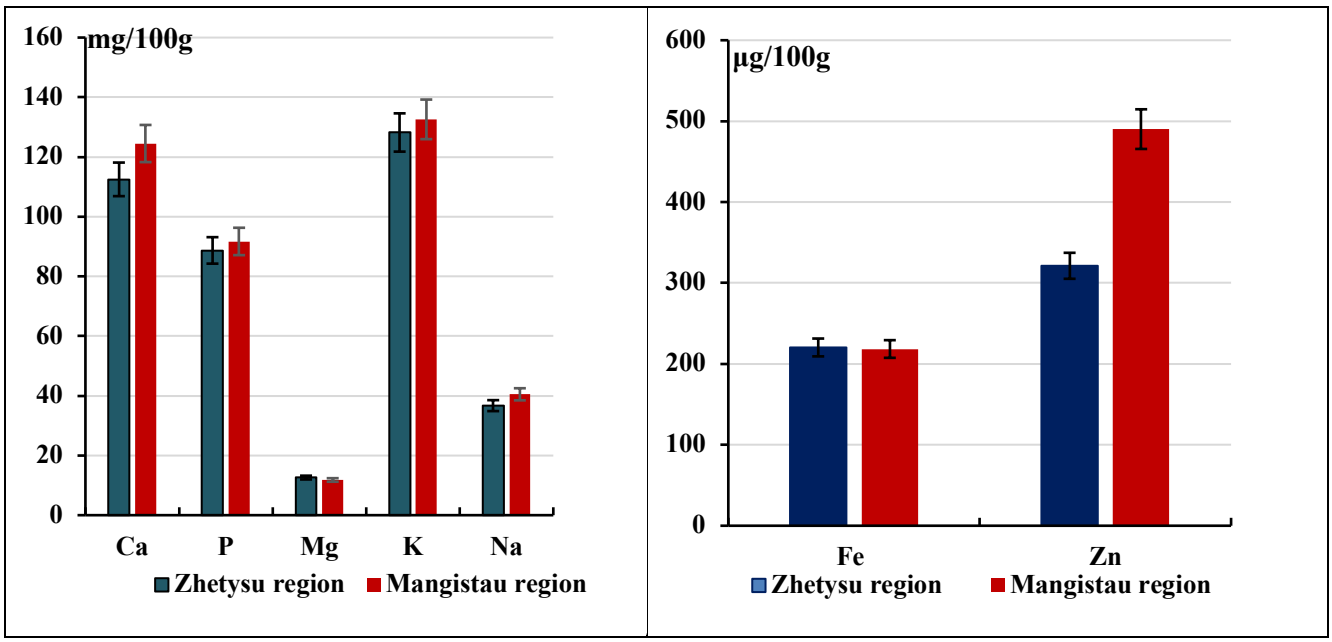


Figure 3 Composition of Macro- (A) and Microelements (B) in Camel Milk.

The results of the analysis of macro- and microelements in camel milk (Figure 3) indicate that, in general, no significant differences were observed between the regions in terms of mineral content, except for calcium (Ca) and zinc (Zn).

The calcium and zinc content in camel milk samples from the Mangystou region was notably higher than those from the Jetisu region. Specifically, the calcium content in the camel milk samples from the Jetisu region was 112.50 mg/100 g, while in the samples from the Mangystou region, it was 124.50 mg/100 g. On average, these values are consistent with the findings of the scholars of Dikhanbayeva et al. **[19]**. Notably, the elevated calcium levels in camel milk from the Mangystou region may be attributed to the conditions in which the camels are kept. According to Mostafidi et al. **[37]**, camels living in desert conditions tend to have higher calcium content in their milk than those in nourishing conditions.

The phosphorus content in milk is influenced by factors such as the animals' feeding diet, breed, and stage of lactation. Our study found no significant differences in phosphorus content between the two samples, with an average phosphorus content of 90.10 mg/100 g, which is practically comparable to the findings of the study Al-Otaibi and el-Demerdash **[38]**.

Magnesium is present in milk in modest quantities and is crucial in maintaining the normal function of the nervous system and heart muscles. It also exhibits a vasodilating effect, stimulates bile secretion, and enhances intestinal motor activity, facilitating the removal of toxins from the body. On average, the magnesium (Mg) content in both camel milk samples was 12.20 mg/100 g. Our results are similar to those reported by researchers The phosphorus content in milk is influenced by factors such as the animals' feeding diet, breed, and stage of lactation. Our study found no significant differences in phosphorus content between the two samples, with an average phosphorus content of 90.10 mg/100 g, which is practically comparable to the findings of the study Al-Otaibi and el-Demerdash **[38]**. Still, they exceed the data presented by author Soliman **[39]**.

The average potassium content in both camel milk samples was 130.40 mg/100 g. These findings align with those of studies The phosphorus content in milk is influenced by factors such as the animals' feeding diet, breed, and stage of lactation. Our study found no significant differences in phosphorus content between the two samples, with an average phosphorus content of 90.10 mg/100 g, which is practically comparable to the findings of study Al-Otaibi and el-Demerdash **[38]** and Dikhanbayeva et al. **[19]**, but are lower than the results reported by the Soliman **[39]**.

The average sodium content in both camel milk samples was 38.60 mg/100 g. Our data show lower sodium levels compared to previous studies by authors Al-Otaibi and el-Demerdash **[38]** and Shamsia **[23]**, but are consistent with the findings of Kazakh scientists Dikhanbayeva et al. **[19]**.

Iron, an essential trace element in milk, is noteworthy for its significance. Khaskheli et al. **[40]** emphasize that camel milk is notably richer in iron than cow's milk. Our study revealed an average iron content of 219 µg/100g in both samples, comparable to the data reported by researcher Soliman **[39]**.

In the Mangystou region, the zinc content in camel milk samples was 490.15 µg/100 g, which is 34.40% higher than in camel milk samples from the Jetisu region. The Mangystou region is an industrial and mining area

responsible for 25% of Kazakhstan's oil production (almost 20 million tons). In line with the findings of scientific research by Meldebekova et al. **[41]**, it is plausible that emissions from these industries have influenced the increase in zinc content in camel milk in the Mangystau region.

Vitamins: Vitamins are integral to camel milk, encompassing both water-soluble and fat-soluble varieties **[42]**. Table 4 presents a comparative examination of vitamin content in camel milk samples from the Jetisu and Mangystou regions.

Table 4 Comparative Analysis of the Vitamin Composition of Camel Milk.

Vitamins, mg/100 g	Jetisu region	Mangystou region
A	0.063 ±0.005	0.074 ±0.003
B1	0.077 ±0.002	0.069 ±0.003
C	5.37 ±0.25	6.41 ±0.30[b]

Note: a, b - means significantly differ from other season samples of the indicated region ($p<0.05$). All values are expressed as the mean of ±SD (Standard Deviation).

According to the results in Table 4, no discernible differences were observed between the regions regarding vitamin A content. Specifically, in camel milk samples from the Jetisu region, vitamin A was found to be 0.063 mg/100 g. In contrast, in samples from the Mangystou region, it measured 0.074 mg/100 g, respectively. Our findings surpass previous studies, such as Konuspayeva et al. **[31]**, who reported vitamin A content in camel milk as 12.60 µg/100 ml, and Haddadin et al. **[42]** where vitamin A content was noted as 201 µg/l. Nevertheless, our data align with the findings of Jordanian scientists **[43]**.

The vitamin B1 content in Jetisu camel milk samples measured 0.077 mg/100g, and in Mangystou samples, it was 0.069 mg/100 g, respectively. These results were lower than Kazakh scientists' findings Dikhanbayeva et al. **[19]** for Kazakh camel milk. Wang et al. **[44]** also noted that the vitamin B1 content in camel milk was lower compared to that in cow's milk.

Regarding vitamin C, Jetisu camel milk samples showed a content of 5.37 mg/100 g, while Mangystou samples registered 6.41 mg/100 g. These findings were lower than those reported by Kazakh scientists Konuspayeva et al. **[45]**, but are consistent with the results presented by the authors of Stahl et al. **[46]**. On average, the vitamin C content in Mangystou camel milk is 16.20 % higher than that in Jetisu camel milk. This disparity is likely attributed to feeding conditions, as animals predominantly acquire vitamins through their diet. Additionally, factors such as age, time of year, lactation period, and the microflora of camel rumen and intestines may contribute to these variations. Notably, the high vitamin C content in Mangystou camel milk is crucial given the predominantly desert conditions in this region, where fruits and vegetables are scarce.

CONCLUSION

Based on the above results, the following conclusions can be drawn:

1. Comparative analysis showed subtle differences in the biochemical composition of camel milk samples obtained from the Jetisu and Mangystou regions;
2. Camel milk samples from the Mangisatu region contained more protein and fat compared to camel milk samples from the Jetisu region their content was 3.65-3.81% and 4.72-5.75%;
3. Regarding amino acid composition, camel milk samples from the Jetisu region had more essential amino acids per 100 g of protein than camel milk samples from the Jetisu region: 41.29 g versus 38.20 g, respectively;
4. A study of fatty acid composition showed that camel milk samples from the Jetisu region contained 64.291% saturated fatty acids, 26.531% monounsaturated fatty acids, and 9.18% polyunsaturated fatty acids, while camel milk samples from the Mangystou region had 62.135% saturated fatty acids, 31.886 % monounsaturated fatty acids, and 5.494% polyunsaturated fatty acids, respectively;
5. Mineral composition, including calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), iron (Fe), and zinc (Zn) content. The results of the analysis showed the following values for the content of mineral elements in 100 g of camel milk: calcium (Ca): 112.50 mg in the Jetisu region and 124.5 mg in the Mangystou region; phosphorus (P): 88.70 mg in Jetisu region and 91.70 mg in Mangystou region; magnesium (Mg): 12.60 mg in Jetisu region and 11.80 mg in Mangystou region; potassium (K): 128.2 mg in Jetisu region and 132.6 mg in Mangystou region; sodium (Na): 36.70 mg in Jetisu region and 40.50 mg in Mangystou region; iron (Fe): 220.32 µg in the Jetisu region and 321.24 µg in the Mangystou region; zinc (Zn): 218.42 mcg in Jetisu region and 490.15 mcg in Mangystou region. Camel milk from the Mangystou region has a higher content of calcium, phosphorus, magnesium, potassium, sodium, iron and zinc compared to samples from the Jetisu region;
6. Analyzing the vitamin composition of camel milk from the above-mentioned regions, we can conclude that camel milk samples from the Mangystau region are a rich source of vitamin C, its content was 6.41 mg/100.

These findings represent a valuable contribution to the limited body of information on camel milk's nutritional and biological value from the western and southern regions of Kazakhstan, particularly the Jetisu and Mangystou regions. Given that camel milk serves as a staple food in these areas, its unique chemical composition and nutritional and biological values can be advantageous. This distinctive profile not only enhances the potential of dairy producers but also provides the opportunity to produce healthy and high-quality dairy products in Kazakhstan.

REFERENCES

1. Bureau of National statistics of Agency for Strategic planning and reforms of the Republic of Kazakhstan. Retrieved from https://stat.gov.kz/ru/industries/business-statistics/stat-forrest-village-hunt-fish/spreadsheets/
2. El-Hatmi, H. E.-H. (2015). Comparison of composition and whey protein fractions of human, camel, donkey, goat and cow milk. In Mljekarstvo (Vol. 65, Issue 3, pp. 159–167). Croatian Dairy Union. https://doi.org/10.15567/mljekarstvo.2015.0302
3. Kumar, D., Verma, A. K., Chatli, M. K., Singh, R., Kumar, P., Mehta, N., & Malav, O. P. (2016). Camel milk: alternative milk for human consumption and its health benefits. In Nutrition & Food Science (Vol. 46, Issue 2, pp. 217–227). Emerald. https://doi.org/10.1108/nfs-07-2015-0085
4. Ho, T. M., Zou, Z., & Bansal, N. (2022). Camel milk: A review of its nutritional value, heat stability, and potential food products. In Food Research International (Vol. 153, p. 110870). Elsevier BV. https://doi.org/10.1016/j.foodres.2021.110870
5. Konuspayeva, G., Lemarie, É., Faye, B., Loiseau, G., & Montet, D. (2008). Fatty acid and cholesterol composition of camel's (Camelus bactrianus, Camelus dromedaries and hybrids) milk in Kazakhstan. In Dairy Science and Technology (Vol. 88, Issue 3, pp. 327–340). Springer Science and Business Media LLC. https://doi.org/10.1051/dst:2008005
6. Konuspayeva, G., Faye, B., & Bengoumi, M. (2022). Mineral status in camel milk: a critical review. In Animal Frontiers (Vol. 12, Issue 4, pp. 52–60). Oxford University Press (OUP). https://doi.org/10.1093/af/vfac044
7. ISO 8968-1:2014 | IDF 20-1:2014. Milk and milk products. Determination of nitrogen content. Part 1: Kjeldahl principle and crude protein calculation
8. ISO 19662:2018 | IDF 238:2018. Milk. Determination of fat content. Acido-butyrometric (Gerber method)
9. SST 34304-2017 Milk and milk products. Method for determination of lactose and galactose content
10. SST 3624-92 Milk and milk products. Titrimetric methods of acidity determination
11. SST 26781-85 Milk. Method of pH measuring
12. SST R 55569-2013 Feedstuffs, compound feeds, feed raw materials. Determination of proteinogenic amino acids using capillary electrophoresis
13. SST 32915-2014 Milk and milk products. Determination of fatty acid content by gas chromatography method
14. SST 54635-2011 Functional food products. Method of vitamin A determination
15. SST EN 14122-2013 Foodstuffs. Determination of vitamin B by HPLC
16. SST R EN 14130-2010 Foodstuffs. Determination of vitamin C by HPLC
17. SST 34141-2017 Food products, feed, food raw. Determination of arsenic, cadmium, mercury and lead by the method of inductively coupled plasma-mass spectrometry
18. Ishill, S., Hoshino, B., & Nurtazin, S. T. (2017). Properties and Seasonal Variation of Milk Produced by One-humped Camels (Camelus dromedarius) and Two-humped camels (C. bactrianus) in the Republic of Kazakhstan. In Journal of Arid Land Studies (Vol. 26, pp. 219–222). The Japanese Association for Arid Land Studies. https://doi.org/10.14976/jals.26.4_219
19. Dikhanbayeva, F., Zhaxybayeva, E., Dimitrov, Z., Baiysbayeva, M., Yessirkep, G., & Bansal, N. (2021). Studying the effect of the developed technology on the chemical composition of yogurt made from camel milk. In Eastern-European Journal of Enterprise Technologies (Vol. 3, Issue 11 (111), pp. 36–48). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2021.235831
20. Elhosseny, M., Gwida, M., Elsherbini, M., Samra, M. B., & Ashmawy, A. M. (2018). Evaluation of physicochemical properties and microbiological quality of camel milk from Egypt. In Journal of Dairy, Veterinary & Animal Research (Vol. 7, Issue 3). MedCrave Group, LLC. https://doi.org/10.15406/jdvar.2018.07.00197
21. Ahmed, A., & Sayed, R. (2014). Nutritional value and sanitary evaluation of raw Camels milk. In Emirates Journal of Food and Agriculture (Vol. 26, Issue 4, p. 317). Pensoft Publishers. https://doi.org/10.9755/ejfa.v26i4.16158

22. Alaoui Ismaili, M., Saidi, B., Zahar, M., Hamama, A., & Ezzaier, R. (2019). Composition and microbial quality of raw camel milk produced in Morocco. In Journal of the Saudi Society of Agricultural Sciences (Vol. 18, Issue 1, pp. 17–21). Elsevier BV. https://doi.org/10.1016/j.jssas.2016.12.001
23. Shamsia, S. M. (2009). Nutritional and therapeutic properties of camel and human milks. In International Journal of Genetics and Molecular Biology (Vol. 1, Issue 2, pp. 052–058). Academic Journals
24. Sanayei, S., Jahadi, M., Fazel, M., & Janigorban, M. (2015). Physico-Chemical Characteristics of Raw Milk of One-Humped Camel from Khur and Biabanak in Isfahan Province of Iran. In Journal of Food Biosciences and Technology, (Vol. 5, Issue 2, pp. 67–72) Islamic Azad University, Science and Research Branch
25. Shuiep, E. S., El Zubeir, I. E. M., El Owni, O. A. O., & Musa, H. H. (2008). Influence of season and management on composition of raw camel (Camelus dromedarius) milk in Khartoum state, Sudan. In Tropical and Subtropical Agroecosystems (Vol. 8, Issue 1, pp. 101–106). Universidad Autonoma de Yucatan.
26. Fouzia, R., Noureddine, S., & Mebrouk, K. (2013). Evaluation of the factors affecting the variation of the physicochemical composition of Algerian camel's raw milk during different seasons. In Advances in Environmental Biology (Vol. 7, Issue 14, pp. 4879–4884). American-Eurasian Network for Scientific Information Publications (AENSI).
27. Sahel, N., Chougrani, F., Cheriguene, A., & Hamani, Z. (2023). Microbiological and Physico-Chemical Characteristics of Camel Milk from Southwestern Algeria. In Pakistan Journal of Agricultural Research (Vol. 36, Issue 4). Researchers Links Ltd. https://doi.org/10.17582/journal.pjar/2023/36.4.377.388
28. Cherifa, B., Oumelkheir, S., & Amar, E. (2018). Influence of feeding on some physicochemical and biochemical characteristics of camel milk (Camelus dromadarius). In Emirates Journal of Food and Agriculture (Vol. 30, Issue 4, p. 251). Faculty of Food and Agriculture, United Arab Emirates University. https://doi.org/10.9755/ejfa.2018.v30.i4.1658
29. Ren, W., Li, Y., Yin, Y., & Blachier, F. (2013). Structure, Metabolism and Functions of Amino Acids: An Overview. In Nutritional and Physiological Functions of Amino Acids in Pigs (pp. 91–108). Springer Vienna. https://doi.org/10.1007/978-3-7091-1328-8_7
30. Barłowska, J., Szwajkowska, M., Litwińczuk, Z., & Król, J. (2011). Nutritional Value and Technological Suitability of Milk from Various Animal Species Used for Dairy Production. In Comprehensive Reviews in Food Science and Food Safety (Vol. 10, Issue 6, pp. 291–302). Wiley. https://doi.org/10.1111/j.1541-4337.2011.00163.x
31. Konuspayeva, G., Faye, B., & Mussaad, A. (2014). Some lipid components of the camel milk and blood in intensive farm in Saudi Arabia. In Emirates Journal of Food and Agriculture (Vol. 26, Issue 4, p. 349). Faculty of Food and Agriculture, United Arab Emirates University. https://doi.org/10.9755/ejfa.v26i4.17276
32. Teng, F., Wang, P., Yang, L., Ma, Y., & Day, L. (2017). Quantification of Fatty Acids in Human, Cow, Buffalo, Goat, Yak, and Camel Milk Using an Improved One-Step GC-FID Method. In Food Analytical Methods (Vol. 10, Issue 8, pp. 2881–2891). Springer Science and Business Media LLC. https://doi.org/10.1007/s12161-017-0852-z
33. Karaman, A. D., Yildiz Akgül, F., Öğüt, S., Seçilmiş Canbay, H., & Alvarez, V. (2022). Gross composition of raw camel's milk produced in Turkey. In Food Science and Technology (Vol. 42). FapUNIFESP (SciELO). https://doi.org/10.1590/fst.59820
34. Dreiucker, J., & Vetter, W. (2011). Fatty acids patterns in camel, moose, cow and human milk as determined with GC/MS after silver ion solid phase extraction. In Food Chemistry (Vol. 126, Issue 2, pp. 762–771). Elsevier BV. https://doi.org/10.1016/j.foodchem.2010.11.061
35. Zhumabay, A., Serikbayeva, A., Kozykan, S., Yusof, Y. A., & Kozhakhmetova, A. (2023). Determination of the fatty and amino acid composition of camel milk, milk powder and shubat. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 918–928). HACCP Consulting. https://doi.org/10.5219/1931
36. Benmeziane – Derradji, F. (2021). Evaluation of camel milk: gross composition—a scientific overview. In Tropical Animal Health and Production (Vol. 53, Issue 2). Springer Science and Business Media LLC. https://doi.org/10.1007/s11250-021-02689-0
37. Mostafidi, M., Moslehishand, M., Piravivanak, Z., & Pouretedai, Z. (2016). Evaluation of mineral content and heavy metals of dromedary camel milk in Iran. In Food Science and Technology (Vol. 36, Issue 4, pp. 717–723). FapUNIFESP (SciELO). https://doi.org/10.1590/1678-457x.16116
38. Al-Otaibi, M., & El-Demerdash, H. (2013). Nutritive Value and Characterization Properties of Fermented Camel Milk Fortified with some Date Palm Products Chemical, Bacteriological and Sensory Properties. In International Journal of Nutrition and Food Sciences (Vol. 2, Issue 4, p. 174). Science Publishing Group. https://doi.org/10.11648/j.ijnfs.20130204.13

39. Soliman, G. Z. A. (2005). Comparison Of Chemical And Mineral Content Of Milk From Human, Cow, Buffalo, Camel And Goat In Egypt. In The Egyptian Journal of Hospital Medicine (Vol. 21, Issue 1, pp. 116–130). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/ejhm.2005.18054

40. Khaskheli, M., Arain, M. A., Chaudhry, S., Soomro, A. H., & Qureshi, T. A. (2005). Physico-chemical quality of camel milk. In Journal of Agriculture and Social Sciences (Vol. 1, Issue 2, pp 164–166). Friends Science Publishers.

41. Meldebekova, A., Konuspayeva, G., Diacono, E., & Faye, B. (n.d.). Heavy Metals and Trace Elements Content in Camel Milk and Shubat from Kazakhstan. In Impact of Pollution on Animal Products (pp. 117–123). Springer Netherlands. https://doi.org/10.1007/978-1-4020-8359-4_12

42. Haddadin, M. S., Gammoh, S. I., & Robinson, R. K. (2007). Seasonal variations in the chemical composition of camel milk in Jordan. In Journal of Dairy Research (Vol. 75, Issue 1, pp. 8–12). Cambridge University Press (CUP). https://doi.org/10.1017/s0022029907002750

43. Aludatt, M. H., Ereifej, K., Alothman, A. M., Almajwal, A., Alkhalidy, H., Al-Tawaha, A. R., & Alli, I. (2010). Variations of physical and chemical properties and mineral and vitamin composition of camel milk from eight locations in Jordan. In Journal of Food, Agriculture and Environment (Vol. 8, Issue 3, pp. 8–12). WFL Publisher Ltd.

44. Wang, S. Y., Liang, J. P., Shao, W. J., & Wen, H. (2011). Mineral, vitamin and fatty acid contents in the camel milk of dromedaries in the Anxi Gansu China. In Journal of Camel Practice and Research (Vol. 18, Issue 2, pp. 273–276) Camel Publishing House.

45. Konuspayeva, G., Faye, B., & Loiseau, G. (2011). Variability of vitamin C content in camel milk from Kazakhstan. In Journal of Camelid Science (Vol. 4, Issue 1, pp. 63–69) International society of Camelid Research and Development.

46. Stahl T, Sallmann HP, Duehlmeier R, et al. (2006) Selected vitamins and fatty acid patterns in dromedary milk and colostrum. Journal of Camel Practice and Research (Vol. 13, Issue 1, pp. 53–57) Camel Publishing House.

Funds:

This work was not supported by external grants.

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Aidana Yessenova**, Almaty Technological University, Department of Department of Food Safety and Quality, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77474846380
E-mail: essenova_06.07@mail.ru
ORCID: https://orcid.org/0000-0002-6101-1446

Fatima Dikhanbayeva, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77477555985
E-mail: fatima6363@mail.ru
ORCID: https://orcid.org/0000-0003-4257-3774

Elmira Assembayeva, Almaty Technological University, Department of Food Biotechnology, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77074575972
E-mail: elmiraasembaeva@mail.ru
ORCID: https://orcid.org/0000-0001-7964-7736

Dinara Nurmukhanbetova, Educational program Restaurant and hotel business, Narxoz University, Zhandosov 55, 050035, Almaty, Republic of Kazakhstan,
Tel.: +77073709726
E-mail: dinara.nurmukhanbetova@narxoz.kz
ORCID: https://orcid.org/0000-0002-8939-6325

Elmira Zhaxybayeva, Design and Technology Institute, Kyzylorda University named Korkyt Ata, Ayteke Bi Street 29A, 120000, Kyzylorda, Republic of Kazakhstan,
Tel.: +77475896132
E-mail: zhelya90@gmail.com
ORCID: https://orcid.org/0000-0002-0383-4946

potravinárstvo®
OPEN ACCESS
Received: 15.1.2024
Revised: 3.4.2024
Accepted: 16.4.2024
Published: 20.5.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 439-452
https://doi.org/10.5219/1954
ISSN: 1337-0960 online
www.potravinarstvo.com

Quality and safety of pork meat after cooling and treatment with lactic starters

Volodymyr Vovkotrub, Olha Iakubchak, Nataliia Vovkotrub, Larysa Shevchenko, Tetiana Lebedenko, Nataliia Holembovska, Oksana Pylypchuk, Alina Omelian

ABSTRACT

Cooling the pork half-carcasses in a refrigerating chamber with showering had no significant impact on their surface temperature. Still, it reduced the core temperature of the meat in 1 hour after cooling compared with air-cooling. pH-value of all pork half-carcasses that were subjected to cooling with the showering method, as well as the final processing of the pork with suspensions of *Lactobacillus sakei* and *Leuconostoc carnosum* in 1 hour and on the 4th day of storage in a chilled condition was within the limits typical for fresh and high-quality meat. The greatest weight loss of the pork half-carcasses occurred during the first 24 hours when they were being cooled. The weight loss of the pork half-carcasses in a chilled condition during 1 day when they were being cooled in a refrigerating chamber without the use of showering was 2.27%, when they were being cooled with the use of showering – 1.65%, when they were being cooled with the use of showering and final processing with SafePro® B-SF-43 (*Leuconostoc carnosum*) – 1.61% and SafePro® B-2 (*Lactobacillus sakei*) – 1.25% in comparison with the output of a hot carcass. Microorganism cultures of strains SafePro® B-SF-43 and SafePro® B-2, when they are applied at a dose of 10^6/cm^2, had contributed to a colonization of the meat with the lactic-acid microorganisms and a significant decrease in the number of QMAFAnM in the neck and spine areas in 1 hour after cooling. *S. aureus, Salmonella* spp., and *L. monocytogenes* were not detected in the meat of the pork half-carcasses in all processing options during 4 days of storage in a chilled condition.

Keywords: quality, safety, pork, storage, stiving

INTRODUCTION

A wide range of factors determines the quality and safety of pork: genetic potential, feeding and rearing conditions of pigs, and conditions of stunning, slaughtering, and post-slaughter ablution of pork carcasses **[1]**, **[2]**. Nowadays, enterprises specializing in meat processing use many technologies to improve the quality of the final product due to the speed and method of animal slaughter, cooling, and further storage of the carcasses. At the same time, an important criterion is the sensory properties of the meat products that consumers prefer **[3]**, **[4]**.

In the last few years, research on pork cooling has focused on accelerated cooling to minimize carcass weight loss due to evaporation and rates of microbial contamination. Furthermore, the accelerated cooling of the pork carcasses can also be used to improve the physicochemical properties of the meat due to a decrease in metabolic rate, in particular, glycolysis in post-slaughter tissues. In turn, slowing glycolysis leads to less loss of droplet moisture in the muscles and less meat with PSE signs **[5]**.

Understanding the mechanisms underlying the development of water loss in droplet evaporation when the pork carcasses are being cooled will make it possible to reduce the weight loss and preserve the meat quality. It is understood that early post-slaughter processes, including the reduction rate and extent of the pH value, the proteolysis, and even the protein oxidation, are the main processes influencing the moisture-holding capacity of the meat. The cell structures, including the intra- and extra-myofibrillar spaces, retain a significant part of the

muscle water. During the meat's ageing processes, the water's space, which is contained in the myofibrils, decreases, and the fluid can be displaced into the extra-myofibrillar spaces, where it is lost as droplets. The lateral reduction of the myofibrils during the stiffening can be transmitted throughout the cell if the proteins that bind the myofibrils together and the myofibrils with the cell membrane (such as desmin) are not degraded. Recent figures point to the fact that the degradation of key cytoskeletal proteins by calpain proteinases plays a certain part in determining the moisture-holding capacity of the muscles **[6]**. The microbial contamination of the meat, which occurs during the slaughtering of animals and subsequent operations of primary processing of the pork carcasses, also impacts this process. At the same time, exogenous and endogenous contamination with the bacteria occurs, the sources of which can be the skin cover, the contents of the alimentary tract, air, equipment, vehicles, tools, hands, clothes, and shoes of workers who have contact with the meat, as well as the water, which is used for the pork carcasses to be processed **[7]**.

This microflora causes meat spoilage and can harm the consumer's body. The main human pathogens in terms of microbial hazards, which were found in the animal meat, include *E. coli, Salmonella* spp*., Campylobacter* spp*., Listeria monocytogenes, Clostridium botulinum/perfringens, Staphylococcus aureus* and *Bacillus cereus* **[8]**, **[9]**. Several scientists attach particular importance to the cross-over contamination of meat products with pathogenic microorganisms, particularly *Listeria monocytogenes* **[10]**, **[11]**. A significant number of chemicals, in particular, organic acids **[12]**, bacteriocins, essential oils **[13]**, as well as ionizing radiation, hydrostatic pressure, electric fields, sonication, and microwaves **[14]**, are used to reduce the contamination risks of the meat with the microflora. However, these reduction methods of the microbial contamination of the meat have several disadvantages; firstly, they change the organoleptic properties, which affects this product's attractiveness to the consumer.

One of the promising reduction methods for the microbial contamination of the meat is using the microbial cultures of lactic acid microorganisms, which can produce the bacteriocins that can inhibit the growth and reproduction of various bacteria. The action mechanism of the bacteriocins is based on the ability to interact with the cell surface to increase the permeability of its membrane, suppress the formation of the cell wall components, and synthesize the nucleic acids and protein **[15]**.

Among the most common and promising microorganisms capable of producing bacteriocins in the meat industry are bacteria of the genus *Lactobacillus*, in particular, *Lactobacillus sakei*. It is a facultative heterofermenter capable of producing alcohol or lactic acid from sugars. *L. sakei* is used in Europe to produce traditional dry sausages as a starter and can be used to preserve fresh meat **[16]**. Sakacin P, produced by *Latilactobacillus sakei*, has antibacterial activity against *Listeria monocytogenes* and *Bacillus cereus* **[17]**, **[18]**. The lactic acid microorganism *Leuconostoc carnosum*, which produces leucocin, is also widely known. Its effectiveness has been proven against developing *Listeria monocytogenes* in many meat products **[19]**, **[20]**.

Scientific Hypothesis

Cooling the pork half-carcasses with the use of stiving and final processing of the half-carcass surface by the suspensions of lactic-acid microorganisms *Leuconostoc carnosum* or *Lactobacillus sakei* will reduce the weight loss of the pork half-carcasses due to moisture evaporation and preserve the quality and safety of the meat when stored.

MATERIAL AND METHODOLOGY

Samples

The study material was the pork half-carcasses obtained after the primary processing in the LLC "Antonivsky meat-processing plant" conditions, Kyiv region, Ukraine. The animals were slaughtered in compliance with the current "Rules for pre-slaughter veterinary inspection of animals and veterinary-sanitary examination of meat and meat products" **[21]**, good hygienic practice (GMP), after which the pork half-carcasses were transported to the specialized refrigerating chambers – the lactic acid microorganisms *Leuconostoc carnosum* and *Lactobacillus sakei* produced by Chr. Hansen (LLC "Chr. Hansen Ukraine") was used for the study.

Chemicals

Media and diagnostic tests manufactured by HiMedia (India) were used for microbiological studies.

Medium M091 Plate Count Agar was used to determine QMAFAnM. For recovery and quantitative counting of the bacteria of the genus *Lactobacillus* – Lactobacillus MRS Agar M641, for recovery of pathogenic and non-pathogenic staphylococci – Baird Parker, Agar M043, for *Salmonella* – Bismuth Sulphite Agar M027 and Xylose Lysine Deoxycholate Agar M031, for *L. monocytogenes* – Agar Palcam, Agar Oxford.

Animals, Plants, and Biological Materials

For research, 20 heads of young fattening pigs of the large white breed aged 6 months, who were slaughtered from a private farm in the Kyiv region, Ukraine, were used. Pork half carcasses weighing 43-44 kg were selected to form groups in the experiment.

Instruments

pH meter for meat EZODO MP-103M Taiwan, GOnDO'Electronic Co., Ltd.

Petrie dishes.

Disposable microbiological tubes.

Laboratory Methods

The weight of the pork half-carcasses was determined with the use of industrial scales TV4-1500 in the conditions of the meat-processing plant with an accuracy of 0.1 kg.

The washings from the surface of the pork half-carcasses in the neck and spine area were made to determine the quantity of mesophilic aerobic and facultatively anaerobic microorganisms (QMAFAnM), the bacteria of the genera *Salmonella* spp*., S. aureus, Lactobacillus, L. monocytogenes*, fungi, and mold. For this purpose, consistent nine-fold dilutions were prepared in a sterile physiological solution.

The quantity of the microorganism was determined in colony-forming units (CFU), and the results were expressed in lg CFU/cm^2 of the surface. The recovered microorganisms were identified by genus and species using current methods.

The meat of the slaughtered animals (color, smell, consistency, cooking test) was organoleptically evaluated according to DSTU 7992:2015 **[22]**.

Description of the Experiment

Sample preparation: Meat sampling from slaughtered animals and preparation for microbiological tests were carried out according to the requirements of DSTU 8381:2015 **[23]**. Washings from the meat surface of the slaughtered animals, stored in the refrigeration chambers of the meat-processing plant, were sampled with a sterile swab according to the requirements of DSTU ISO 17604:2014 **[24]**.

Number of samples analyzed: 10 half-carcasses of each group, 40 half-carcasses total. The parameters of the pork half-carcasses cooled in the refrigerating chamber (control 1) were compared with the pork half-carcasses, cooled in the refrigerating chamber with the use of stiving (control 2), cooled in the refrigerating chamber with the use of stiving, and processed with the starter of culture SafePro® B-SF-43 (*Leuconostoc carnosum*) (experimental 1), as well as cooled in the refrigerating chamber with the use of stiving and processed with the starter of culture SafePro® B-2 (*Lactobacillus sakei*) (experimental 2).

Number of repeated analyses: From 5 to 10 samples were used in each experiment.

Number of experiment replications: 1

Design of the experiment: The pork half-carcasses obtained after slaughtering were divided into 4 groups, each containing 10 half-carcasses (Table 1).

Table 1 Determination experiment scheme of a cooling effect on pork half-carcasses with stiving method.

Group	Experiment conditions
Control 1	Carcass ablution without showering
Control 2	Carcass ablution with showering
Experimental 1	Carcass ablution with stiving and processing by starter of culture SafePro® B-SF-43 (*Leuconostoc carnosum*) in a dose of 10^6/cm^2
Experimental 2	Carcass ablution with stiving and processing by a starter of culture SafePro® B-2 (*Lactobacillus sakei*) in a dose of 10^6/cm^2

Experimental groups 1 and 2 were processed with the microbial starters at the rate of 10^6/cm^2 of the lactic acid microorganisms, which were applied to the surface of the pork half-carcasses after the end of the stiving process. All pork half-carcasses were stored in a refrigerator at 3 ±1 °C. The study results were recorded in 1 hour after cooling, in a day, and on the 4th day of storage of the pork half-carcasses in refrigerators.

Statistical Analysis

The obtained results were statistically processed using the ANOVA program, and the table data are presented as x ±SD (mean ± standard deviation). The normality of data distribution was confirmed using the program R-3.6.3 for Windows. The difference between the groups was considered probable using the Tukey test at $p \leq 0.05$ (considering the Bonferroni correction).

RESULTS AND DISCUSSION

The cooling rate of pork carcasses impacts the meat's sensory properties **[25]**, **[26]**. The rapid decrease of the core temperature of the meat determines the intensity of post-slaughter metabolism **[27]** and the formation of volatile and non-volatile compounds in it, which create the aroma of the final product and its appeal to the consumer **[28]**. The temperature of the meat surface of the pork half-carcasses that were not subjected to stiving 1 hour after cooling probably did not differ from similar indicators of the pork half-carcasses that were cooled with striving. At the same time, the core temperature of the pork half-carcasses was probably lower ($p \leq 0.05$) in the case of cooling with the use of stiving of both control group 2 and both experimental groups, in comparison with the data of the pork half-carcasses of control group 1 (Table 2).

Table 2 Temperature and pH-value of pork half-carcasses in 1 h after cooling, x ±SD, n = 5.

Group	The surface temperature of half-carcass, °C	The core temperature of half-carcass, °C	pH-value of meat, un.
Control 1	11.93 ±0.60^{a}	28.12 ±1.55^{a}	6.84 ±0.05^{a}
Control 2	12.19 ±1.33^{a}	24.66 ±2.47^{b}	6.67 ±0.13^{a}
Experimental 1	11.68 ±0.75^{a}	24.60 ±2.12^{b}	6.70 ±0.13^{a}
Experimental 2	11.85 ±1.24^{a}	23.82 ±1.15^{b}	6.80 ±0.11^{a}

Note: different letters of upper indices $^{a, b}$ indicates the values that were significantly different in one column of the table ($p \leq 0.05$) according to the comparison results with the use of the Tukey test.

Our data are compliant with the studies **[29]**, which showed that jet cooling (stiving) significantly accelerates the decrease of the core temperature of the pork carcass muscles and can be combined with other cooling phases of the meat **[30]**. The pH value of the meat of all pork half-carcasses subjected to cooling with the stiving method 1 hour after cooling was within the limits characteristic of fresh and high-quality meat (see Table 2). The study results **[31]** point to the fact that the decreased rate of the pH value/temperature in the muscles of the slaughtered animals significantly impacts the proteolysis of the muscle protein by increasing the activity of proteases and degrading several proteins.

Reaching the cooling temperature of the carcass core meat up to 2 °C depends on its weight, and for this purpose, it is averagely necessary from 17 hours to more than 27 hours for a carcass weight of 95 kg if the carcass weight is more than 105 kg **[32]**. In our experiment, the weight of the pork carcass did not exceed 95 kg in all cases (Table 4), which did not require a longer cooling process for the pork half-carcasses.

The surface temperature and the core thickness of the pork half-carcasses on the 4th day of storage probably did not differ between the groups (Table 3).

Table 3 Temperature and pH-value of pork half-carcasses on the 4th day of storage, x ±SD, n = 5.

Group	The surface temperature of half-carcass, °C	The core temperature of half-carcass, °C	pH-value of meat, un.
Control 1	3.40 ±0.32	3.30 ±0.89	6.37 ±0.17
Control 2	3.48 ±0.15	3.24 ±0.09	6.43 ±0.29
Experimental 1	3.24 ±0.22	3.62 ±0.86	6.73 ±0.22
Experimental 2	3.52 ±0.31	3.80 ±0.50	6.41 ±0.29

It is known that the pH value of the meat has a significant impact on its tenderness **[33]**, **[34]** in particular, through the regulation of the phosphorylation intensity of the muscle protein **[35]**, **[36]**. On the 4th day of storage, the pH value of the pork meat did not depend on the cooling method of the pork half-carcasses, as well as on the processing of the half-carcass surface with the starters of the lactic-acid microorganisms, and was within the parameters of the quality meat in all cases. Organoleptic indicators: color, consistency, smell, transparency, and aroma of meat broth obtained from the pork half-carcasses of the control and experimental groups did not differ on the 4th day of storage. The meat was pink, characteristic of pork, quite dense and springy when cut, with a specific smell characteristic of fresh meat (Figure 1).

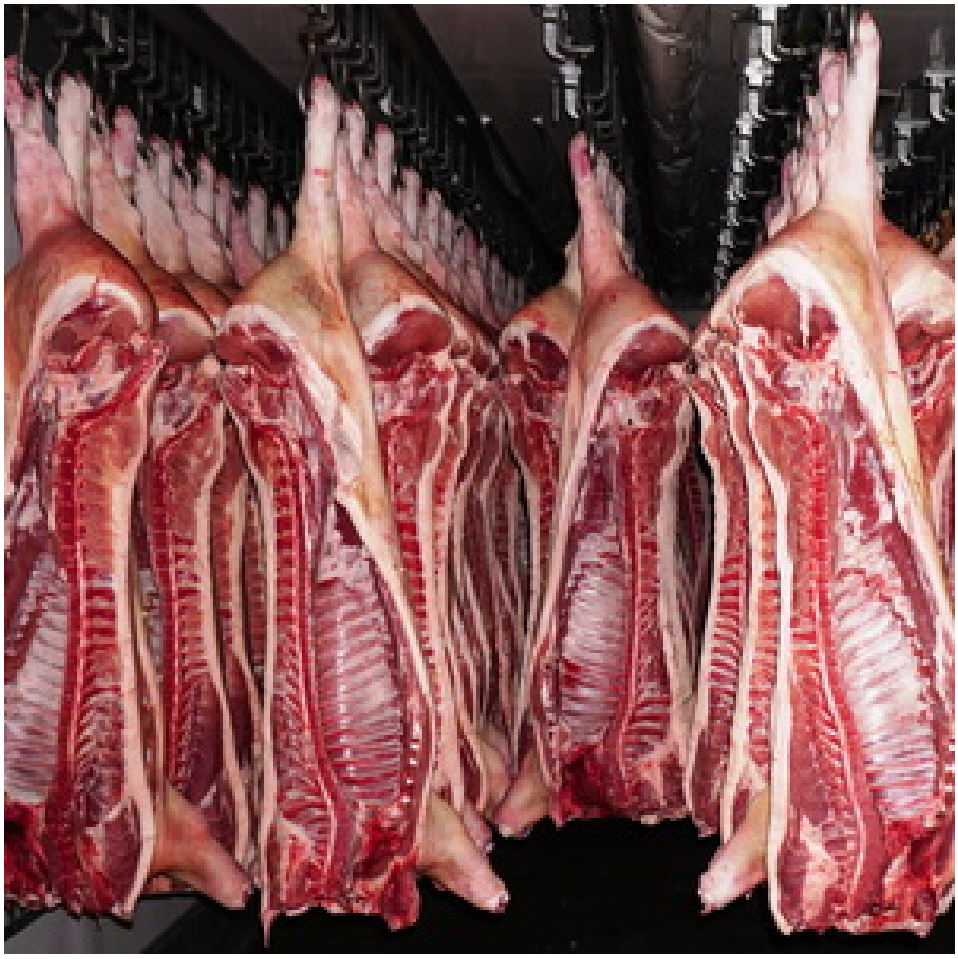


Figure 1 Storage of the exsperimentals groups of pork half carcasses for 4 days in the refrigerator.

During the storage of the pork half-carcasses in the refrigerating chambers, the process of drying, which is associated with moisture loss in the meat. The cooling rate of the carcasses of the slaughtered animals significantly impacts their weight loss due to the droplet evaporation **[37]** and thus, on the economic indicators of meat production **[38]**. As can be seen from the obtained data, the average weight of the paired carcass did not differ between the control and experimental groups (Table 4).

Table 4 Weight of pork half-carcass when stored in a chilled condition, kg, x ±SD, n = 10.

Group	Paired carcass	In a day of storage	In 4 days of storage
Control 1	44.01 ±5.09	43.01 ±4.98	42.72 ±4.93
Control 2	42.95 ±6.20	42.26 ±6.31	41.89 ±6.16
Experimental 1	43.31 ±3.89	42.71 ±3.71	42.35 ±3.68
Experimental 2	44.80 ±4.20	44.24 ±4.16	43.96 ±4.12

The biochemical processes and structural changes that occur in the muscles during the first 24 hours after slaughter play an important role in the meat's final quality and taste properties and are affected by the cooling processes to which the carcasses are subjected after slaughter. For the pork, due to exposure to high muscle temperatures and low pH value, a faster cooling process is required to reduce the development of pale, soft, and exudative (PSE) pork with a recommended core muscle temperature of 10 °C at 12h and 2-4 °C by 24h. Cooling with stiving is a system in which chilled water is applied to the carcasses at the early stage of post-mortem cooling. It is used to control carcass shrinkage and to improve the cooling rate through evaporative cooling **[39]**.

The greatest weight loss of the pork half-carcasses occurred during the first 24 hours when they were being cooled both without the use of stiving and with the use of stiving and the final processing of the pork half-carcasses with the suspensions of the lactic-acid microorganisms. The storage of the pork in a chilled condition for 1 day contributed to a decrease ($p \leq 0.05$) in the total half-carcass weight of control group 1 by 2.27%, control group 2 – by 1.65%, experimental group 1 – by 1.61%, and experimental group 2 – by 1.25%, in comparison with the output of the paired carcasses (Figure 2-5). Our experiment results comply with the data obtained for different cooling methods of the pork carcasses **[40]**, as well as for cooling methods of the beef carcasses **[41]**.

On the 4th day of storage of the pork in a chilled condition, the shrinkage of the pork half-carcasses of control group 1 was 2.93%, control group 2 – 2.50%, experimental group 1 – 2.22%, and experimental group 2 – 1.87%, in comparison with similar indicators of the paired carcasses (Figure 2-5). Such difference ($p \leq 0.05$) in the moisture loss of the pork half-carcasses is due to the accelerated cooling with the use of stiving and the decrease in the intensity of the moisture evaporation **[42]**.

In the period from 1 to 4 days, the intensity of the weight loss of the pork half-carcasses was in all cases lower ($p \leq 0.05$) than in the first 24 hours after slaughter (Figure 2-5). This is due to the achievement of the stable core temperature of the cooled half-carcasses and the decrease in the intensity of the evaporation process in the refrigerating chamber.

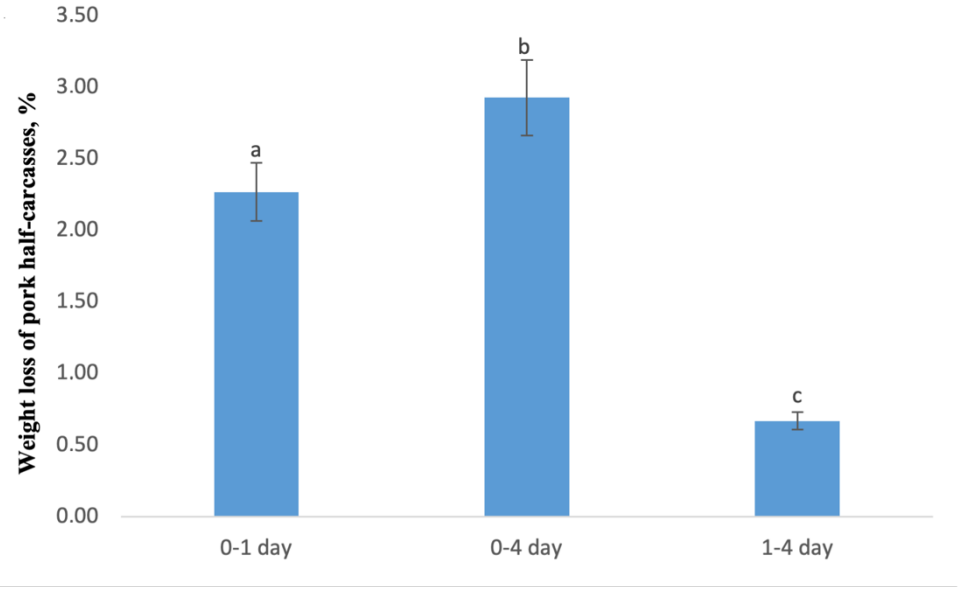


Figure 2 Weight loss of pork half-carcasses during traditional cooling, x ±SD, n = 10. Note: here and elsewhere, different upper indices [a, b, c] indicates the values that were significantly different in one column of the table ($p \leq 0.05$) according to the comparison results with the use of the Tukey test.

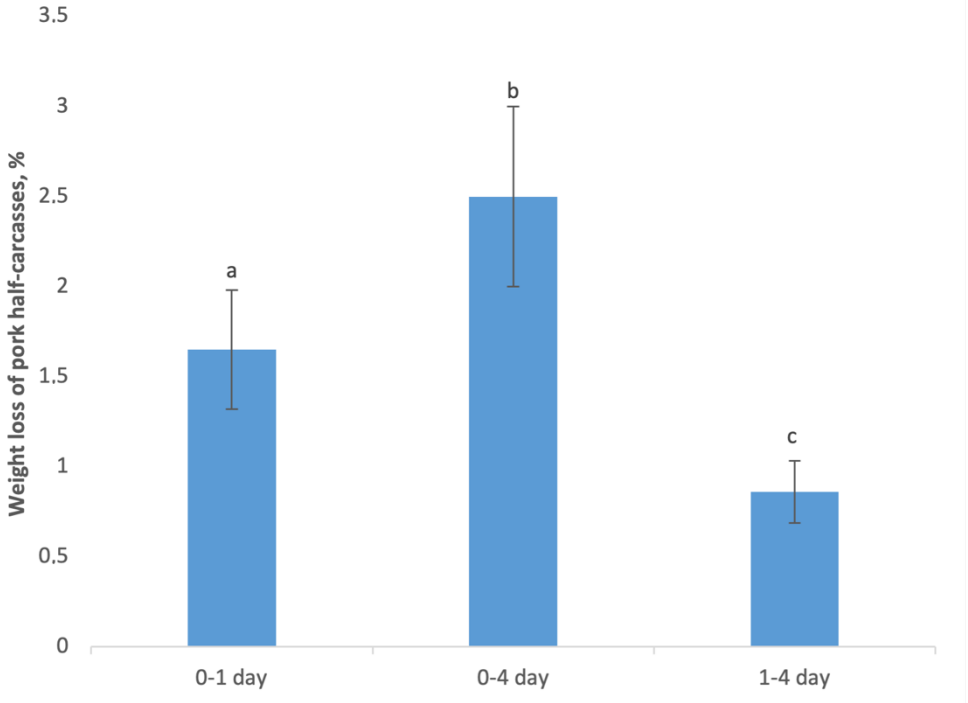


Figure 3 Weight loss of pork half-carcasses after cooling with the use of stiving, x ±SD, n = 10. Note: see Figure 2.

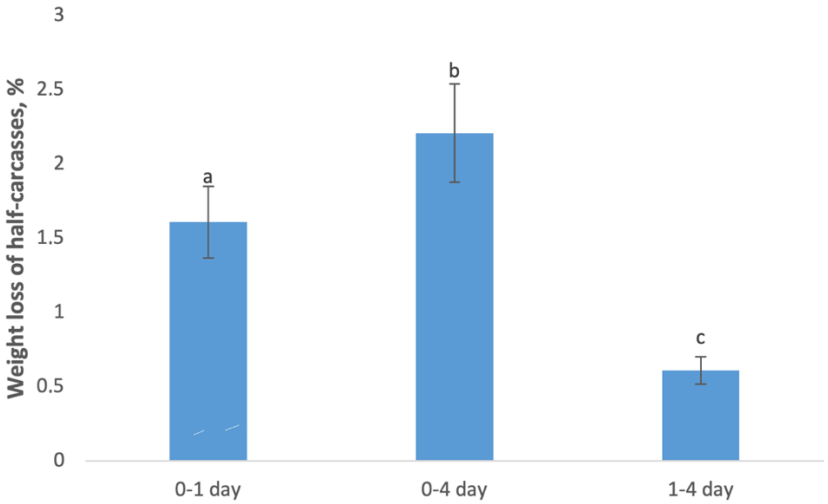


Figure 4 Weight loss of pork half-carcasses after cooling with the use of stiving and final processing with suspension of culture *Leuconostoc carnosum* (SafePro® B-SF-43), x ±SD, n = 10. Note: see Figure 2.

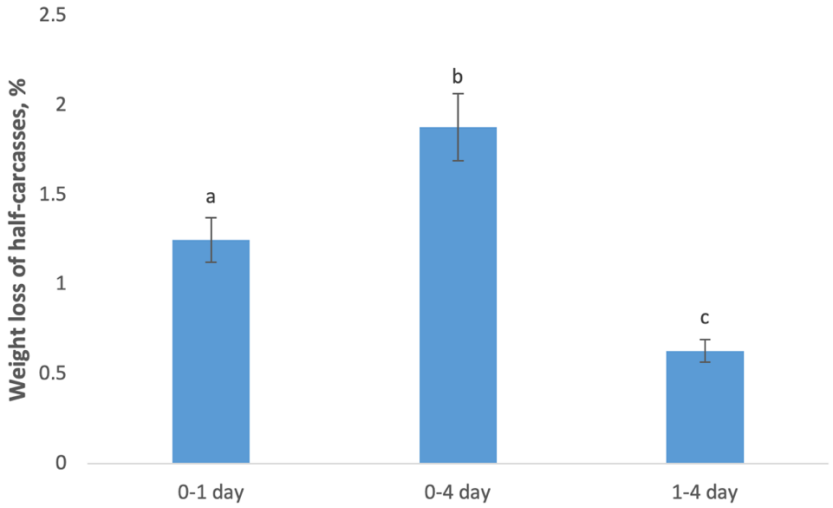


Figure 5 Weight loss of pork half-carcasses after cooling with the use of stiving and final processing with suspension of culture *Lactobacillus sakei* (SafePro® B-2), x ±SD, n = 10. Note: see Figure 2.

It's worth mentioning that meat is the main product through which food-origin pathogenic microorganisms are spreading among people **[43]**, **[44]** and depends on the observance of good hygiene practices at the enterprises for the production and circulation of meat products **[45]**, **[46]**. Microbiological study data obtained from the neck and spine areas of the pork half-carcasses 1 hour after cooling point to the fact that the pork of both the control and experimental groups met the requirements of the current regulations. However, QMAFAnM in the neck and spine areas of the pork half-carcasses of the experimental groups was an order of magnitude lower ($p \leq 0.05$) in comparison with the control groups (Table 5). In this connection, it's worth mentioning that the main microorganisms in the pork meat samples of the experimental groups were the lactic acid bacteria, which were used to process these half-carcasses. At the same time, the number of lactic acid bacteria was <1 log CFU/cm^3 in the pork half-carcasses of the control groups, which were cooled without stiving and with the use of striving. This amount of the lactic-acid microorganisms in the meat of the pork half-carcasses, which were subject to the final processing with the suspensions of the lactic-acid microorganisms, is associated with the ability of *Lactobacillus sakei* **[47]**, **[48]** and *Leuconostoc carnosum* **[49]**, **[50]** to withstand the temperature regime of the refrigerating chamber, the reduced pH-value of the meat and to use its nutritional ingredients for their development.

The initial contamination of the half-carcasses with the mold fungi and yeast was practically at the same level in the meat samples of the control and experimental pork groups in the neck area. Still, in the spine area of both control pork groups, it exceeded ($p \leq 0.05$) the similar values of both experimental options, where the final processing of the pork half carcasses with the starters of the lactic acid microorganisms was used (Table 5).

Table 5 Microbiological indicators of pork half-carcasses in 1 h after cooling, x ±SD, n = 5, log CFU/cm^2.

Group	QMAFAnM	Lactic-acid bacteria	Mold fungi and yeast
		Neck	
Control 1	3.05 ±0.70^{a}	<1^{c}	0.58 ±0.11^{a}
Control 2	2.85 ±0.34^{ab}	<1^{c}	0.68 ±0.07^{b}
Experimental 1	2.33 ±0.33^{b}	5.67 ±0.96^{a}	0.56 ±0.04^{a}
Experimental 2	2.39 ±0.07^{b}	6.07 ±0.41^{b}	0.54 ±0.09^{a}
		Spine	
Control 1	3.11 ±0.22^{a}	<1^{c}	0.68 ±0.11^{b}
Control 2	3.08 ±0.12^{a}	<1^{c}	0.73 ±0.13^{b}
Experimental 1	2.19 ±0.23^{b}	5.54 ±0.14^{a}	0.34 ±0.09^{a}
Experimental 2	2.39 ±0.21^{b}	5.98 ±0.18^{b}	0.34 ±0.08^{a}

Note: different upper indices $^{a, b, c}$ indicate the values that were significantly different in one column of the table within one sampling area ($p \leq 0.05$) according to the comparison results with the use of the Tukey test.

One of the promising directions of the safety provision of meat products is the use of natural antimicrobial agents in the technological process, in particular, bacteriocins, which are produced by lactic acid microorganisms **[51]**, **[52]**. Therefore, the processing of the pork half-carcasses with the suspension of the lactic acid microorganisms, which are capable of forming these substances, makes it possible to predict the reduction in the spread risk of microbial pathogens in the meat. No pathogenic microorganisms, in particular, *S. Aureus, L. monocytogenes,* and *Salmonella* spp. were detected in the meat of the paired pork half-carcasses, as well as during 4 days of storage in a chilled condition, which indicates their safety for the consumer (Tables 5-6).

Table 6 Microbiological indicators of pork half-carcasses on the 4th day of storage, x ± SD, n = 5, log CFU/cm^2.

Group	QMAFAnM	Lactic-acid bacteria	Mold fungi and yeast
		Neck	
Control 1	3.63 ±0.26^{a}	2.50 ±0.29^{a}	2.30 ±0.12^{a}
Control 2	3.69 ±0.37^{a}	2.80 ±0.38^{a}	2.09 ±0.75^{a}
Experimental 1	3.75 ±0.56^{a}	5.17 ±0.35^{b}	1.84 ±0.75^{a}
Experimental 2	3.80 ±0.56^{a}	5.73 ±0.34^{b}	1.89±0.70^{a}
		Spine	
Control 1	3.75 ±0.48^{a}	2.40 ±0.27^{a}	1.84 ±0.21^{a}
Control 2	3.66 ±0.52^{a}	2.39 ±0.27^{a}	1.79 ±0.17^{a}
Experimental 1	3.40 ±0.23^{a}	5.43 ±0.37^{b}	1.20 ±0.11^{b}
Experimental 2	3.62 ±0.60^{a}	5.82 ±0.54^{b}	1.24 ±0.08^{b}

Note: different upper indices $^{a, b}$ indicates the values that were significantly different in one column of the table ($p \leq 0.05$) according to the comparison results with the use of the Tukey test.

There was no significant difference in QMAFAnM on the 4th day of storage both in the washings from the meat surface of the experimental groups, which were subjected to the final processing with the suspension of the lactic-acid microorganisms and in the control groups of the pork half-carcasses, which were cooled without and with the use of striving. The quantity of the lactic acid bacteria on the surface of the pork half-carcasses did not differ significantly between the control groups, which indicates the absence of the influence of the stiving process on this indicator (Table 6).

At the same time, it's worth mentioning that the number of the lactic-acid bacteria in the washings from the neck and spine of the pork half-carcasses of the experimental groups, which were subjected to the final processing with the suspensions of the lactic-acid bacteria of strains SafePro® B-SF-43 and SafePro® B-2, was higher ($p \leq 0.05$) by almost 3 orders of magnitude, in comparison with both control groups. There was also an increase ($p \leq 0.05$) in the number of molds and yeasts in the meat of the pork half-carcasses of all experimental groups. Still, their number was significantly lower ($p \leq 0.05$) in the washings from the spine surface of the half-carcasses of the experimental groups.

This probably happened due to the processing of the pork half-carcasses with the microbial starters, the microorganisms that colonized the meat surface, and their ability to synthesize the bacteriocins **[53]**, **[54]**, microflora **[55]**.

The effectiveness of using suspensions of lactic acid bacteria strains SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) for the processing of pork carcasses is also related to the ability of these microorganisms to ferment glucose and synthesize lactic acid, which lowers the pH value of the environment and acts as a preservative that prevents the development and reproduction of pathogenic microflora and microflora capable of causing meat spoilage. This, in turn, reduces the intensity of destruction of muscle fibers and increases the shelf life of pork in a chilled form **[56]**. At the same time, *Lactobacillus sakei* and *Leuconostoc carnosum* are facultative anaerobes that can withstand the temperature of 2 °C typical of a cold room, which allows their growth in such a nutrient-rich environment as meat.

Several studies have proven that *L. sakei* suppresses the growth of *Colletotrichum gloeosporioides*, *Botrytis cinerea*, *Penicillium expansum,* and *Aspergillus flavus* due to the synthesis of 3-phenyllactic acid. *L. carnosum* is known to be able to synthesize, in addition to lactic acid, hydrogen peroxide and more than 4 heat-stable bacteriocins, including leucocin, mesentericin B105, mesentericin Y105 and/or mesentericin-like protein. In addition, *L. carnosum* can synthesize aromatic four-carbon metabolites, such as acetoin and butanediol from pyruvate, making it promising for fermented meat products **[57]**, **[58]**. In addition, *Lactobacillus sakei* and *Leuconostoc carnosum* strains SafePro® B-2 and SafePro® B-SF-43 do not form mucus, do not ferment starch, and withstand a significant concentration of table salt in an environment, which reaches 5-6%, which makes their use suitable for production of fermented sausage products, which contain pork.

Thus, the use of suspensions of lactic acid bacteria strains SafePro® B-2 (*Lactobacillus sakei*) and SafePro® B-SF-43 (*Leuconostoc carnosum*) is promising not only for processing pork half-carcasses but also for further study of the quality and safety of products made from this pork, their shelf life and appeal to consumers.

The study of the safety and quality of pork meat after cooling and treatment with lactic acid starters opens a wide range of promising directions for future research. Here are some ideas to explore:

Study of microbiological safety: Studies can focus on the detection and study of microorganisms on the surface of pork meat after treatment with lactic acid starters. It is important to investigate how these starter cultures affect the level of bacterial contamination and the safety of meat consumption.

Evaluation of meat quality: Research can be directed to evaluating the organoleptic properties (such as taste, aroma, and texture) of pork meat after treatment with lactic acid starters. This will help determine whether the meat retains its quality and appeal to consumers.

Study of effects on shelf life: Studies may include analyzing the shelf life of pork meat after treatment with lactic acid starters. This will help determine how effectively starter cultures increase the shelf life of meat and prevent it from spoiling.

Effectiveness of antimicrobial protection: It is important to investigate how effectively lactic acid starters reduce the risk of the spread of pathogenic microorganisms on the surface of pork meat and ensure its safety for consumption.

Development of new processing technologies: Research can be directed to developing new processing technologies for pork meat using lactic starters, which provide optimal results in terms of product quality and safety.

Consumer health impact studies: Research may include an analysis of the human health impact of consuming pork treated with lactic acid starters. This will help identify potential risks or benefits for consumers.

In general, research into the safety and quality of pork meat after cooling and treatment with lactic acid starters has great potential for developing new technologies that provide quality and safe meat for consumers.

CONCLUSION

Cooling of the pork half-carcasses in the refrigerating chamber with the use of stiving after 24 hours of storage reduces the weight loss of the pork half-carcasses by 0.62%, in combination with the final processing of the half-carcasses with the suspension of SafePro® B-SF-43 (*Leuconostoc carnosum*) – by 0.66% and SafePro® B-2 (*Lactobacillus sakei*) – by 1.02%, in comparison with the air cooling in the refrigerating chamber. Cooling of the pork half-carcasses in the refrigerating chamber with the use of stiving on the 4th day of storage makes it possible to reduce the shrinkage, on average, by 0.46%. The use of the lactic-acid starters of the microorganisms of strains SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) for the processing of the half-carcass surface reduces this indicator, on average, by 0.71% and by 1.06%. The microorganism cultures of strains SafePro® B-SF-43 (*Leuconostoc carnosum*) and SafePro® B-2 (*Lactobacillus sakei*) when applied to the surface of the pork half-carcasses at a dose of $10^6/cm^2$ contribute to the colonization of the meat with the useful lactic-acid microflora, which has a positive impact on the quality and safety indicators, and can be a promising technology element for extending the shelf life of the meat.

REFERENCES

1. Austrich-Comas, A., Jofré, A., Gou, P., & Bover-Cid, S. (2023). Assessing the impact of different technological strategies on the fate of salmonella in chicken dry-fermented sausages using challenge testing and predictive models. In Microorganisms (Vol. 11, Issue 2, p. 432). MDPI AG. https://doi.org/10.3390/microorganisms11020432
2. Azhar, N. S., Md Zin, N. H., & Tengku Abdul Hamid, T. H. (2017). Lactococcus lactis strain a5 produces nisin-like bacteriocin active against gram-positive and negative bacteria. In Tropical Life Sciences Research (Vol. 28, Issue 2, pp. 107–118). Penerbit Universiti Sains Malaysia. https://doi.org/10.21315/tlsr2017.28.2.8
3. Balan, P., Farouk, M. M., Stuart, A. D., Kemp, R., Staincliffe, M., Craige, C., & Kim, Y. H. B. (2019). Effects of electrical stimulation and pre-rigor conditioning temperature on the aging potential of hot-boned beef M. longissimus lumborum. In Animal Science Journal (Vol. 90, Issue 8, pp. 1050–1059). Wiley. https://doi.org/10.1111/asj.13217
4. Bal'-Prylypko, L. V., Derevyanko, L. P., Slobodyanyuk, N. M., Starkova, E. R., & Androshchiuk, O. S. (2018). Using of the Ampullaria glauca snails' caviar for correction of the effects of the ionizing radiation exposure in a small dose. In Nuclear Physics and Atomic Energy (Vol. 19, Issue 2, pp. 159–165). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/jnpae2018.02.159
5. Bodie, A. R., O'Bryan, C. A., Olson, E. G., & Ricke, S. C. (2023). Natural Antimicrobials for *Listeria Monocytogenes* in Ready-to-Eat Meats: Current Challenges and Future Prospects. In Microorganisms (Vol. 11, Issue 5, p. 1301). MDPI AG. https://doi.org/10.3390/microorganisms11051301
6. Bredholt, S., Nesbakken, T., & Holck, A. (2001). Industrial application of an antibacterial strain of Lactobacillus sakei as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats. In International Journal of Food Microbiology (Vol. 66, Issue 3, pp. 191–196). Elsevier BV. https://doi.org/10.1016/s0168-1605(00)00519-5
7. Zheplinska, M., Mushtruk, M., Vasyliv, V., Sarana, V., Gudzenko, M., Slobodyanyuk, N., Kuts, A., Tkachenko, S., & Mukoid, R. (2021). The influence of cavitation effects on the purification processes of beet sugar production juices. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 18–25). HACCP Consulting. https://doi.org/10.5219/14
8. Candeliere, F., Raimondi, S., Spampinato, G., Tay, M. Y. F., Amaretti, A., Schlundt, J., & Rossi, M. (2020). Draft genome sequences of 12 leuconostoc carnosum strains isolated from cooked ham packaged in a modified atmosphere and from fresh sausages. In C. Putonti (Ed.), Microbiology Resource Announcements (Vol. 9, Issue 2). American Society for Microbiology. https://doi.org/10.1128/mra.01247-19
9. Shevchenko, L. V., Dobrozhan, Y. V., Mykhalska, V. M., Osipova, T. Y., & Solomon, V. V. (2019). Contamination of hen manure with nine antibiotics in poultry farms in Ukraine. In Regulatory Mechanisms in Biosystems (Vol. 10, Issue 4, pp. 532–537). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/021978
10. Chen, H., Tian, F., Li, S., Xie, Y., Zhang, H., & Chen, W. (2012). Cloning and heterologous expression of a bacteriocin sakacin P from *Lactobacillus sakei* in *Escherichia coli*. In Applied Microbiology and Biotechnology (Vol. 94, Issue 4, pp. 1061–1068). Springer Science and Business Media LLC. https://doi.org/10.1007/s00253-012-3872-z
11. Chen, L., Bai, Y., Everaert, N., Li, X., Tian, G., Hou, C., & Zhang, D. (2019). Effects of protein phosphorylation on glycolysis through the regulation of enzyme activity in ovine muscle. In Food Chemistry (Vol. 293, pp. 537–544). Elsevier BV. https://doi.org/10.1016/j.foodchem.2019.05.011
12. Vasyliv, V., Mushtruk, M., Zheplinska, M., Mukoid, R., & Tkachenko, S. (2021). Method of Electrohydraulic Activation of Water-Lime Suspension in Sugar Production. In Lecture Notes in Mechanical Engineering (pp. 664–673). Springer International Publishing. https://doi.org/10.1007/978-3-030-91327-4_64
13. Costa, J. C. C. P., Bover-Cid, S., Bolívar, A., Zurera, G., & Pérez-Rodríguez, F. (2019). Modelling the interaction of the sakacin-producing *Lactobacillus sakei CTC494* and *Listeria monocytogenes* in filleted gilthead sea bream (Sparus aurata) under modified atmosphere packaging at isothermal and non-isothermal conditions. In International Journal of Food Microbiology (Vol. 297, pp. 72–84). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2019.03.002
14. Cherednichenko, O., Bal-Prylypko, L., Paska, M., & Nikolaenko, M. (2021). Expediency of creation of technology of production of meat products of long term of storage of the combined structure. In IOP Conference Series: Earth and Environmental Science (Vol. 723, Issue 3, p. 032086). IOP Publishing. https://doi.org/10.1088/1755-1315/723/3/032086

15. Geeraerts, W., Pothakos, V., De Vuyst, L., & Leroy, F. (2018). Variability within the dominant microbiota of sliced cooked poultry products at expiration date in the Belgian retail. In Food Microbiology (Vol. 73, pp. 209–215). Elsevier BV. https://doi.org/10.1016/j.fm.2018.01.019
16. Gonzalez-Fandos, E., Herrera, B., & Maya, N. (2009). Efficacy of citric acid against *Listeria monocytogenes* attached to poultry skin during refrigerated storage. In International Journal of Food Science & Technology (Vol. 44, Issue 2, pp. 262–268). Wiley. https://doi.org/10.1111/j.1365-2621.2007.01673.x
17. Kochkodan, O., Slobodianiuk, N., Kovshun, L., & Hyzhan, O. (2020). Molecular Interactions in Binary Surfactant Solutions: Effect of Ionic Counterpart. In Journal of the Mexican Chemical Society (Vol. 64, Issue 3). Sociedad Quimica de Mexico, A.C. https://doi.org/10.29356/jmcs.v64i3.1153
18. Cherednichenko, O., & Bal-Prylypko, L. (2020). Rationale and economic feasibility of improving the technology of long-term storage of meat products. In IOP Conference Series: Earth and Environmental Science (Vol. 548, Issue 2, p. 022053). IOP Publishing. https://doi.org/10.1088/1755-1315/548/2/022053
19. Janßen, D., Dworschak, L., Ludwig, C., Ehrmann, M. A., & Vogel, R. F. (2020). Interspecies assertiveness of *Lactobacillus curvatus* and *Lactobacillus sakei* in sausage fermentations. In International Journal of Food Microbiology (Vol. 331, p. 108689). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2020.108689
20. Rules of pre-slaughter veterinary inspection of animals and veterinary-sanitary examination of meat and meat products. Order of the Head of the State Department of Veterinary Medicine dated June 7, 2002 No. 28; registered in the Ministry of Justice of Ukraine dated June 21, 2002 No. 524/6812. 2002. 46 p.
21. DSTU 7992:2015. Meat and meat raw materials. Methods of sampling and organoleptic assessment of freshness.
22. DSTU 8381:2015. Meat and meat products. Organization and methods of microbiological research.
23. DSTU ISO 17604:2014 (ISO 17604:2003, IDT). Microbiology of food products and animal feed. Sampling of animal carcasses for microbiological analysis.
24. Suchenko, Y., Suchenko, V., Mushtruk, M., Vasyliv, V., & Boyko, Y. (2017). Changing the quality of ground meat for sausage products in the process of grinding. In Eastern-European Journal of Enterprise Technologies (Vol. 4, Issue 11 (88), pp. 56–63). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2017.108876
25. Ugnivenko, A., Getya, A., Nosevych, D., Antoniuk, T., Kruk, O., Slobodyanyuk, N., Ivaniuta, A., Omelian, A., Gryshchenko, S., & Israelian, V. (2022). The study of "muscle eye" in bulls of Ukrainian black-spotted dairy-meat breed as a factor in improving the properties of meat products. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 519–529). HACCP Consulting. https://doi.org/10.5219/1762
26. Khatri, Y., & Huff-Lonergan, E. (2023). Effect of postmortem pH on the physical, biochemical, and sensory characteristics of bovine longissimus thoracis et lumborum muscle. In Food Science & Nutrition (Vol. 11, Issue 4, pp. 2002–2011). Wiley. https://doi.org/10.1002/fsn3.3235
27. Lonergan, S. M., Huff-Lonergan, E., & Hunt, M. C. (2018). Understanding postmortem biochemical processes and post-harvest ageing factors to develop novel smart-aging strategies. In Meat Science (Vol. 144, pp. 74–90). Elsevier BV. https://doi.org/10.1016/j.meatsci.2018.04.031
28. Kumariya, R., Garsa, A. K., Rajput, Y. S., Sood, S. K., Akhtar, N., & Patel, S. (2019). Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. In Microbial Pathogenesis (Vol. 128, pp. 171–177). Elsevier BV. https://doi.org/10.1016/j.micpath.2019.01.002
29. Martín, I., Rodríguez, A., Delgado, J., & Córdoba, J. J. (2022). Strategies for Biocontrol of *Listeria Monocytogenes* Using Lactic Acid Bacteria and Their Metabolites in Ready-to-Eat Meat- and Dairy-Ripened Products. In Foods (Vol. 11, Issue 4, p. 542). MDPI AG. https://doi.org/10.3390/foods11040542
30. Morey, A., Bowers, J. W., Bauermeister, L. J., Singh, M., Huang, T. S., & McKee, S. R. (2014). Effect of salts of organic acids on *Listeria monocytogenes,* shelf life, meat quality, and consumer acceptability of beef frankfurters. In Journal of Food Science (Vol. 79, Issue 1). Wiley. https://doi.org/10.1111/1750-3841.12220
31. Nastasijevic, I., Milanov, D., Velebit, B., Djordjevic, V., Swift, C., Painset, A., & Lakicevic, B. (2017). Tracking of *Listeria monocytogenes* in meat establishment using Whole Genome Sequencing as a food safety management tool: a proof of concept. In International Journal of Food Microbiology (Vol. 257, pp. 157–164). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2017.06.015
32. Odetokun, I. A., Ballhausen, B., Adetunji, V. O., Ghali-Mohammed, I., Adelowo, M. T., Adetunji, S. A., & Fetsch, A. (2018). *Staphylococcus aureus* in two municipal abattoirs in Nigeria: Risk perception, spread and public health implications. In Veterinary Microbiology (Vol. 216, pp. 52–59). Elsevier BV. https://doi.org/10.1016/j.vetmic.2018.01.022
33. Zheplinska, M., Mushtruk, M., Vasyliv, V., Kuts, A., Slobodyanyuk, N., Bal-Prylypko, L., Nikolaenko, M., Kokhan, O., Reznichenko, Y., & Salavor, O. (2021). The micronutrient profile of medicinal plant extracts.

In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 528–535). HACCP Consulting. https://doi.org/10.5219/1553

34. Pandey, N., Malik, R., Kaushik, J., Singroha, G. (2013). Gassericin A: a circular bacteriocin produced by the lactic acid bacteria *Lactobacillus gasseri*. In World Journal of Microbiology and Biotechnology (Vol. 29, Issue 11, pp. 1977–1987). Springer Science and Business Media LLC. https://doi.org/10.1007/s11274-013-1368-3

35. Rogoskii, I., Mushtruk, M., Titova, L., Snezhko, O., Rogach, S., Blesnyuk, O., Rosamaha, Y., Zubok, T., Yeremenko, O., & Nadtochiy, O. (2020). Engineering management of starter cultures in study of temperature of fermentation of sour-milk drink with apiproducts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 1047–1054). HACCP Consulting. https://doi.org/10.5219/1437

36. Raimondi, S., Luciani, R., Sirangelo, T. M., Amaretti, A., Leonardi, A., Ulrici, A., Foca, G., D'Auria, G., Moya, A., Zuliani, V., Seibert, T. M., Søltoft-Jensen, J., & Rossi, M. (2019). Microbiota of sliced cooked ham packaged in a modified atmosphere throughout the shelf life. In International Journal of Food Microbiology (Vol. 289, pp. 200–208). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2018.09.017

37. Mushtruk, M., Deviatko, O., Ulianko, S., Kanivets, N., & Mushtruk, N. (2021). An Agro-Industrial Complex Fat-Containing Wastes Synthesis Technology in Ecological Biofuel. In Lecture Notes in Mechanical Engineering (pp. 361–370). Springer International Publishing. https://doi.org/10.1007/978-3-030-77823-1_36

38. Raimondi, S., Spampinato, G., Candeliere, F., Amaretti, A., Brun, P., Castagliuolo, I., & Rossi, M. (2021). Phenotypic Traits and Immunomodulatory Properties of *Leuconostoc carnosum* Isolated From Meat Products. In Frontiers in Microbiology (Vol. 12). Frontiers Media SA. https://doi.org/10.3389/fmicb.2021.730827

39. Rani, Z. T., Mhlongo, L. C., & Hugo, A. (2023). Microbial profiles of meat at different stages of the distribution chain from the abattoir to retail outlets. In International Journal of Environmental Research and Public Health (Vol. 20, Issue 3, p. 1986). MDPI AG. https://doi.org/10.3390/ijerph20031986

40. Bayer, O. V., Kaminska, O. V., Shevchenko, L. V., Mykhalska, V. M., Stupak, O. M., Bondarets, O. V., & Dobrozhan, Yu. (2019). Development and evaluation of the suitability of the method for determining the content of egg coccidiostatics using ultra performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS). In Methods and Objects of Chemical Analysis: Vol. Vol. 14, No.1 (pp. 43–51). Taras Shevchenko National University of Kyiv. https://doi.org/10.17721/moca.2019.43-51

41. Richardson, E. L., Fields, B., Dilger, A. C., & Boler, D. D. (2018). The effects of ultimate pH and color on sensory traits of pork loin chops cooked to a medium-rare degree of doneness. In Journal of Animal Science (Vol. 96, Issue 9, pp. 3768–3776). Oxford University Press (OUP). https://doi.org/10.1093/jas/sky258

42. Rivas, L., Dupont, P. Y., Wilson, M., Rohleder, M., & Gilpin, B. (2019). An outbreak of multiple genotypes of *Listeria monocytogenes* in New Zealand linked to contaminated ready-to-eat meats retrospective analysis using whole-genome sequencing. In Letters in Applied Microbiology (Vol. 69, Issue 6, pp. 392–398). Oxford University Press (OUP). https://doi.org/10.1111/lam.13227

43. Rybarczyk, A., Karamucki, T., Pietruszka, A., Rybak, K., & Matysiak, B. (2015). The effects of blast chilling on pork quality. In Meat Science (Vol. 101, pp. 78–82). Elsevier BV. https://doi.org/10.1016/j.meatsci.2014.11.006

44. Bober, A., Liashenko, M., Protsenko, L., Slobodyanyuk, N., Matseiko, L., Yashchuk, N., Gunko, S., & Mushtruk, M. (2020). Biochemical composition of the hops and quality of the finished beer. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 307–317). HACCP Consulting. https://doi.org/10.5219/1311

45. Sikes, A. L., Jacob, R., D'Arcy, B., & Warner, R. (2017). Very fast chilling modifies the structure of muscle fibres in hot-boned beef loin. In Food Research International (Vol. 93, pp. 75–86). Elsevier BV. https://doi.org/10.1016/j.foodres.2016.12.027

46. Pylypchuk, O., Tyshchenko, L., Israelian, V., & Mushtruk, N. (2022). Influence of parameters of marinating meat semi-finished products on the quality of the finished product. In Animal Science and Food Technology (Vol. 13, Issue 2). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(2).2022.44-52

47. Holembovska, N., Tyshchenko, L., Slobodyanyuk, N., Israelian, V., Kryzhova, Y., Ivaniuta, A., Pylypchuk, O., Menchynska, A., Shtonda, O., & Nosevych, D. (2021). Use of aromatic root vegetables in the technology of freshwater fish preserves. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 296–305). HACCP Consulting. https://doi.org/10.5219/1581

48. Tomović, V. M., Petrović, L. S., & Džinić, N. R. (2008). Effects of rapid chilling of carcasses and time of deboning on weight loss and technological quality of pork semimembranosus muscle. In Meat Science (Vol. 80, Issue 4, pp. 1188–1193). Elsevier BV. https://doi.org/10.1016/j.meatsci.2008.05.013

49. Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. In Frontiers in Bioengineering and Biotechnology (Vol. 9). Frontiers Media SA. https://doi.org/10.3389/fbioe.2021.612285

50. Wen, X., & James S., D. (2012). Survival of *Campylobacter jejuni* and *Salmonella enterica Typhimurium* in Vacuum-Packed, Moisture-Enhanced Pork. In Journal of Food Protection (Vol. 75, Issue 3, pp. 576–579). Elsevier BV. https://doi.org/10.4315/0362-028x.jfp-11-343

51. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/1711

52. Zagorec, M., & Champomier-Vergès, M.-C. (2017). Lactobacillus sakei: A Starter for Sausage Fermentation, a Protective Culture for Meat Products. In Microorganisms (Vol. 5, Issue 3, p. 56). MDPI AG. https://doi.org/10.3390/microorganisms5030056

53. Zdolec, N., Kotsiri, A., Houf, K., Alvarez-Ordóñez, A., Blagojevic, B., Karabasil, N., Salines, M., & Antic, D. (2022). Systematic review and meta-analysis of the efficacy of interventions applied during primary processing to reduce microbial contamination on pig carcasses. In Foods (Vol. 11, Issue 14, p. 2110). MDPI AG. https://doi.org/10.3390/foods11142110

54. Ugnivenko, A., Kos, N., Nosevych, D., Mushtruk, M., Slobodyanyuk, N., Zasukha, Y., Otchenashko, V., Chumachenko, I., Gryshchenko, S., & Snizhko, O. (2022). The yield of adipose tissue and by-products in the course of the slaughter of inbred and outbred bulls of the Ukrainian beef breed. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 307–319). HACCP Consulting. https://doi.org/10.5219/1758

55. Zwirzitz, B., Wetzels, S. U., Dixon, E. D., Stessl, B., Zaiser, A., Rabanser, I., Thalguter, S., Pinior, B., Roch, F.-F., Strachan, C., Zanghellini, J., Dzieciol, M., Wagner, M., & Selberherr, E. (2020). The sources and transmission routes of microbial populations throughout a meat processing facility. In npj Biofilms and Microbiomes (Vol. 6, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41522-020-0136-z

56. Candeliere, F., Raimondi, S., Spampinato, G., Tay, M. Y. F., Amaretti, A., Schlundt, J., & Rossi, M. (2021). Comparative Genomics of Leuconostoc carnosum. In Frontiers in Microbiology (Vol. 11). Frontiers Media SA. https://doi.org/10.3389/fmicb.2020.605127

57. Vovkotrub, V., Iakubchak, O., Horalskyi, L., Vovkotrub, N., Shevchenko, L., Shynkaruk, N., Rozbytska, T., Slyva, Y., Tupitska, O., & Shtonda, O. (2023). The microscopic structure of pork neck after cooling with showering stiving and processing by culture Lactobacillus sakei. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 759–776). HACCP Consulting. https://doi.org/10.5219/1905

58. Zagorec, M., & Champomier-Vergès, M.-C. (2017). Lactobacillus sakei: A Starter for Sausage Fermentation, a Protective Culture for Meat Products. In Microorganisms (Vol. 5, Issue 3, p. 56). MDPI AG. https://doi.org/10.3390/microorganisms5030056

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Dr. for Olga Iakubchak

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

Ethical committee approval. The study protocols and experimental procedures were approved by the Commission on Bioethics National University of Life and Environmental Sciences of Ukraine. Keeping laying hens in the vivarium at the Faculty of Veterinary Medicine, at the National University of Life and Environmental Sciences of Ukraine was conducted by Council Directive 1999/74/EC. The data acquisition was performed by Law of Ukraine „On Protection of Animals from Cruelty" No 3447-IV from 04.08.2017 and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes of 1986.

Contact Address:

Volodymyr Vovkotrub, National University of Life and Environmental Sciences of Ukraine, Department of Veterinary Hygiene, Faculty of Veterinary Medicine, Vystavkova Str. 16, 03127, Kyiv, Ukraine,
E-mail: vetmed_bcrda@ukr.net
ORCID: https://orcid.org/0009-0007-2760-4559

Olha Iakubchak, National University of Life and Environmental Sciences of Ukraine, Department of Veterinary Hygiene, Faculty of Veterinary Medicine, Vystavkova Str. 16, 03127, Kyiv, Ukraine,
E-mail: yakubchak_om@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-9390-6578

Nataliia Vovkotrub, Bila Tserkva National Agrarian University, Faculty of Veterinary Medicine, Department of Propaedeutics and Internal Diseases of Animals and Poultry named after V.I. Levchenko, pl. Soborna, 8/1, 09117, Bila Tserkva, Ukraine,
E-mail: nataliia.vovkotrub@btsau.edu.ua
ORCID: https://orcid.org/0000-0003-3297-454X

Larysa Shevchenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Veterinary Hygiene, Vystavkova Str. 16, 03127, Kyiv, Ukraine,
E-mail: shevchenko_laris@ukr.net
ORCID: http://orcid.org/0000-0001-7472-4325

Tetiana Lebedenko, Odesa National University of Technology, Faculty of Innovative Food Technologies and Restaurant, Hotel Business, Hotel and Restaurant Business Departments, Odesa, 112 Kanatna Street, 65039, Ukraine,
E-mail: RGSiT_ONAHT@i.ua
ORCID: https://orcid.org/0000-0001-8385-4674

Nataliia Holembovska, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technology of meat, fish and marine products, Vystavkova Str. 16, 03127, Kyiv, Ukraine,
E-mail: natashagolembovska@gmail.com
ORCID: https://orcid.org/0000-0001-8159-4020

***Oksana Pylypchuk**, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Management of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Vystavkova Str. 16, 03127, Kyiv, Ukraine,
E-mail: pilipchuk_os@ukr.net
ORCID: https://orcid.org/0000-0002-2757-6232

Alina Omelian, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technologies of Meat, Fish and Seafood, Vystavkova Str. 16, 03127, Kyiv, Ukraine,
E-mail: alina_omelian@nubip.edu.ua
ORCID: https://orcid.org/0000-0001-9004-5250

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 2.2.2024
Revised: 20.3.2024
Accepted: 16.4.2024
Published: 20.5.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 453-466
https://doi.org/10.5219/1958
ISSN: 1337-0960 online
www.potravinarstvo.com

A flour composite mixture for gluten-free confectionery

Tsira Khutsidze, Eliza Pruidze, Maria Silagadze, Eliso Dzneladze, George Pkhakadze, Irma Berulava

ABSTRACT

The article is devoted to the development of a recipe for a gluten-free flour composite mixture based on buckwheat, rice, and corn flours for the preparation of confectionery products, as well as the enhancement of its nutritional value using locally produced flaxseed and chickpea flours. For the production of gluten-free sugar cookies, two types of gluten-free flour composite mixture have been developed: a) rice, buckwheat, and flaxseed flour and b) rice, corn, and chickpea flour. The optimal flour ratio is 40:30:30. The physic-chemical and organoleptic indicators of gluten-free sugar cookies obtained using the gluten-free flour composite mixture align with the indicators specified in the standard. The developed gluten-free composite flour mixture is characterized by a fairly high satisfaction level with the balanced nutrition formula regarding basic nutrients. The levels of protein, carbohydrate, and fat satisfaction increased by 64, 37, and 118%, respectively, with the addition of flax to the rice and buckwheat mixture. Adding chickpeas to the rice and corn mixture increased protein and fat satisfaction by 35.5 and 27%, respectively, while carbohydrates remained almost unchanged.

Keywords: gluten-free flour confectionery, rice, buckwheat, flax, chickpea

INTRODUCTION

The number of people suffering from celiac disease and gluten sensitivity is increasing every year worldwide, including Georgia. The most common treatment for such people is a lifelong gluten-free diet, which has increased demand for gluten-free flour products. A significant shortcoming of available gluten-free products is their low protein content and high levels of fat and salt. To comply with recommendations regarding nutrition, it is necessary to develop the products and change the recipe **[1]**.

The number of products intended for gluten-free diets in Georgia is still small, mainly represented by imported products, which are expensive. In addition to bakery products, thanks to their convenience, unique taste, and texture, gluten-free flour confectionery such as cookies, cakes, muffins, and crackers are of great interest. Scientists have recently developed technologies for producing gluten-free flour products, but more is needed. There is evidence in the literature about the preparation of gluten-free flour products, their nutritional value, and their sensory properties. The problems of knowledge and information in this area of research have been identified, and the need for further research is noted.

The main way to produce gluten-free products is to completely replace gluten-containing raw materials with non-toxic grain crops. In addition, rice, buckwheat, and corn are mostly used as gluten-free flour. The nutritional value of their products is because they consist mostly of starch-containing products. Therefore, two or three gluten-free raw materials should be combined, or the recipe's nutritional value should be increased using raw materials rich in essential amino acids, vitamins, mineral substances, and dietary fibers. For this purpose, it is promising to use non-traditional local raw materials, the right selection of which provides the normalization of the functions of the digestive system and, in general, metabolism in the bodies of patients with celiac disease **[2]**, **[3]**, **[4]**.

Based on the above, developing a gluten-free flour composite mixture recipe using local, non-traditional raw materials is relevant.

The novelty of the research lies in the development of a recipe of a gluten-free composite flour mixture based on buckwheat, rice, and corn flours and increasing its nutritional value using flaxseed and chickpea flours.

Flour products are primarily made of wheat flour. The production of gluten-free products is a big challenge for manufacturers. The main fraction of gluten (glutenin and gliadin) plays a decisive role in forming technological properties that determine the dough's water absorption capacity, viscosity, and elasticity. Consequently, gluten removal poses a serious problem for manufacturers. The main task is to find a suitable substitute for gluten. Food safety, acceptability, and availability are also big concerns because they should comply with the FDA-approved recommendations **[5]**.

Although the number of gluten-free products on the market has increased, there are still some gaps in their nutritional value and organoleptic indicators. Commercially viable gluten-free products are low in nutritional value, especially in protein and dietary fiber content, and have a high glycemic index. On the other hand, from an organoleptic viewpoint, gluten-free products do not have the appropriate texture, mechanical properties, taste, and aroma. This results from selecting raw materials containing many carbohydrates **[6]**.

The composition of gluten-free flour dominates in forming gluten-free products' texture and organoleptic indicators. However, other factors such as grain milling methods, flour particle sizes, and processing may have a specific influence. Gluten-free cookies, cakes, cupcakes, and crackers are inferior to wheat-based products in terms of consumer properties. Further research is needed to obtain more delicious and edible gluten-free products **[7]**, **[8]**.

The goal of the work is to develop a recipe for a gluten-free flour composite mixture for the preparation of flour confectionery.

To improve the structure and quality of gluten-free flour products and to extend their shelf life, pseudocereals (amaranth, buckwheat), completely ground grains, a dietary fibre obtained from secondary products of fruit and vegetable processing, and alternative flours (chia seeds, chestnut, etc.) are used. The reason for this is their ability to absorb water and form a gel, resulting in the texture's formation and thickening **[9]**.

Various scientists have investigated the possibility of using nontraditional raw materials with low starch content to increase the nutritional value of gluten-free products **[9]**. Flax and chickpea additives have been common in Georgia since ancient times, and in recent years, farmers' interest has grown **[10]**.

Flaxseed flour contains a large amount of easily digestible protein, omega-3 oils, vitamins of group B, and other useful substances. Flax occupies the first place in dietary nutrition. The gastrointestinal tract does not absorb many substances in flaxseed flour, but they remove toxins and bad cholesterol. These include cellulose, phenolic polymers, lignin, etc. Phytoestrogens contained in flour ensure the metabolism of hormones, maintain their natural level, and also have an antioxidant effect **[11]**, **[12]**, **[13]**, **[14]**.

Non-traditional flour is an additional source of protein and a valuable alternative to a nutrient-rich and useful raw material in the production of flour products. Legumes like chickpeas have become more relevant in baking because they have properties beneficial to human health. It reduces obesity and type 2 diabetes. It is a source of bioavailable protein. The chemical composition of chickpea flour is characterized by the content of starch and proteins, 37% and 23%, respectively, and the amount of fat, which is equal to 5%. The albumin and globulin fractions predominate among proteins. It can be used to develop innovative products of high biological value **[15]**. Adding chickpea flour to food increases its nutritional value and maybe a new way to reduce the amount of acrylamide in the product **[14]**.

Numerous studies have been conducted by various researchers on the possibility of using non-traditional raw materials such as amaranth **[15]**, grape seed **[5]**, flax seeds **[8]**, **[11]**, soya **[9]**, chickpea **[6]**, **[10]**, and chestnut flours **[14]** to increase the nutritional value and quality of gluten-free products.

Scientific Hypothesis

The quality indicators of gluten-free flour pastry products prepared using the gluten-free composite mixture obtained from rice, buckwheat, and corn flour align with those specified in the standard. Adding flax and chickpea flour increases the mix's nutritional value.

MATERIAL AND METHODOLOGY

Samples

The objects of the study are control samples of sugar cookies **[16]**, in which wheat flour is entirely replaced by a basic gluten-free flour composite mixture, and test samples, in which gluten-free flour composite mixtures completely replace wheat flour. The basic gluten-free flour composite mixture was prepared by mixing rice and buckwheat flour or rice and corn flour. In contrast, the gluten-free flour composite mixtures were made by adding

flaxseed or chickpea flour to the essential composite mixtures. Flax and chickpeas are grown on various farms in western Georgia. We bought the rest of the raw materials, such as rice, buckwheat, sugar, molasses, and essences, at the Kutaisi market. We obtained the flour by grinding these raw materials in the laboratory and then sifted into a sieve with mesh sizes of 0.5 mm.

Chemicals

All reagents used were of U.S.P. purity or higher. All solvents, including water, were used with the LC/MS label.

Animals, Plants and Biological Materials

Rice (*Oryza sativa*), buckwheat (*Fagopyrum esculentumi*), flax (*Linum usitatissimum*) chickpea (*Cicer arietinum L.*), corn (*Zea mays*).

Instruments

The mass of the sample was determined by an electronic digital analytical balance SF-400C model (Toms, Qilin, China).

For the control and experimental baking of sugar cookies, we used an electric oven with a proofer FDE-903-HR Primax Fast Line Combi Oven (Primax, Via Gemona, San Vito al Tagliamento (PN), Italy).

Laboratory Methods

Organoleptic and physico-chemical research methods according to the ISO-International Organization for Standardization were used.

Organoleptic indicators were determined on a 10-point scale according to the following characteristics: surface condition, colour, taste, smell, sectional view, and shape **[17]**, where 1 means extreme dislike, and 10 means extreme like. At least 5 testers took part in the assessment of organoleptic indicators. They were selected from Akaki Tsereteli State University staff working at the Department of Food Technology and students. Final scores were calculated as the arithmetic mean for each characteristic.

To evaluate the quality of the finished products, we measured the moisture content of cookies, alkalinity, swelling capacity, and organoleptic indicators.

We determined the moisture content of the sugar cookies by drying up the test sample at a temperature of 130 ^{0}C to a constant weight. The initial mass of the sample and the mass after drying were determined with a weighing accuracy of 0.01 g. We calculated the mass loss by the difference in mass of the test sample before drying and after drying according to the state standard GOST 5900-2014 **[18]** and expressed the result as a percentage:

$$W\ \% = \frac{M_1 - M_2}{M_1}\ x\ 100\ \%, \quad (1)$$

Where:

W % is the sample's moisture content (as a percentage), M1 is the sample's initial mass before drying (in grams), and M2 is the sample's mass after drying (in grams).

The alkalinity of cookies was determined by neutralizing the alkaline substances in the sample with a solution of sulfuric acid (Sulphuric acid, H_2SO_4, LenReaktiv, ≥99.0%) with a concentration of 0.1 mol.dm^{-3} in the presence of the indicator bromothymol blue (Bromothymol blue, $C_{27}H_{28}Br_2O_5S$, LenReaktiv, ≥99.5 %) until the formation of the yellow body. The method determines the alkalinity of flour confectionery made using chemical leavening agents according to the state standard GOST 5898-87 **[19]**.

To determine the cookies' swelling capacity, we immersed the finished products in water at 20 °C for 2 minutes and determined the mass increase using an analytical digital scale. The formula expresses the swelling capacity of cookies, GOST 10114-80 **[20]**.

$$\Psi\ \% = \frac{M_2}{M_1}\ x\ 100\ \%, \quad (3)$$

Where:

Ψ % – the swelling capacity of cookies (as percentage); M_1 – the mass of swelled cookies (in grams); M_2 – the mass of dry cookies (in grams).

Organoleptic indicators were determined on a 10-point scale according to the following characteristics: surface condition, color, taste and smell, appearance of the fracture, and shape, GOST 24901-2014 **[17]**.

The baking temperature of sugar cookies in an electric oven was 230-240 °C. The baking time was 5 minutes. The moisture content of the sugar dough was 16-16.5%. We determined the readiness of cookies based on

moisture and appearance. The moisture content of the finished products was 9.5-10 %. For all samples, the baking temperature and duration were not changed.

Description of the Experiment

Sample preparation: Due to its high fat and sugar content, we prepared the sugar cookie dough in two stages. In the first stage, we prepared an emulsion to obtain a homogeneous dispersion system to dissolve fat and water. Emulsion plays a special role in determining the quality of cookies since it largely determines the technological process of product preparation. We used an emulsifier to obtain an emulsion and chummed all the raw materials provided by the recipe except flour.

Number of samples analyzed: we analyzed 10 samples.

Number of repeated analyses: All measurements of instrument readings were performed two times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was three times.

Design of the experiment: At the beginning of the experiment, we determined the content of moisture content, alkalinity, and swelling capacity of gluten-free flour composite mixtures. The physic-chemical parameters (moisture content, alkalinity, and swelling capacity) of sugar cake with flaxseed and chickpea flours were studied. Content of key nutrients (proteins, fats, and carbohydrates) in gluten-free flour composite mixture Based on the data obtained, we have developed the recipe for gluten-free flour composite mixtures for gluten-free confectionery.

Statistical Analysis

A statistical analysis of the obtained data is carried out for the analysis of the physicochemical indicators (moisture, ability to fight, alkalinity) of the test samples of the sugar cookies; the reliability of the obtained data was evaluated by the mathematical statistics methods using the Windows IBM SPSS Statistics software program (version 20.0, IBM, Armonk, New York, USA). We calculated the arithmetic average of the measured value. Then, we computed the error of each measurement and calculated the squared errors to compute the absolute measurement error. We selected the reliability value $p = 0.95$ **[21]**. Based on the number of measurements and the reliability value, the Student's coefficient equals $t = 3.75$ (Figures 1, 2, 3 and 4) **[21]**. We used statistical functions of the average arithmetic value and standard error to describe the ordered sample.

The results were interpreted graphically using Microsoft Excel. In Tables 1, 2, 3 and 4, and Figures 1, 2, 3 and 4, the data of typical tests are presented, and each value is an average of at least three determinations.

RESULTS AND DISCUSSION

The characterization of gluten-free raw materials and literary data showed that to develop a recipe for gluten-free flour confectionery, it is necessary to use two types of flour simultaneously. Accordingly, we prepared a composite mixture using two flours. Therefore, based on the purpose of the research, we conducted the study in two stages: in the first stage, we made two recipes of a main composite mixture from rice, buckwheat, and corn flours with different ratios of components: the first: rice + buckwheat, and the second: rice + corn. We calculated the recipe for a 100 kg mixture and changed the components' ratio. We considered five options for each when preparing both mixtures. In the second stage, to increase the nutritional value, we added flaxseed flour to the basic composite mixture of rice and buckwheat and chickpea flour to the mixture of rice and corn flour.

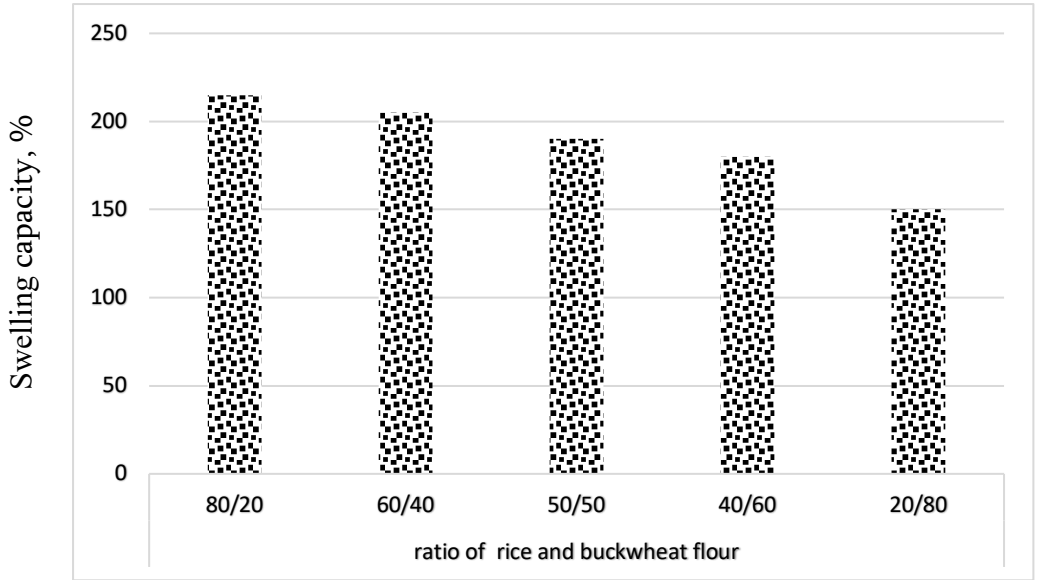
To develop a gluten-free composite mixture recipe, we prepared sugar cookies called "Jubilee" **[16]**, in which wheat flour was completely replaced by a flour composite mixture obtained with different ratios of components.

To prepare the sugar cookies, we kneaded the dough on emulsion according to the generally accepted technology **[30]**, **[31]**, **[32]**, **[33]**. We kneaded and shaped the dough using a composite mixture. During the work, it was revealed that increasing the amount of rice flour in the rice and buckwheat made it easier to shape the dough. The study results are presented in Table 1 and shown in Figure 1.

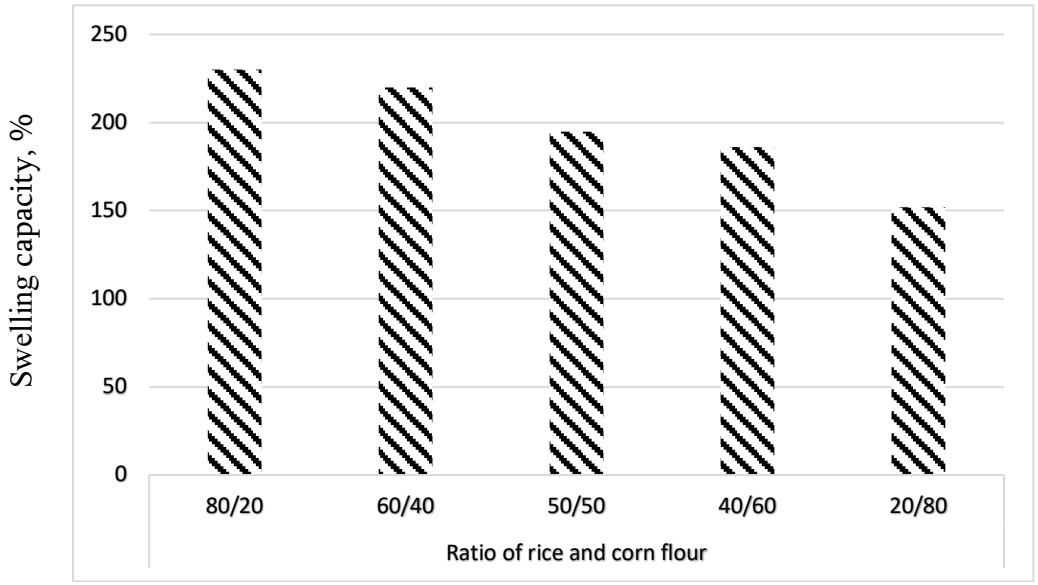
Table 1 Physic-chemical indicators of gluten-free sugar cookies.

Quality indicators	The ratio of rice and buckwheat flours					The rice and cornmeal mixture				
	80:20	60:40	50:50	40:60	20:80	80:20	60:40	50:50	40:60	20:80
Alkalinity, degrees	1.0 ±0.1	1.1 ±0.1	1.3 ±0.1	1.4 ±0.1	1.4 ±0.1	1.0 ±0.1	1.1 ±0.1	1.1 ±0.1	1.0 ±0.1	1.1 ±0.1
Moisture content,%	9.0 ±0.3	9.2 ±0.2	9.4 ±0.2	9.5 ±0.2	10.0 ±0.2	8.0 ±0.2	8.1 ±0.2	8.0 ±0.2	8.0 ±0.2	8.0 ±0.2

Note: $p < 0.05$. All values are expressed as the mean ±SD (standard deviation).



a)



b)

Figure 1 The swelling capacity of gluten-free sugar cookies. Note: a) based on rice and buckwheat flours; b) based on rice and corn flours.

The research determined that the swelling capacity of cookies obtained from the composite mixture of rice and buckwheat increases with an increase in rice flour. This is probably related to rice flour's higher starch content than buckwheat flour. The moisture content of the products increases with the increase in buckwheat flour, while the alkalinity remains almost unchanged.

The moisture and alkalinity of the sugar cookies obtained from the mixture of rice and corn did not change and were 8.0% and 1 degree, respectively. As the proportion of cornmeal decreased, swelling capacity increased due to corn and rice's different starch contents and properties.

The organoleptic evaluation of the finished products revealed that the gluten-free sugar cookies based on rice and buckwheat had a straight, round shape. As the amount of buckwheat flour in the mixture increases, its specific aroma becomes more robust. For example, when the mixture contained 80% buckwheat flour, the product had a pronounced taste and smell of buckwheat. The taste and smell could have been more pronounced when adding 50-60% buckwheat flour. The aroma and smell of rice flour were enhanced at lower levels of buckwheat flour in the mixture. All products had an even brownish-gold color.

All the products based on the rice and corn mixture had a regular shape and a smooth surface with small swellings. Products with a high cornmeal content had a pronounced bitter taste of corn. The color varied from yellowish gold to dark brown. By increasing the proportion of rice flour, the characteristic taste of corn flour decreased. However, products with a high content of rice flour had a typical rice taste. The surface of the products was slightly rough, of a light cream color, and had a crunchy consistency.

Thus, to prepare sugar cookies, we have chosen two essential flour composite mixtures: 1. rice and buckwheat and 2. rice and corn, in which the ratio of components is 50:50.

In the next stage, flaxseed flour was added to the basic composite mixture of rice and buckwheat, and chickpea flour was added to the mix of corn and rice. We prepared the mixture with different ratios of components. To determine the optimal ratio of the components in the gluten-free mixture, we prepared sugar cookies, to this, we calculated the recipe for sugar cookies per 1 kg of finished product. We took the products obtained from the basic composite mixture as a control.

The optimal moisture content of the dough and the amount of water to be added were determined experimentally. To obtain satisfactory-quality gluten-free cookies, it is necessary to prepare a dough with a moisture content of 30%, while the moisture content of a sugar dough made of second-grade wheat flour is 18-20%. This is due to the high water absorption capacity of the gluten-free raw material compared to wheat flour. The dough became dense and sticky by further increasing the moisture content of the semi-finished product. Dough-forming was complicated.

Thus, when preparing sugar cookies from non-traditional raw materials, one must consider their ability to absorb water and select the dough's moisture content.

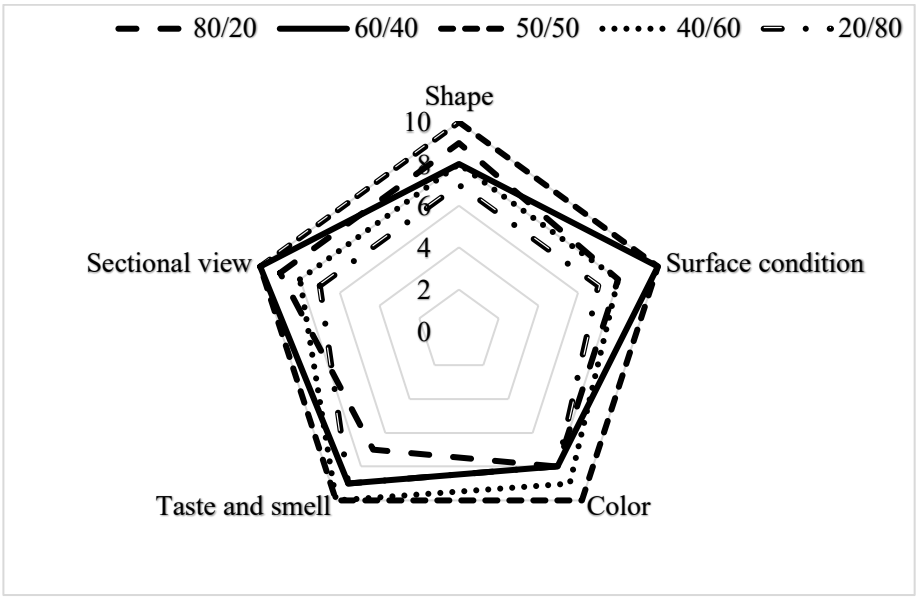
The physico-chemical and organoleptic characteristics of sugar cookies are presented in Table 2 and shown in Figure 2 and Figure 3.

Table 2 The physicochemical parameters of sugar cake with adding flaxseed and chickpea flours.

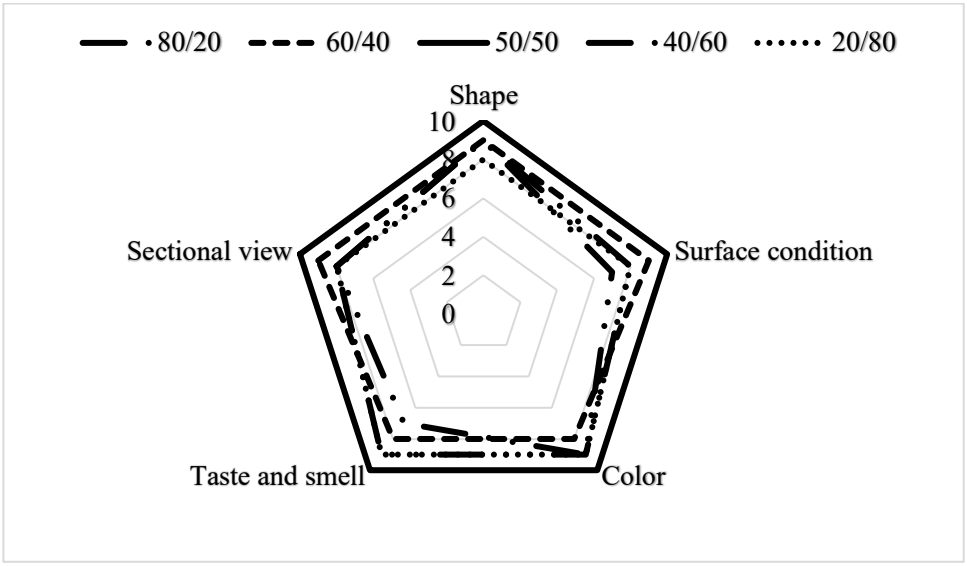
Quality indicators	Rice flour + buckwheat flour + flaxseed flour					Rice flour+ corn flour+ chickpea flour				
	control sample	40:40:20	40:30:30	30:40:30	30:30:40	control sample	40:40:20	40:30:30	30:40:30	30:30:40
Alkalinity, degrees	1.3 ±0.1	1.1 ±0.3	1.0 ±0.3	0.9 ±0.4	0.8 ±0.3	1.1 ±0.2	1.1 ±0.2	1.2 ±0.4	1.2 ±0.3	1.2 ±0.3
Moisture content,%	9.4 ±0.5	9.8 ±0.4	10.0 ±0.5	9.5 ±0.5	10.0 ±0.3	10.0 ±0.3	9.8 ±0.2	10.0 ±0.3	9.5 ±0.4	10.0 ±0.3
Swelling capacity,%	190 ±2.0	185 ±2.3	180 ±1.9	180 ±1.9	180 ±2.0	195 ±2.1	195 ±2.3	200 ±2.3	195 ±1.9	200 ±2.1

Note: $p < 0.05$. All values are expressed as the mean ±SD (standard deviation).

The obtained results show that the physicochemical indicators of the gluten-free sugar cookies using the composite mixture obtained with gluten-free flours agree with the indicators specified in the standard.



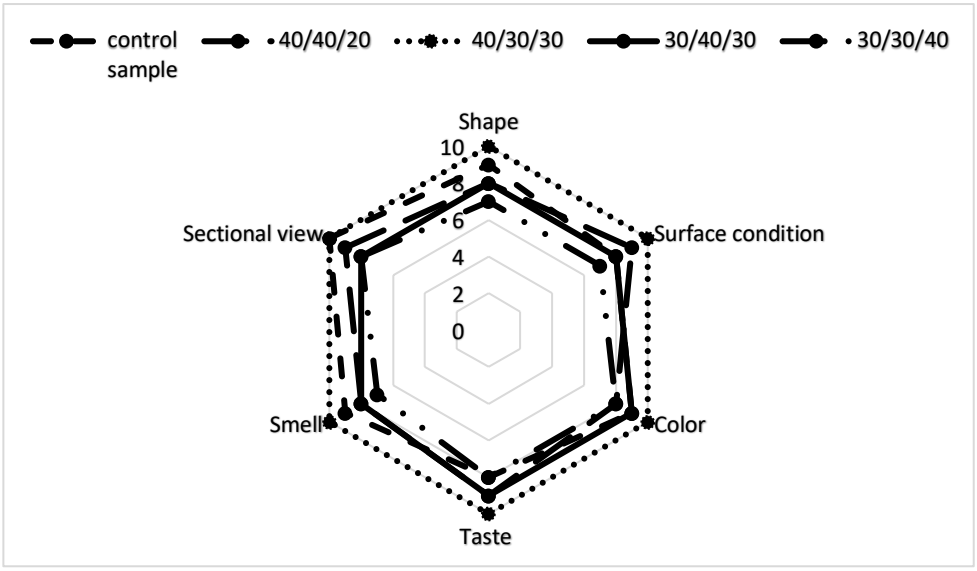
a)



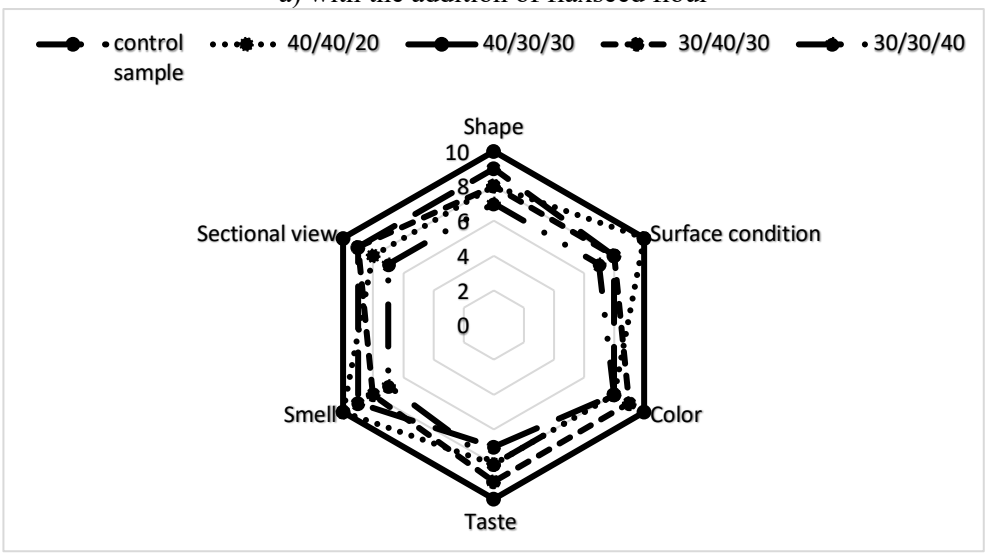
b)

Figure 2 Organoleptic evaluation of cookies obtained from a composite mixture of gluten-free flour. Note: a) rice and corn; b) rice and buckwheat.

By increasing the dosage of flaxseed flour in the composite mixture of rice, buckwheat and flaxseed flour, the nut flavour in the finished products becomes stronger, and cookies become softer and fatter due to the lipids contained in the flaxseed flour. The color of the products remains almost unchanged. The addition of flaxseed flour reduces strength and alkalinity, but the moisture remains almost unchanged. In the composite mixture of rice, corn, and chickpea flours, increasing the share of chickpea flour increased the finished products' swelling capacity due to the cellulose and protein content.



a) with the addition of flaxseed flour



b) with the addition of chickpea flour

Figure 3 Organoleptic indicators of sugar cookies with the addition of flaxseed and chickpea flour.

Based on the conducted studies, two gluten-free composite flour mixtures have been proposed: one made from rice, buckwheat, and flaxseed flours and the other from rice, corn, and chickpea flours in a ratio of 40:30:30. In order to use in the research, we prepared 1 kg of composite mixture with the given ratio of components.

Based on the ratio of the components, we calculated the recipe for 1 t of gluten-free flour composite mixture, presented in Table 3.

Table 3 Recipe for a gluten-free composite flour mixture.

Name of raw material	Dry substances,%	consumption of raw material, kg		consumption of raw material, kg	
		on 1 ton of finished products		on 1 ton of finished products	
		in nature	in dry substances	in nature	in dry substances
Rice flour	86.0 ±0.1	400.0 ±5.0	344.0 ±4.3	400 ±5.0	344.0 ±4.3
Buckwheat flour	88.9 ±0.1	300.0 ±5.0	266.7 ±4.4	-	-
Flaxseed flour	93.1 ±0.1	300.0 ±5.0	279.3 ±4.7	-	-
Corn flour	88.9 ±0.1	-	-	300 ±5.0	266.7 ±4.4
Chickpea flour	86.0 ±0.1	-	-	300 ±5.0	258.0 ±4.3

Note: p <0.05. All values are expressed as the mean ±SD (standard deviation).

Various scientists have used the gluten-free raw materials selected for our gluten-free flour blends. For example, a review article by the authors presents the preparation of bread, cakes, pates, and snacks for the dietary nutrition of patients with celiac disease using rice, buckwheat, corn, and quinoa flour **[22]**. Also, 14 recipes for instant pasta using various structure formers have been developed from a mixture of rice, buckwheat, and soy flour **[23]**. A gluten-free bread recipe was developed using corn, buckwheat, and plantain flour in the following ratio 40:40:20 **[24]**. Scientists developed eight recipes that used 100% beans, chickpeas, peas, kiwicha, quinoa, lentils, corn, and bean flour **[25]**. A team of scientists created gluten-free cookies from the following mixture of flours: corn, rice, and soy flour **[26**]. According to the authors, non-puffing popcorn flour (RPF raw popcorn flour), puffing corn corn meal (PPF) and a mixture of them (1:1), in addition to corn flour as a control sample, were used to prepare recipes for gluten-free cakes and cookies **[27]**. In a study by other researchers, wheat flour was replaced with defatted flour made from flax, sesame, chia, and poppy seeds to increase the nutritional value of traditional commercial cookies **[28]**. Gluten-free biscuits have been developed using composite rice-chickpea flour with acacia, apricot, or karaya gum exudate. The approximate composition of rice, chickpeas, and their compound flours was measured **[29]**. Based on the results of the analysis of the literature data, it was established that the rational ratio of corn and rice is 80:20. This proportion ensures proper physicochemical indicators and high organoleptic indicators of finished gluten-free bakery products. It was established that the manufactured gluten-free bakery products exceeded the control sample regarding physico-chemical parameters, namely, porosity, acidity, dimensional stability, and moisture content of the crumb **[34]**. The appropriateness of our choice of buckwheat and flax as enrichments is confirmed by scientists who developed a recipe for gluten-free muffins enriched with buckwheat, almond, and flax flour. The mentioned additives significantly increase the protein content compared to products prepared with wheat flour **[35]**. The obtained data on the physiochemical parameters of sugar cookies are consistent with the data of other authors in the scientific literature, according to which the moisture content of muffins made from flax and chickpea flour does not change compared to control samples made from wheat flour **[35]**. According to other authors, replacing wheat flour with chickpea flour reduced the springiness, cohesiveness, chewiness, springiness, and specific volume of the cake **[36]**. Some authors' articles found that the rational ratio of corn and rice is 80:20. This proportion ensures proper physical and chemical characteristics and high organoleptic characteristics of finished gluten-free bakery products. It has been established that the manufactured gluten-free bakery products are superior to the control sample in terms of physicochemical parameters, namely porosity, acidity, dimensional stability, and crumb moisture **[28]**. Gluten-free rice flour biscuits were made using chestnut flour (0, 30, 40 and 50%) and date seed flour (0, 10 and 20%). %) and modified starch (0.3, 0.5, 0.6, and 0.9%). In this work, the physicochemical, rheological, and sensory properties of the prepared preparations were studied. The results showed that the dough's moisture content, specific volume, and viscosity were lowest in the control and highest in the variant containing 20% date seed flour, 30% chestnut flour, and 0.9% modified starch **[37]**. Other studies have shown that amaranth and its compounds have more viscous properties and improved water-holding capacity than wheat flour. Differences were also found in the geometric and textural properties of the dough and cookies. However, amaranth-oatmeal cookies were acceptable in color, taste, and texture with no significant differences in sensory quality compared to wheat flour cookies **[15]**. Chickpeas, almonds, and flax seeds were used in high concentrations to formulate a gluten-free muffin recipe for the first time. These muffins were more nutritious than the traditional recipes available. No health hazards were found in these cupcakes. Some sensory characteristics of the muffins were slightly lower and comparable to wheat/rice based muffins **[35]**. Gluten-free biscuits have been developed using composite rice-chickpea flour with acacia, apricot or karaya gum exudate. The approximate composition of rice, chickpeas and their compound flours was measured. Replacing rice flour with chickpea flour up to 20% (by weight) significantly impacted its immediate composition and adhesive properties. The addition of exudate further improved the stickiness and viscoelasticity of the dough **[29]**. Lentil biscuits were developed that contained the highest amount of protein and fiber, but lower fat and carbohydrate content compared to other samples. In terms of color, the corn biscuits were the lightest, with greater brightness, less redness, and more yellowness **[25]**. Some researchers prepared different dough samples with different concentrations of flaxseed meal (i.e. 15 g, 17 g, 20 g and 22.5 g) and thus optimized them by rheological testing of the dough samples. Cookies prepared from different dough samples with different flaxseed concentrations were tested for rheological properties, and the texture profile of the formulations was analyzed **[38]**. Our results on the chemical composition of sugar cookies are consistent with those of other scientists, who believe that replacing wheat flour with gluten-free rice, almond, chickpea, and flax flour increased the protein content. cake by 2-3 times, from 4.25 g in the control to 14.64 g in the test sample

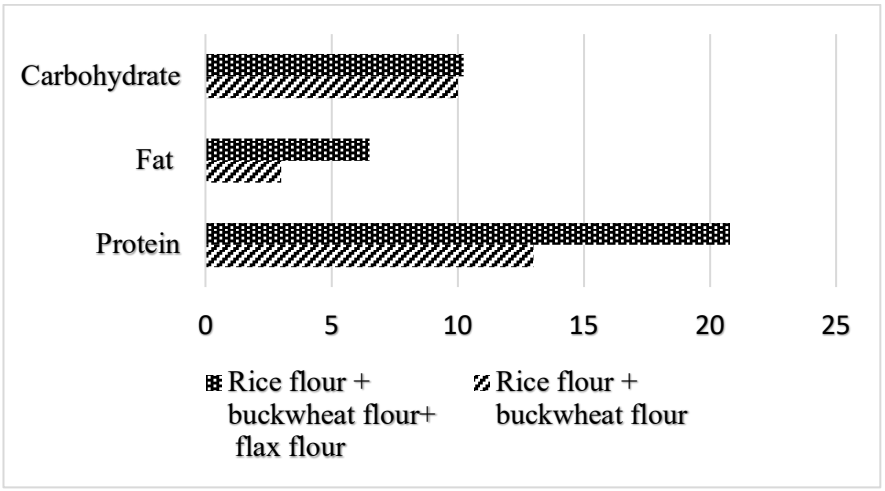
[35]. According to the authors, moisture, protein, ash, and fat increased due to increasing chickpea flour substitution rates. However, the carbohydrate content was reduced compared to other control biscuits. Thus, the nutritional value of sweet cookies was increased compared to control samples **[40]**. According to some scientists, biscuits with higher protein content showed higher acceptability than biscuits with higher starch content and no added protein **[41]**. Flaxseed is an excellent source of nutrients, including protein, soluble and insoluble dietary fiber, and omega-3 fatty acids. Flaxseed proteins can improve the biological parameters of bread and unleavened flatbreads (chapatis) **[42]**. Scientists have determined the optimal amount of ground flaxseed flour substitute in baked goods and assessed the effect of flaxseed on the sensory and nutritional qualities of these baked goods. Flaxseed, when replacing flour by 30-50%, significantly increased the nutritional quality of several nutrients without affecting the overall acceptability of baked goods **[43]**. The addition of chickpea flour at low doses (2% by weight) increases bread volume by 20%. It reduces crumb hardness by 40% due to increased gas retention (no holes within the pores) and superior homogeneity of the starch-protein network. The combination of chickpea meal and pumpkin seed has many benefits reflected in the improved nutritional quality of the final product, including increased protein and dietary fiber content, improved mineral and fatty acid profile, increased total phenolic content, and antioxidant activity compared to the control sample **[6]**. To determine the nutritional value of a gluten-free composite flour mixture, we calculated the content of proteins, fats, carbohydrates, and the satisfaction level of the daily need for nutrients (following the balanced nutritional formula) **[39]**. The results are shown in Table 4 and Figure 4.

Table 4 Content of key nutrients, proteins, fats and carbohydrates in gluten-free flour composite mixture.

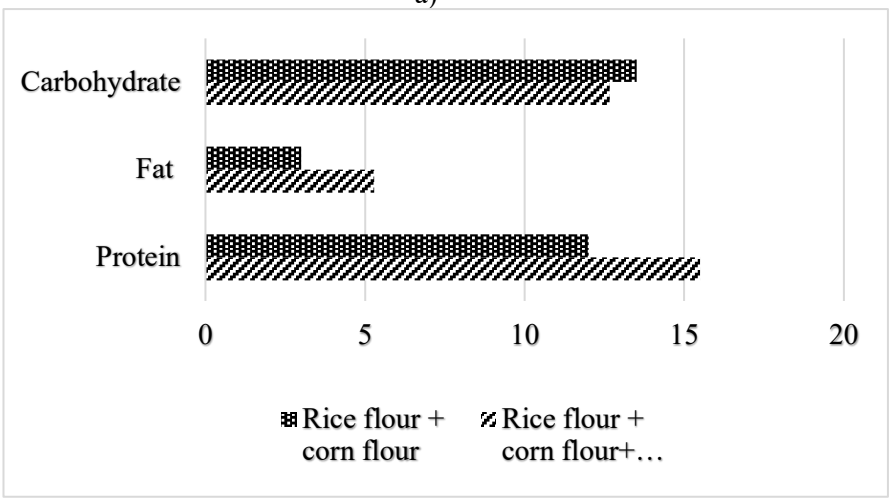
Key nutrients	Norm of the daily need	Rice flour + buckwheat flour	Rice flour + buckwheat flour + flaxseed flour	Rice flour+ corn flour	Rice flour+ corn flour+ chickpea flour
Proteins, g	90	12.2 ±1.05	18.7 ±1.03	10.8 ±1.05	13.93 ±1.05
Carbohydrates, g	400-500	45.0 ±1.23	46.08 ±1.25	58.5 ±1.22	56.67 ±1.16
Fats, g	90	2.3 ±0.93	5.88 ±0.95	2.3 ±0.92	4.75 ±0.87
Nutritional value, kkal		249.5 ±1.21	312.04 ±1.20	297.9 ±1.22	325.15 ±1.23

Note: $p < 0.05$. All values are expressed as the mean ±SD (standard deviation).

As can be seen from the table data, in gluten-free composite mixtures with the addition of flax and chickpea flours, the protein content increased by 53.3 and 28.7%, respectively, and the fat content increased by 2.5 times and 2 times, respectively. The carbohydrate content remained virtually unchanged or decreased slightly, and also, the nutritional value of the flour composition mixture increased by 25.1% with the addition of flax flour, and by 9.1% with the addition of chickpea flour.



a)



b)

Figure 4 The satisfaction levels of key nutrients in a gluten-free flour composite mixture. Note: a) In a composite mixture of rice, buckwheat, and flaxseed flour, b) In a composite mixture of rice, corn, and chickpea flour.

The developed gluten-free composite flour mixture is characterized by a reasonably high satisfaction level with the balanced nutrition formula. As seen in Figure 4 (a), adding flax to the rice-buckwheat mixture increased the protein satisfaction level by 64%, the carbohydrate satisfaction level by 37%, and the fat satisfaction level by 118%. Figure 4 (b) illustrates that with the addition of chickpeas to the rice-corn mixture, the protein satisfaction level increased by 35.5%. At the same time, carbohydrates remained almost unchanged, and the fat satisfaction level increased by 27%.

CONCLUSION

Research has led to the developing of a gluten-free flour blend designed specifically for making gluten-free sugar cookies.

- The following flours are recommended for the flour mixture to produce gluten-free sugar cookies: rice, buckwheat, and corn flour. To increase the nutritional and biological values of the products, flaxseed, and chickpea flours were added to the gluten-free composite mixture;
- Two gluten-free flour composite mixtures have been developed: a) rice, buckwheat, and flaxseed flours and b) rice, corn, and chickpea flours. The optimal ratio of flours is 40:30:30, respectively.
- The physicochemical and organoleptic indicators of gluten-free sugar cookies using the composite mixture obtained with gluten-free flours are consistent with the indicators specified in the standard;
- The developed gluten-free composite flour mixture is characterized by a reasonably high satisfaction level with the balanced nutrition formula regarding proteins, fats, and carbohydrates of the primary nutrients. The levels of protein, carbohydrate, and fat satisfaction increased by 64, 37, and 118%, respectively, with the addition of flax to the rice and buckwheat mixture. Adding chickpeas to the rice and corn mixture increased protein and fat satisfaction levels by 35.5 and 27%, respectively, while carbohydrates remained almost unchanged.

REFERENCES

1. Simón, E., Molero-Luis, M., Fueyo-Díaz, R., Costas-Batlle, C., Crespo-Escobar, P., & Montoro-Huguet, M. A. (2023). The Gluten-Free Diet for Celiac Disease: Critical Insights to Better Understand Clinical Outcomes. In Nutrients (Vol. 15, Issue 18, p. 4013). MDPI AG. https://doi.org/10.3390/nu15184013
2. Sapone, A., Bai, J. C., Ciacci, C., Dolinsek, J., Green, P. H., Hadjivassiliou, M., Kaukinen, K., Rostami, K., Sanders, D. S., Schumann, M., Ullrich, R., Villalta, D., Volta, U., Catassi, C., & Fasano, A. (2012). Spectrum of gluten-related disorders: consensus on new nomenclature and classification. In BMC Medicine (Vol. 10, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/1741-7015-10-13
3. Aljada, B., Zohni, A., & El-Matary, W. (2021). The Gluten-Free Diet for Celiac Disease and Beyond. In Nutrients (Vol. 13, Issue 11, p. 3993). MDPI AG. https://doi.org/10.3390/nu13113993
4. Yamsaengsung, R., Berghofer, E., & Schoenlechner, R. (2012). Physical properties and sensory acceptability of cookies made from chickpea addition to white wheat or whole wheat flour compared to gluten-free amaranth or buckwheat flour. In International Journal of Food Science & Technology (Vol. 47, Issue 10, pp. 2221–2227). Wiley. https://doi.org/10.1111/j.1365-2621.2012.03092.x
5. Hamdi, A. M., & Ahmed, S. H. (2021). Effect of Adding Grape Seed Powder on The Chemical Composition and Rheological Properties of Local Wheat Flour. In Tikrit journal for agricultural sciences (Vol. 21, Issue 4, pp. 112–121). Tikrit University. https://doi.org/10.25130/tjas.21.4.12
6. Tomić, J., Škrobot, D., Popović, L., Dapčević-Hadnađev, T., Čakarević, J., Maravić, N., & Hadnađev, M. (2022). Gluten-Free Crackers Based on Chickpea and Pumpkin Seed Press Cake Flour: Nutritional, Functional and Sensory Properties. In Food Technology and Biotechnology (Vol. 60, Issue 4, pp. 488–498). Faculty of Food Technology and Biotechnology, University of Zagreb. https://doi.org/10.17113/ftb.60.04.22.7655
7. Xu, J., Zhang, Y., Wang, W., & Li, Y. (2020). Advanced properties of gluten-free cookies, cakes, and crackers: A review. In Trends in Food Science & Technology (Vol. 103, pp. 200–213). Elsevier BV. https://doi.org/10.1016/j.tifs.2020.07.017
8. Terentyev, S. E., Labutina, N. V., Romanova, I. N., Tryabas, Y. A., Sharipov, F. F., & Dyakonova, M. A. (2022). Features of the use of seeds of various agricultural crops in the production of bakery products. In IOP Conference Series: Earth and Environmental Science (Vol. 1045, Issue 1, p. 012098). IOP Publishing. https://doi.org/10.1088/1755-1315/1045/1/012098
9. Silagadze, M. A., Gachechiladze, S. T., Pruidze, E. G., Khetsuriani, G. S., Khvadagiani, Kh. B., & Pkhakadze, G. N. (2017). Development of new-generation dietary bread technologies by using soya processing products. In Annals of Agrarian Science (Vol. 15, Issue 2, pp. 177–180). Elsevier BV. https://doi.org/10.1016/j.aasci.2017.05.018
10. Sadunishvili, T., Maisaia, I. (2022). Georgia is the country of the ancient Agriculture. Matsne. In History, Archaeology, Ethnology and Art History Series (Vol. 2, pp. 126–138). http://macne.org.ge/index.php/macne/article/view/74/115. In Georgian
11. Wirkijowska, A., Zarzycki, P., Sobota, A., Nawrocka, A., Blicharz-Kania, A., & Andrejko, D. (2020). The possibility of using by-products from the flaxseed industry for functional bread production. In LWT (Vol. 118, p. 108860). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.108860
12. Kajla, P., Sharma, A., & Sood, D. R. (2014). Flaxseed—a potential functional food source. In Journal of Food Science and Technology (Vol. 52, Issue 4, pp. 1857–1871). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-014-1293-y
13. Kaur, P., Waghmare, R., Kumar, V., Rasane, P., Kaur, S., & Gat, Y. (2018). Recent advances in utilization of flaxseed as potential source for value addition. In OCL (Vol. 25, Issue 3, p. A304). EDP Sciences. https://doi.org/10.1051/ocl/2018018
14. Rachwa-Rosiak, D., Nebesny, E., & Budryn, G. (2015). Chickpeas-Composition, Nutritional Value, Health Benefits, Application to Bread and Snacks: A Review. In Critical Reviews in Food Science and Nutrition (Vol. 55, Issue 8, pp. 1137–1145). Informa UK Limited. https://doi.org/10.1080/10408398.2012.687418
15. Inglett, G. E., Chen, D., & Liu, S. X. (2015). Physical properties of gluten-free sugar cookies made from amaranth–oat composites. In LWT - Food Science and Technology (Vol. 63, Issue 1, pp. 214–220). Elsevier BV. https://doi.org/10.1016/j.lwt.2015.03.056
16. Smirnova M. K., & Abramova G. G. (2021). Recipes for cookies, cookiess and waffles. Moscow, "Book on Demand" (552 p.). Ripol Classic.
17. GOST 24901-2014. (2014). Cookiess. General specifications. Moscow: Standartinform.
18. GOST 5900-2014. (2019). Confectionery. Methods for determination of moisture and solids. Moscow: Standartinform.

19. GOST 5898-87. (2012). Confectionery. Methods for determination of acidity and alkalinity.) Moscow: Standartinform.
20. GOST 10114-80. (2012). Bisquits. Method for determination of swelling in water. Moscow: Standartinform.
21. Gaydyshev I. P. (2001). Analysis and data processing. Special guide. Saint Petersburg, Piter.
22. Abid, J., Ayub, M. U., Ahmed, S., Ali, H. U., Zeb, M., Zahid, R. S., & Khan, S. (2022). A study on rice and buckwheat based diet and its role in celiac disease: an overview. In International Journal Of Community Medicine And Public Health (Vol. 9, Issue 12, p. 4699). Medip Academy. https://doi.org/10.18203/2394-6040.ijcmph20223233
23. Cyganova, T. B., Nikolaeva, Y. V., Tarasova, V. V., Vadovskij, I. K., Rechkin, I. V., & Smirnov, D. A. (2022). Gluten-free raw materials in the technology of instant pasta. In IOP Conference Series: Earth and Environmental Science (Vol. 1052, Issue 1, p. 012021). IOP Publishing. https://doi.org/10.1088/1755-1315/1052/1/012021
24. Utarova, N., Kakimov, M., Gajdzik, B., Wolniak, R., Nurtayeva, A., Yeraliyeva, S., & Bembenek, M. (2024). Development of Gluten-Free Bread Production Technology with Enhanced Nutritional Value in the Context of Kazakhstan. In Foods (Vol. 13, Issue 2, p. 271). MDPI AG. https://doi.org/10.3390/foods13020271
25. Silva-Paz, R. J., Silva-Lizárraga, R. R., Jamanca-Gonzales, N. C., & Eccoña-Sota, A. (2024). Evaluation of the physicochemical and sensory characteristics of gluten-free cookies. In Frontiers in Nutrition (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fnut.2023.1304117
26. Man, S., Paucean, A., & Muste, S. (2014). Preparation and Quality Evaluation of Gluten-Free Biscuits. In Bulletin UASVM Food Science and Technology (Vol. 71, Issue 1, pp. 38–44). University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.
27. Utilization of Popcorn for Gluten Free Cake and Cookies Manufacturing. (2020). In Middle East Journal of Applied Sciences. Current Research Web. https://doi.org/10.36632/mejas/2020.10.4.71
28. Martínez, E., García-Martínez, R., Álvarez-Ortí, M., Rabadán, A., Pardo-Giménez, A., & Pardo, J. E. (2021). Elaboration of Gluten-Free Cookies with Defatted Seed Flours: Effects on Technological, Nutritional, and Consumer Aspects. In Foods (Vol. 10, Issue 6, p. 1213). MDPI AG. https://doi.org/10.3390/foods10061213
29. Hamdani, A. M., Wani, I. A., & Bhat, N. A. (2020). Gluten free cookies from rice-chickpea composite flour using exudate gums from acacia, apricot and karaya. In Food Bioscience (Vol. 35, p. 100541). Elsevier BV. https://doi.org/10.1016/j.fbio.2020.100541
30. Pruidze E., Khutsidze T. (2022). Technology of Flour Confectionery products. In Kutaisi (225 p.). Akaki Tsereteli State University Publishing House.
31. Zubchenko A. V. (1999). Technology of confectionery production. 3rd ed. Voronezh, Voronezh State University).
32. Mamchenko T. V. (2015). Technology of flour confectionery products production. Bryansk: "Bryansk State Agricultural University", (98 p.). UDC 641/642. BBK 36.84.
33. Matveeva T. V., Koryachkina S. A. (2011). Flour confectionery products with functional purpose. Oriol, Gosuniversitet UNPK. Retrieved from http://oreluniver.ru/ file/chair/thkimp/study/matveeva_much_kond.pdf.
34. Gorach, O., Oksana, D., & Rezvykh, N. (2024). Innovative Technology for the Production of Gluten-free Food Products of a New Generation. In Current Nutrition & Food Science (Vol. 20, Issue 6, pp. 734–744). Bentham Science Publishers Ltd. https://doi.org/10.2174/0115734013280307231123055025
35. Jabeen, S., Khan, A. U., Ahmad, W., Ahmed, M.-D., Ali, M. A., Rashid, S., Rashid, A., & Sharifi-Rad, J. (2022). Development of Gluten-Free Cupcakes Enriched with Almond, Flaxseed, and Chickpea Flours. In A. Ali (Ed.), Journal of Food Quality (Vol. 2022, pp. 1–11). Hindawi Limited. https://doi.org/10.1155/2022/4049905
36. Herranz, B., Canet, W., Jiménez, M. J., Fuentes, R., & Alvarez, M. D. (2016). Characterisation of chickpea flour-based gluten-free batters and muffins with added biopolymers: rheological, physical and sensory properties. In International Journal of Food Science & Technology (Vol. 51, Issue 5, pp. 1087–1098). Wiley. https://doi.org/10.1111/ijfs.13092
37. Mohammadi, M., Khorshidian, N., Yousefi, M., & Khaneghah, A. M. (2022). Physicochemical, Rheological, and Sensory Properties of Gluten-Free Cookie Produced by Flour of Chestnut, Date Seed, and Modified Starch. In S. He (Ed.), Journal of Food Quality (Vol. 2022, pp. 1–10). Hindawi Limited. https://doi.org/10.1155/2022/5159084
38. Bashir, Sh., Yaseen, M., Sharma, V., & Purohit, S. (2020). Rheological and Textural Properties of Gluten Free Cookies based on Pearl Millet and Flaxseed. In Biointerface Research in Applied Chemistry (Vol. 10, Issue 5, pp. 6565–6576). AMG Transcend Association. https://doi.org/10.33263/BRIAC105.65656576

39. Skurikhin I. M., & Volgareva M. N. (1987). Chemical Composition of Foods: Book 1: Reference Tables for Essential Nutrient Content and Energy Value of Food products. In Agropromizdat (224 p.). Retrieved from https://www.centrmag.ru/catalog/product/himicheskij-sostav-pishhevyh-produktov-kniga-1-spravochnye/.
40. Abdulquader, E. A., Ahmad, El-H., Rabou, M. A., & Abdrabou, E. (2017). Effect of Enriched Gluten Free Biscuits with Chickpea Flour or Kareish Cheese on Chemical, Nutritional Value, Physical and Sensory Properties. In Alexandria Journal of Agricultural Sciences (Vol. 62, Issue 1, pp. 93–101). Alexandria University.
41. Mancebo, C. M., Rodriguez, P., & Gómez, M. (2016). Assessing rice flour-starch-protein mixtures to produce gluten free sugar-snap cookies. In LWT - Food Science and Technology (Vol. 67, pp. 12. https://doi.org/10.1016/j.lwt.2015.11.045
42. Hussain, S., Anjum, F. M., Alamri, M. S., Mohamed, A. A., & Nadeem, M. (2013). Functional flaxseed in baking. In Quality Assurance and Safety of Crops & Foods (Vol. 5, Issue 4, pp. 375–385). Codon Publications. https://doi.org/10.3920/QAS2012.0207
43. Lipilina, E., & Ganji, V. (2009). Incorporation of ground flaxseed into bakery products and its effect on sensory and nutritional characteristics – a pilot study. In Journal of Foodservice (Vol. 20, Issue 1, pp. 52–59). Wiley. https://doi.org/10.1111/j.1748-0159.2008.00124.x
44. Bird, L. G., Pilkington, C. L., Saputra, A., & Serventi, L. (2017). Products of chickpea processing as texture improvers in gluten-free bread. In Food Science and Technology International (Vol. 23, Issue 8, pp. 690–698). SAGE Publications. https://doi.org/10.1177/1082013217717802

Funds:

This work was not supported by external funding.

Acknowledgments:

This work was supported by Akaki Tsereteli State University, Kutaisi, Georgia

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Tsira Khutsidze**, Akaki Tsereteli State University, Faculty of Engineering and Technology, Department of Food Technology, 59 Tamar Mephe str., 4600 Kutaisi, Georgia, Tel.: +995551412585,
E-mail: cirakh@gmail.com
ORCID: https://orcid.org/0000-0001-5748-856X

Eliza Pruidze, Akaki Tsereteli State University, Faculty of Engineering and Technology, Department of Food Technology, 59 Tamar Mephe str., 4600 Kutaisi, Georgia, Tel.: +995577315849,
E-mail: eliza.pruidze@atsu.edu.ge
ORCID: https://orcid.org/0009-0001-5232-5419

Maria Silagadze, Akaki Tsereteli State University, Faculty of Engineering and Technology, Department of Food Technology, 59 Tamar Mephe str., 4600 Kutaisi, Georgia, Tel.: +995593671752,
E-mail: mariasilagadze@yahoo.com
ORCID: https://orcid.org/0000-0003-1179-2659

Eliso Dzneladze, Batumi Shota Rustaveli State University. Doctor of Technical Sciences, Head of the Secretariat of the Academic Council, Senior Lecturer. Georgia, Batumi, 6010, Ninoshvili/Rustaveli str. 35/32, Tel.: +995(422) 271786,
E-mail: bsu-samdivno@bsu.edu.ge
ORCID: https://orcid.org/0009-0005-5187-8731

George Pkhakadze, Agricultural University of Georgia, Faculty of Natural Sciences, 240 Davit Agmashenebeli Highway, 0159 Tbilisi, Tel.: +995591900567,
E-mail: georgepkhakadze86@gmail.com
ORCID: https://orcid.org/0000-0002-4467-5312

Irma Berulava, Akaki Tsereteli State University, Faculty of Engineering and Technology, Department of Food Technology, 59 Tamar Mephe str., 4600 Kutaisi, Georgia, Tel.: + 995593532506,
E-mail: irma.berulava@atsu.edu.ge
ORCID: https://orcid.org/0000-0001-8312-6802

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 21.2.2024
Revised: 30.4.2024
Accepted: 1.6.2024
Published: 10.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 468-479
https://doi.org/10.5219/1967
ISSN: 1337-0960 online
www.potravinarstvo.com

The rheological characteristics of the dough for semi-finished biscuits with the addition of crickets flour

Olha Sereda, Oxana Melnyk, Tetiana Marenkova, Olena Koshel, Sergii Bokovets

ABSTRACT

The article proposes to study the influence of new protein-containing raw materials, namely cricket flour (CF) *Acheta domesticus* on the quality of whipped flour semi-finished product (WFP) research methods. The study used generally accepted methods for determining the rheological properties of the dough: the dependence of viscosity on shear stress, temperature, and rate of deformation of the dough, as well as the elasticity of finished products and physicochemical properties of the finished semi-finished product. The mass fraction of moisture was determined by drying to constant weight. The mass fraction of ash not dissolved in 10% hydrochloric acid in absolute dry matter was determined using the dry method in a muffle furnace. The mass fraction of protein was determined by the Kjeldahl method. Alkalinity was determined by the titrometric method. The mass fraction of total sugar was determined by the iodometric method. During the manufacture of WFP, cricket flour was added to 5.0-15.0%. The results of rheological parameters showed that adding CF to the dough in an amount of 15.0% significantly affected the viscosity and plasticity of the dough, which led to a decrease in volume and porosity in the finished semi-finished product. According to physicochemical parameters, it was found that with increased BC concentration, the mass fraction of protein increases (from 4.4% in conventional WFP to 62.06% with the addition of CF in the amount of 10.0%). At the same time, the mass fraction of total sugar decreases. The mass fraction of moisture in the finished semi-finished product also changes. The resiliency and elasticity of the finished products were determined by the laboratory method using the "Labor" penetrometer. According to the results of studies of resiliency and elasticity of the finished product, it was determined that the semi-finished product with the addition of 15.0% cricket flour had the lowest indicators compared to the products with the addition of 5.0% and 10.0% cricket.

Keywords: cricket flour, whipped flour semi-finished product, rheological parameters, physico-chemical parameters, resiliency

INTRODUCTION

One of the most popular directions in producing food products is the enrichment of food products using new raw materials, which is connected with consumers' growing interest in products of increased nutritional value with a high content of nutrients. Fortification of food products is carried out due to several factors, such as increasing the nutritional value of products, correcting nutrient and mineral deficiencies, and improving consumer properties. As a result, products with a high content of vitamins, amino acids, and minerals are gaining more and more popularity **[1]**.

Also, special importance is attached to enriching raw materials with a high protein content among food products. After all, protein deficiency in the adult body leads to a decrease in appetite and body weight, increased fatigue and reduced work capacity, damage to the immune system and an increase in the level of

morbidity, a decrease in the activity of enzymes, a violation of the processes of digestion and hematopoiesis, and a negative effect on the liver, cardiovascular, and respiratory systems **[2]**.

In addition, not all raw materials are equally suitable for enrichment. One of the popular products that are subject there is flour confectionery products, namely whipped flour semi-finished products (WFP) because these products are popular among the public but have a high content of carbohydrates and a low content of proteins and biologically active substances. Such foods can be enriched by directly adding innovative ingredients during preparation. Such enrichment will increase the product's nutritional value and influence its rheological, physico-chemical, and sensory properties **[1]**, expanding the range of products of this group.

WFP contains a large amount of carbohydrates, so to improve it, choose products with a high content of protein and biologically active substances, or replace raw materials with a high content of carbohydrates (e.g., sugar or flour) with raw materials with an increased content of functional nutrients.

One of the raw materials that have increased nutritional value and is recognized in the European Union as a product with a high protein content is cricket flour, which is made from the cricket genus of *Acheta domesticus* **[3]**.

In recent years, crickets have attracted the attention of researchers as a source of protein for a growing and increasingly demanding population. Cricket farming is characterized by low environmental costs during cultivation, processing, and use of cricket processing products. The widespread use of crickets in the food industry requires the safe artificial cultivation of various species of crickets, their processing into food products, the labelling and marketing of food products using insect processing products, and consumers' acceptance of this type of food **[4]**.

Most edible insects are collected from the wild and farming them for food is relatively new: breeding crickets is a good example of this new trend.

Insects can be considered a nutritious, healthy basis for obtaining raw materials used in human and animal diets, containing healthy fats, proteins, fiber, vitamins, and minerals such as calcium, iron, and zinc. The nutritional value of insect processing products depends on the insect species, stage of metamorphosis, habitat, feeding habits, and post-harvest processing methods. For example, Chen Xand et al. evaluated the protein content of 100 insect species and found that the protein content ranges from 13% to 77% of the dry matter weight. Insects are often eaten whole but can be processed into powder, pellets, or pastes and added to other foods. In addition, components such as chitin, proteins, and fats can be extracted from insects and used separately as additives in food products **[5]**. In addition, each insect has its taste, which makes it suitable for use in recipes of various foods **[6]**.

Crickets contain a variety of proteins with different molecular weights and solubility. Only ~20% of proteins are soluble in cold water. The rest is a water-insoluble fraction that contains a large amount of muscle proteins **[7]**.

It is known that proteins are biopolymers that consist of amino acid residues connected by peptide bonds. Proteins can perform functions of structure formation, regulation, contraction, or protection. They can serve for transporting, storing, or assembling parts of membranes; they can also be toxins or enzymes. Each cell of a living system can contain thousands of proteins, each performing a unique function. Their structures, as well as their functions, are very different. However, all of them are amino acid polymers located in a linear sequence **[8]**.

Proteins play an important role in the body's normal functioning and are vital for humans **[9]**. The shortage of protein in the human body causes a delay in the growth of tissues, the assimilation of certain substances, and the formation of new cells the growth of tissues, the formation of new cells, and the assimilation of various substances is impossible.

The human body cannot produce protein independently, so it is recommended to consume food of plant and animal origin with a high protein content **[10]**.

Until recently, the public did not perceive eating insects or insect processing products as innovative raw materials that would improve the chemical or nutritional composition of products and dishes. One of the main factors in not accepting insects as food is the need for a legislative and regulatory framework for this type of product. However, in 2022 the European Union issued an Implementing Regulation of the Commission (EU) which allows the use and consumption on the European Union market of frozen, dried, and powdered forms of crickets of the genus *Acheta domesticus* as a novel food product following Regulation (EU) 2015/2283 of the European Parliament and Council and amendments to Commission Executive Regulation (EU) 2017/2470 **[3]**.

Several scientists have investigated the consumer acceptability of products containing raw materials from insects in different countries. They investigated the advantages of using insects and concluded that conventional technological measures, such as masking the content of raw materials from insects in traditional food products, increase consumers' willingness to use such food products **[11]**, **[12]**, **[13]**, **[14]**, **[15]**, **[16]**.

Scientists from the USA **[7]**, Kyrgyzstan, Asia, and Africa are developing technology for obtaining insect flour. In world practice, domestic cricket (*Acheta domesticus*) is usually used to produce insect flour. Crickets are grown on special farms. For example, the Aspire Corporation (USA) grows crickets on large farms. The entire growing process is automated **[17]**. Crickets are raised on vertical farms equipped with sensors and automated systems. With the help of robotic modules, crickets receive a precisely calculated amount of food **[17]**. At the same time, Belgian farmers raised crickets on typical farms north of Brussels. Cowro Valley Farms raises thousands of insects in heated aluminium foil tents with lots of trays and hanging insect cans in California. Insects are fed with alfalfa and beans grown by the enterprise.

Since the cultivation of crickets for further processing and use in food products is becoming increasingly widespread, scientists are increasingly paying attention to designing the composition of products using insect processing products and studying their properties. Cricket flour is used for the production of various food products, including bakery products **[18]**, cookies **[19]**, muffins **[20]**, and pasta **[21]**.

Considering the chemical composition of flour from crickets, it can be used to improve the nutritional value of whipped flour products. After all, in their composition, whipped flour semi-finished products contain a large number of carbohydrates due to the content of a significant amount of sugar in the recipes, which is of technological importance since it gives the finished products high-quality organoleptic and structural properties, and also increases the temperature of pasteurisation of starch grains of flour and denaturation of egg white, while allowing the formed gas bubbles to expand **[22]**, however, makes the products high-calorie with low biological activity.

This work aims to develop a whipped flour semi-finished product with an increased protein content by using cricket flour as an innovative ingredient and studying the rheological properties of the dough and the physicochemical properties of the baked semi-finished product.

According to the set goal, the following tasks were defined:

- investigate the rheological properties of the dough;
- to investigate the resilience and elasticity of the baked biscuit semi-finished product;
- to investigate the physicochemical properties of the ready-to-use flour semi-finished product using cricket flour.

Scientific Hypothesis

Developing a functional product using cricket flour can increase the nutritional and biological value and affect the rheological and physicochemical parameters of the sponge cake and the finished semi-finished product. When cricket flour is added, we expect the physicochemical and rheological indicators to be within the normal range and increase the number of proteins in the sponge cake.

MATERIAL AND METHODOLOGY

Samples

To produce WFP, chicken eggs, sugar, wheat flour, citric acid, and cricket flour were used.

Chemicals

All ingredients (except cricket flour) for preparing the semi-finished flour batter were purchased from the local market and were of analytical quality. Cricket flour was ordered from a European company.

Chicken eggs TM Yasen Svit, sugar TM Komora Krup, wheat flour TM Hutorok, citric acid, flour from crickets TM "SENS Foods" London UK.

Instruments

The dough for the whipped flour semi-finished product was prepared using an Ariete Digimix Professional 1585 mixer and baked in a Liberton LEO-651 electric oven. The rheological properties of the dough for WFP were determined on the "Rheotest-2." The shear rate from 1.9 to 76 s^{-1} was studied using spindle No S07 at 18 revolutions and a temperature of 20 °C.

The elasticity of the finished product was determined using a "Labor" penetrometer. The degree of penetration was measured in 0.1 mm of the distance at which the indicator penetrated the material under study perpendicular to its surface. The research was conducted at 20 °C.

Laboratory Methods

The mass fraction of moisture was determined by drying to constant mass **[23]**. For this model sample, the bags were placed in a drying cabinet heated to 130 °C and dried by long-term drying for 40 minutes. After drying, the boxes with lids were placed in a desiccator for 30 minutes and weighed. For the final result of the mass fraction of moisture in the whipped flour semi-finished product, the average arithmetic value of 4 was taken.

The mass fraction of ash insoluble in 100% hydrochloric acid, based on absolutely dry matter, was determined using the dry method in a muffle furnace **[24]**. The Kjeldahl method determined the mass fraction of protein **[25]**. Alkalinity is using the titration method **[26]**. The iodometric method determined the mass fraction of total sugar **[27]**.

Description of the Experiment

Sample preparation: The whipped flour semi-finished product was prepared in four variations, each differing in the innovative ingredient. Cricket flour was added to the semi-finished product in amounts (5.0%, 10.0%, 15.0%) to replace an equivalent wheat flour. The control sample of the whipped flour semi-finished product was prepared without adding flour from crickets.

According to the technology of preparing a whipped flour semi-finished product, egg yolks were first beaten with granulated sugar until a homogeneous mass was formed and the sugar dissolved. Then egg whites were beaten with citric acid until a lush, smooth structure was formed, after which the beaten yolks were combined with the beaten proteins, and high-grade wheat flour was added (control) or a mixture of wheat flour and cricket flour into the studied samples. The mixed dough was poured into a round mold and baked for 40-50 minutes. at a temperature of 180 °C. After baking, the WFP was cooled for 12 hours at room temperature. According to organoleptic indicators, BC has a powdery appearance, light grey color, pleasant taste, and aroma of walnut **[28]**.

The round sponge cake recipe was chosen (Table 1) as an analogue of the recipe for preparing WFP **[32]**.

Table 1 Recipe for whipped flour semi-finished product using BC in different dosages.

The name of the raw material	Consumption of raw materials for 300 g of products, g			
	Control	5.0%	10.0%	15.0%
Wheat flour of the highest grade	116.82-120.00	110.98-114.00	105.14-108.00	99.30-102.00
Sugar-sand	102.57-105.60	102.57-105.60	102.57-105.60	102.57-105.60
Egg yolks	102.57-108.14	102.57-108.14	102.57-108.14	102.57-108.14
Egg whites	149.45-153.84	149.45-153.84	149.45-153.84	149.45-153.84
Citric acid	0.40-0.46	0.40-0.46	0.40-0.46	0.40-0.46
Crickets flour	-	5.84-6.00	11.68-12.00	17.52-18.00
Total:	491.26	491.26	491.26	491.26
Output of raw materials	300.0	300.0	300.0	300.0

Number of samples analyzed: We analyzed 4 samples.

Number of repeated analyses: The experimental data presented in the paper are averages with repeatability of at least 3 times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was two times.

Design of the experiment: At the beginning of the experiment, the dough's rheological properties and the elasticity of the finished semi-finished product were determined. The content of the mass fraction of moisture, alkalinity, ash, mass fraction of sugar, protein, fat, and chitin was studied. Based on the obtained data, the total energy value of the semi-finished product was determined.

Statistical Analysis

All assays were performed in triplicate and the obtained data are presented as means ± SEM (the standard error of the mean). Sponge cake features were tested through a two-way ANOVA on the flour type, percentage of substitution, and their interaction, with a significance level of $p \leq 0.05$. The accuracy of the measurements was determined with a reliability of d = 0.95. The method's error was estimated by the total error in percent. To determine the optimal concentrations of the main components of the whipped flour semi-finished product, we used mathematical processing of the obtained results by comparing the functions of two variables and probing space parameters. To do this, we first optimally detected the parameter space on an uneven rectangular network and then mathematically processed experimental and calculated (theoretical) values of the function F over the entire parameter space. Control experiments were performed at arbitrarily selected points in the parameter space to test the likelihood of consistent F values. Based on experimental data within the framework of the paired model, correlation and regression analysis methods were used to determine the optimal: concentrations of the components of the semi-finished product model for modeling shear stress, shear rate, and viscosity. The empirical data was approximated using the MathCAD, MathLAB application program package, and the Excel spreadsheet package. Excel provides new tools for creating and designing spreadsheets and powerful tools for

analyzing business information. It is a tool that allows you to organize, process, organize, analyze and graphically present different types of data in the way you want. From the point of view of informatics, most of the documents that research engineers work with belong to the category of tabular documents, where the smallest structural unit of information exists at the intersection of columns and rows a necessary condition, and therefore the simplest. and the most efficient is processed by the table processor. Spreadsheets are the backbone of any spreadsheet. Accordingly, the empirical data were approximated using an Excel spreadsheet.

RESULTS AND DISCUSSION

WFP refers to foam structure systems characterized by a certain viscosity of the initial mass. High structural viscosity determines the dough's mechanical strength, creating an elastic frame and giving the system a solid body's physical and chemical properties. The stability of the dispersed system, which is in the WFP, is largely determined by the viscosity of the initial solutions and their resistance to the action of loads. The study of previous studies on the use of cricket flour in the production of flour confectionery **[29]**, shows that introducing cricket flour into the production technology of WFP can affect the stability of the dough system. That is why the dependence of the effective viscosity of WFP samples with different amounts of cricket flour on the deformation rate and tangential shear stress was investigated.

Viscosity is an important technological characteristic of dough for WFP. The viscosity value evaluates the intensity of the formation and destruction of the structure **[30]**. According to the study's results, the effective viscosity of the dough with the addition of cricket flour affects the structure of the dough. As a result, the system undergoes certain changes. Viscosity is the final change in the characteristic that describes the equilibrium state between the restoration and destruction of the structure in a steady flow. In turn, the effective viscosity is determined through the tangent of the pseudo-Newtonian flow angle. In this study, the effective viscosity was measured using a rotary viscometer. Previously, it was established that the effective dough viscosity decreased with increasing shear rate for WFP using spelt flour **[32]**. Also, effective viscosity decreased when wheat flour was completely replaced by rice flour using the WFP technology. However, a mixture of rice flour and sesame meal flour in a ratio of 70:30% increased viscosity indicators **[33]**.

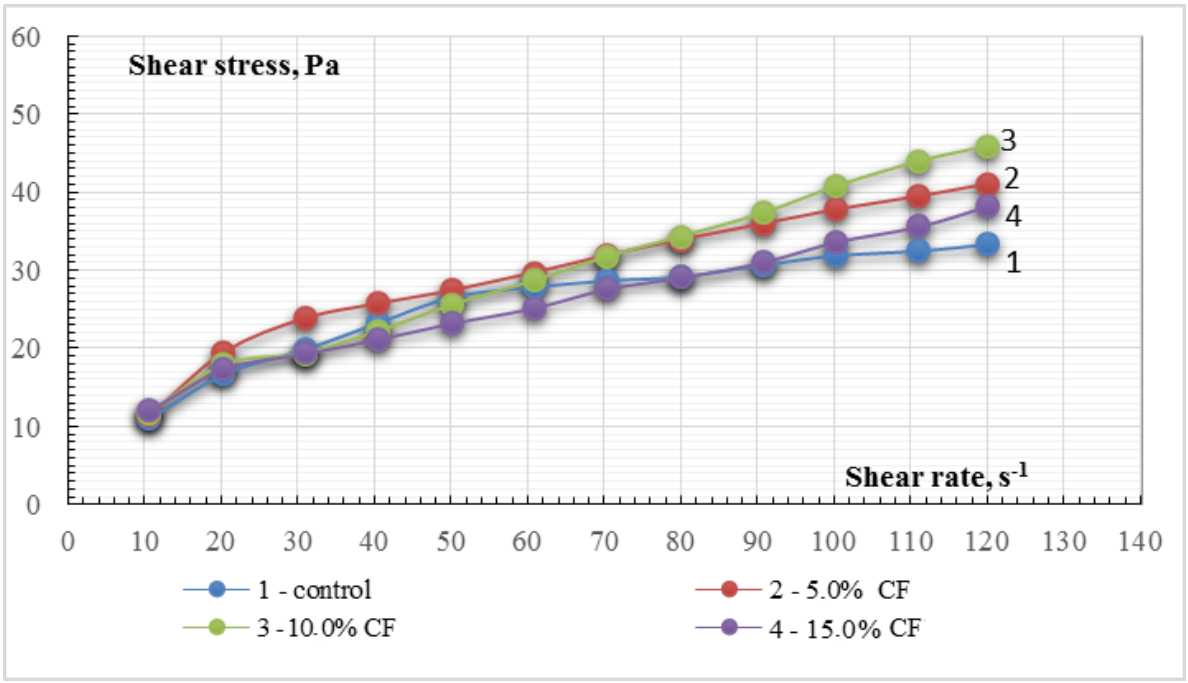


Figure 1 Dependence of shear stress on shear rate for a dough using CF.

Figure 1 shows that as the shear rate increases, the shear stress for all samples increases while the viscosity of these systems decreases. According to these data, it can be concluded that the dough for WFP belongs to non-Newtonian bodies. These systems are characterised by the ultimate shear stress, which can correspond to the elastic deformation component.

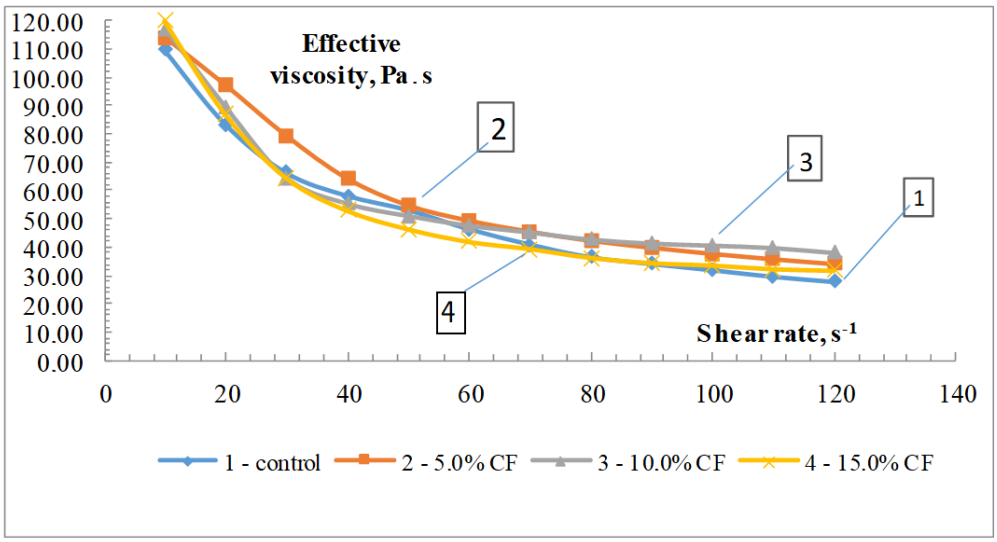


Figure 2 Dependence of effectiveness is cozily on shear rate for dough using CF.

In Figure 2, the analysis of the viscosity curves shows that no significant changes in the viscosity of the dough due to the addition of different amounts of CF were detected; a slight difference was noticeable at the minimum shear rate, the shear stress increased for the sample with the addition of 5.0% cricket flour by 10 ±0.5%, for the sample with 10.0% CF by 13 ±0.5%, for the sample with the addition of 15.0% CF by 15 ±0.5% compared to the control sample, as a result, when more CF is added, the shear stress increases. This suggests that cricket flour has a fairly high moisture-binding capacity. At high speed, the shear strength of the dough was constant for all samples except the control.

Sponge cake belongs to non-Newtonian liquids. This means that the decrease in viscosity has the effect of increasing the shear rate. In this study, the most intensive increase in effective viscosity was noted in the dough with the addition of cricket flour in the amount of 10.0% at a shear rate of 0.8 s^{-1}. However, in the future, the decrease in effective viscosity with increased shear rate is observed less intensively in all samples. The destruction of the dough's foam system can explain this viscosity change with increased shear rate. At a speed of 12.0 s^{-1}, the viscosity of all samples remained at the same level.

Therefore, when studying the rheological parameters of the dough with the addition of cricket flour in the amount of 5.0-15.0%, there are certain changes in the effective viscosity, and it increases. However, an increase in the shear rate in the range of 0.9-12.0 s^{-1} leads to stabilization of the viscosity of the systems of the studied samples. In addition, studies of the effective viscosity of the investigated biscuit dough systems with the addition of different amounts of cricket flour showed that introducing new raw materials does not significantly affect the rheological properties of the dough.

WFP has a foam-like structure characterized by the presence of a porous pulp. Adding a new ingredient to the WFP can lead to the deterioration of its structure and especially affect the elasticity of the pulp. After all, flour from crickets does not contain gluten. Therefore, it is important to determine the effect of CF addition on the elasticity of the WFP depending on the amount of the introduced innovative ingredient. The elasticity of the biscuit affects its aerodynamic properties (lightness, softness, flexibility) and texture. The addition of CF to the WFP formulation significantly increased (p <0.05) the intensity of all model samples compared to the control sample. The greater the elasticity, the lighter and airier the biscuit **[35]**.

The resiliency and elasticity of baked semi-finished products also depend on the drying process and moisture loss during baking. Since the main part of moisture in WFP is bound by gluten flour and starch, replacing part of wheat flour with flour from crickets will affect the quality of the finished product **[36]**.

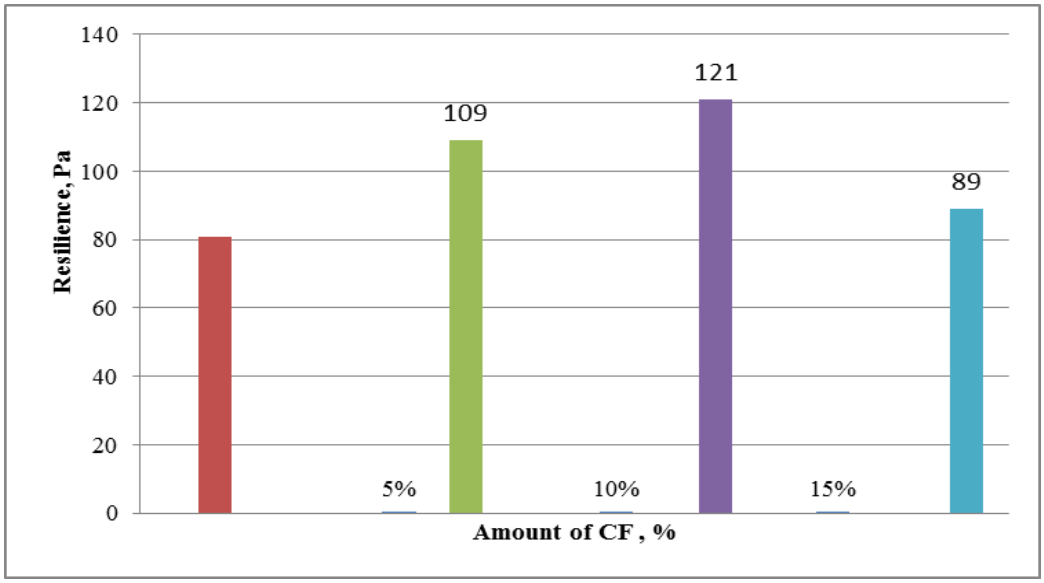


Figure 3 Resilience of biscuit products with the addition of CF.

The resilience is a measure of the ability of the biscuit semi-finished product to recover after compression **[37]**. Low elasticity was found in the control biscuit semi-finished product and semi-finished product with the addition of 15.0% CF (Figure 3). Probably, the increase in elasticity can be associated with a decrease in the volume and total content of starch, and therefore, with the formation of a denser pulp. The elasticity of WFP with adding 5.0% and 10.0% CF is slightly better than the control sample. It is likely that in these models, there is a sufficient amount and significant activity of free moisture, which causes an improvement in elasticity; when the content of cricket flour increases, the amount of protein, which requires an additional amount of moisture, increases, therefore the pulp density increases, accordingly, elasticity decreases.

It is known that physic-chemical parameters characterize the processes occurring during the kneading and baking of semi-finished products **[38]**. The main physic-chemical indicators include a mass fraction of moisture, mass fraction of ash, protein and sugar, alkalinity, and other indicators **[39]**. The ingredients in the recipe begin to closely interact with each other during the formation of the liquid phase of the dough. The stability of the dough structure during kneading and further processing largely depends on its water content and the ratio of free and bound moisture. Depending on the amount of moisture in the dough, during heat treatment of the product, water changes from a liquid phase to a gaseous phase and evaporates at a high temperature **[39]**.

The sugar content in WFP is the highest compared to other types of flour products and is in a ratio of 1:1 to flour.

The sugar content in the dough affects the degree of plasticity since sugar reduces the swelling of proteins and flour starch and significantly affects the structure of the dough and the quality of the final product. The addition of sugar to the flour confectionery recipe depends on the product's recipe, the flour's characteristics, and the temperature of kneading **[40]**.

The protein content in various formulation components determines the mass fraction of protein in WFP. According to the recipe components that are part of the ZBN, egg products up to 6 g **[29]**, wheat flour **[31]**, as well as cricket flour have a certain amount of protein, so the protein content varies depending on the concentration of CF in the WFP recipe **[41]**, **[42]**.

Ash consists mainly of metal oxides and trace elements contained in food products. For some products, the amount of ash affects quality and is a factor to consider in quality analysis.

In turn, the ash content of flour is of particular interest, as it is believed to affect baking quality. An ash content of 1.5-2% is desirable **[31]**.

Table 2 Physicochemical properties of WFP with the addition of different amounts of cricket flour.

Indicator	According to DSTU	control	5.0%	10.0%	15.0%
Mass fraction of moisture, %	16.0 ±3.0%	16.0 ±3.0	25.74 ±3.0	32.58 ±3.0	35.47 ±3.0
The mass fraction of ash not dissolved in 10% hydrochloric acid, in terms of absolute dry matter, %, no more than	0.1	0.01	0.03	0.07	0.1
Mass fraction of protein, %	No less 4.4	4.4 ±2.0	7.8 ±2.0	11.61 ±2.0	17.42 ±2.0
Alkalinity, degree	Not more 2.5	1.1 ±0.25	1.1 ±0.25	1.1 ±0.25	1.1 ±0.25
Mass share of total sugar (in terms of sucrose), %	According to the calculated content according to the recipe	60.0 ±2.5	49.8 ±2.5	31.6 ±2.5	29.4 ±2.5

Because of the importance of general definitions of physicochemical indicators in ZBN, this study analyzed and compared the content of sugar, ash, and protein, as well as determined the mass fraction of moisture and alkalinity of the products (Table 1).

As expected, the composition of the tested samples depends on the ingredients used. The protein content of the samples increased several times compared to the control sample, as cricket flour helps to increase the protein content. The highest values were obtained in the sample with 15.0% cricket flour, while the lowest protein content was found in the sample without adding the tested flour. The increase in protein with the addition of CF was also reported in the technologies of making muffins **[36]**, pancakes **[20]**, and wheat bread **[43]**. A decrease in the amount of sugar indicates a decrease in the amount of carbohydrates; therefore, a decrease in caloric content and an increase in the amount of ash indicates an increase in the content of minerals, as well as macro- and microelements.

Table 3 Comparative analysis of the nutritional value of WFP.

Samples	Moisture, %	Proteins, %	Fats, %	Carbohydrates, %	Ash,%	Chitin, %	Energy value, kcal
Control	16.00 ±3.0	4.4 ±0.14	7.02 ±0.30	60.13 ±0.07	0.00 ±0.00	0.00 ±0.00	321.55
5.0% CF	25.74 ±1.0	7.80 ±0.05	7.95 ±0.10	49.8 ±0.10	0.05 ±0.02	0.31 ±0.01	291.99
10.0%CF	32.58 ±1.0	11.61 ±0.03	8.89 ±0.10	31.6 ±0.07	0.07 ±0.02	0.62 ±0.01	246.53
15.0%CF	35.47 ±1.0	17.42 ±0.04	9.82 ±0.10	29.4 ±0.10	0.09 ±0.02	0.93 ±0.01	269.78

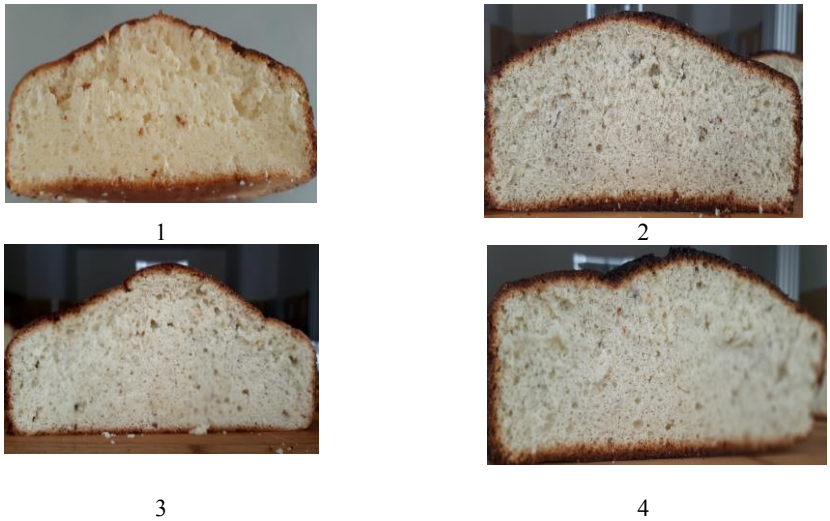
Because of the high protein content in the innovative WFP when CF is added, we note that the nutritional and biological value of the product changes compared to the control sample (Table 2).

Thus, when calculating the nutritional and energy value, we note that the amount of proteins in MPP with the addition of CF in the amount of 5.0% increased by almost 43.0%, with the addition of 10.0%, it increased by almost 62.06%, and with the addition of 15.0% – by 74.7% following the control sample, and the amount of carbohydrates decreased by 17.2%; 47.4%; 51.1%, respectively. The obtained results allow us to conclude that MPP with the addition of CP can be recommended as a product enriched with a protein component.

The organoleptic parameters of baked WPF were discussed in a study published earlier **[28]**. Figure 3 shows the appearance of WPF. Important indicators of products were color, taste, and smell. After all, these indicators affected the quality of the products. When cricket flour was added to BN, the color changed from light green to olive and had a score for the product with the addition of 5.0 and 10.0% − 5, with the addition of 15.0% CF − 3 (p <0.05), the smell was pleasantly nutty with the addition of 10.0% flour and pronounced nutty with the addition of 15.0%. The product's taste was characteristic of a semi-finished biscuit product, but WPF, with the addition of 15.0%, had a more pronounced taste of nuts, so the tasters gave a score of 5 (p <0.05).

Sensory scores were ≥4.75 for samples with 5.0% and 10.0% CF. A chocolate cookie based on CF was accepted with a 0.50 lower sensory score **[41]**. It should be noted that in Ukraine, flour from crickets is a new product on the market; therefore, its acceptance by consumers is related to sensory properties and the important issue of microbiological safety. Microbiological indicators of CF were determined by comparing them with wheat flour **[44]**.

When conducting research among Ukrainian consumers, flour from crickets caused different emotions from negative (i.e. rejection) to positive. Indicators of positive emotions (3.46-2.53) were better when consumers were not told about the CF content in the product. However, 85.0% of the surveyed respondents were ready to consume CF-based WFC for its quality and nutritional characteristics after the tasting.



1 2

3 4

Figure 4 Baked round biscuit with the addition of cricket flour: 1 – control; 2 – with the addition of 5.0% flour from crickets; 3 – with the addition of 10.0% flour from crickets; 4 – with the addition of 15.0% flour from crickets.

CONCLUSION

According to the study's results, when CF is added to the WFP technology, the dough's rheological parameters differ from the control's. In particular, the use of CF in WFP helps to reduce the dough's viscosity. Still, it increases its stability, which in the future will have a positive effect on the processes of forming and baking. Studies of resiliency and elasticity have shown that when CF is added to the sponge cake recipe in the amount of 5.0-10.0%, the resiliency and elasticity of the crumb increase when the ingredient is added in the amount of 15.0%, compared to the main WFP—the value of the data indicators decreases. However, these changes could be more significant and affect the quality of the finished product. In terms of physicochemical properties, the baked innovative WFP differs from the control sample; according to DSTU, the mass part of protein should be at least 4.4%, but in this study, it was determined that the addition of cricket flour to the recipe composition increases the increase in protein by 62.06%. The mass fraction of moisture in the semi-finished product with 5.0-15.0% BC increased from 1.61 to 2.22%; the mass fraction of ash decreased from 0.1 to 0.07%, and the mass fraction of total sugar decreased by 47.4%, respectively. Therefore, flour from crickets is a promising raw material. Its use will expand the range of food and culinary products, increase their nutritional and biological value, and give products new organoleptic characteristics and tastes.

REFERENCES

1. Zielińska, E., Pankiewicz, U., & Sujka, M. (2021). Nutritional, Physiochemical, and Biological Value of Muffins Enriched with Edible Insects Flour. In Antioxidants (Vol. 10, Issue 7, p. 1122). MDPI AG. https://doi.org/10.3390/antiox10071122
2. Stetsenko, N. O. (2023). The problem of protein deficiency in the nutrition of the population of Ukraine and ways to solve it. In Modern engineering and innovative technologies (Issues 28–01, pp. 41–45). Ukrainian Research and Design Institute of Building Materials and Products. https://doi.org/10.30890/2567-5273.2023-28-01-028
3. Commission Implementing Regulation (EU) № 2022.188 of 10 February 2022 authorising the placing on the market of frozen, dried and powder forms of Acheta domesticus as a novel food under Regulation (EU) №2015/2283 of the European Parliament and of the Council, and amending Commission Implementing Regulation (EU) 2017.2470 (Text with EEA relevance).
4. Carcea, M. (2020). Quality and Nutritional/Textural Properties of Durum Wheat Pasta Enriched with Cricket Powder. In Foods (Vol. 9, Issue 9, p. 1298). MDPI AG. https://doi.org/10.3390/foods9091298
5. Xiaoming, C., Ying, F., & Hong, Z. (2008). Review of the nutritive value of edible insects. In Forest insects as food: humans bite back. Proceedings of a workshop on Asia-Pacific resources and their potential for development, p. 19–21.
6. Brynning, G., Bækgaard, J. U., & Heckmann, L.-H. L. (2020). Investigation of Consumer Acceptance of Foods Containing Insects and Development of Non-Snack Insect-based Foods. In Industrial Biotechnology (Vol. 16, Issue 1, pp. 26–32). Mary Ann Liebert Inc. https://doi.org/10.1089/ind.2019.0028

7. Murugu, D. K., Onyango, A. N., Ndiritu, A. K., Osuga, I. M., Xavier, C., Nakimbugwe, D., & Tanga, C. M. (2021). From Farm to Fork: Crickets as Alternative Source of Protein, Minerals, and Vitamins. In Frontiers in Nutrition (Vol. 8). Frontiers Media SA. https://doi.org/10.3389/fnut.2021.704002
8. Ahern, K., Rajagopal, I., & Tan, T. (2018). Biochemistry, free for all. (Version 2.1, p. 3648). Retrieved from http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.
9. Kuchnir, G. V., Levytzkiy, T. R., Ryvak, G. P., Kurylas, L. V., Vilha, O. M., & Fedor, G. Y. (2017). Characteristics of modern methods of determining crude protein in feed and vegetable raw materials. In Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies (Vol. 19, Issue 82, pp. 97–100). Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. https://doi.org/10.15421/nvlvet8220
10. Cakhno, T. V., & Horbach, D. O. (2022). The importance of protein in human life. In Protein as a sports supplement (pp. 103–105). Yuriy Kondratyuk Poltava Polytechnic National University. Retrieved from http://repository.pdmu.edu.ua/handle/123456789/20172.
11. Barsics, F., Caparros Megido, R., Brostaux, Y., Barsics, C., Blecker, C., Haubruge, E., & Francis, F. (2017). Could new information influence attitudes to foods supplemented with edible insects? In British Food Journal (Vol. 119, Issue 9, pp. 2027–2039). Emerald. https://doi.org/10.1108/bfj-11-2016-0541
12. Menozzi, D., Sogari, G., Veneziani, M., Simoni, E., & Mora, C. (2017). Eating novel foods: An application of the Theory of Planned Behaviour to predict the consumption of an insect-based product. In Food Quality and Preference (Vol. 59, pp. 27–34). Elsevier BV. https://doi.org/10.1016/j.foodqual.2017.02.001
13. Pambo, K. O., Okello, J. J., Mbeche, R. M., Kinyuru, J. N., & Alemu, M. H. (2018). The role of product information on consumer sensory evaluation, expectations, experiences and emotions of cricket-flour-containing buns. In Food Research International (Vol. 106, pp. 532–541). Elsevier BV. https://doi.org/10.1016/j.foodres.2018.01.011
14. Lombardi, A., Vecchio, R., Borrello, M., Caracciolo, F., & Cembalo, L. (2019). Willingness to pay for insect-based food: The role of information and carrier. In Food Quality and Preference (Vol. 72, pp. 177–187). Elsevier BV. https://doi.org/10.1016/j.foodqual.2018.10.001
15. Ardoin, R., & Prinyawiwatkul, W. (2020). Product appropriateness, willingness to try and perceived risks of foods containing insect protein powder: A survey of U.S. consumers. In International Journal of Food Science & Technology (Vol. 55, Issue 9, pp. 3215–3226). Wiley. https://doi.org/10.1111/ijfs.14612
16. Nissen, L., Samaei, S. P., Babini, E., & Gianotti, A. (2020). Gluten free sourdough bread enriched with cricket flour for protein fortification: Antioxidant improvement and Volatilome characterization. In Food Chemistry (Vol. 333, p. 127410). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.127410
17. Messina, Gaglio, Morghese, Tolone, Arena, Moschetti, Santulli, Francesca, & Settanni. (2019). Microbiological Profile and Bioactive Properties of Insect Powders Used in Food and Feed Formulations. In Foods (Vol. 8, Issue 9, p. 400). MDPI AG. https://doi.org/10.3390/foods8090400
18. González, C. M., Garzón, R., & Rosell, C. M. (2019). Insects as ingredients for bakery goods. A comparison study of H. illucens, A. domestica and T. molitor flours. In Innovative Food Science & Emerging Technologies (Vol. 51, pp. 205–210). Elsevier BV. https://doi.org/10.1016/j.ifset.2018.03.021
19. Homann, A. M., Ayieko, M. A., Konyole, S. O., & Roos, N. (2017). Acceptability of biscuits containing 10% cricket (Acheta domesticus) compared to milk biscuits among 5-10-year-old Kenyan schoolchildren. In Journal of Insects as Food and Feed (Vol. 3, Issue 2, pp. 95–104). Brill. https://doi.org/10.3920/jiff2016.0054
20. Zielińska, E., Pankiewicz, U., & Sujka, M. (2021). Nutritional, Physiochemical, and Biological Value of Muffins Enriched with Edible Insects Flour. In Antioxidants (Vol. 10, Issue 7, p. 1122). MDPI AG. https://doi.org/10.3390/antiox10071122
21. Carcea, M. (2020). Quality and Nutritional/Textural Properties of Durum Wheat Pasta Enriched with Cricket Powder. In Foods (Vol. 9, Issue 9, p. 1298). MDPI AG. https://doi.org/10.3390/foods9091298
22. Krupa-Kozak, U., Drabińska, N., Rosell, C. M., Piłat, B., Starowicz, M., Jeliński, T., & Szmatowicz, B. (2020). High-Quality Gluten-Free Sponge Cakes without Sucrose: Inulin-Type Fructans as Sugar Alternatives. In Foods (Vol. 9, Issue 12, p. 1735). MDPI AG. https://doi.org/10.3390/foods9121735
23. DSTY 4910:2008 Confectionery products. Methods of determining mass fractions of moisture and dry substances.
24. DSTY 4672:2006 Confectionery products. Methods of determination of ash and metallomagnitic impurities.
25. ISO 5983:1997 Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Kjeldahl method.
26. DSTY 5024:2008 Confectionery products. Methods of determining acidity and alkalinity.

27. DSTY 5059:2008 Confectionery products. Methods of determination of sugars.
28. Sereda, O., & Melnyk, O. (2023). Organoleptic Analysis of Round Sponge Cake with the Addition of Protein-Containing Raw Materials. In Restaurant and hotel consulting. Innovations (Vol. 6, Issue 1, pp. 125–139). Kyiv National University of Culture and Arts. https://doi.org/10.31866/2616-7468.6.1.2023.278476
29. Tsekhmistrenko, S. I., & Tsekhmistrenko, O. S. (2023). Biochemistry of a bird's egg. Teaching. Manual. Retrieved from https://rep.btsau.edu.ua/bitstream/BNAU/8945/1/Biokhimiia_ptashynoho.pdf.
30. Kravchenko, M. F., Romanovska, O. L., & Boruk, S. D. (2015). Rheological properties of biscuit dough with "Zdorovya" flour. In Scientific works of the National Technical University of Ukraine (Vol. 21, Issue 5, pp. 200–205). National Technical University of Ukraine.
31. Nemirich, O. V., Mykhaylenko, V. M., & Berezhna, T. O. (2018). Comparative characteristics of the chemical composition and biological value of gluten and wheat flour. In Techniques and Technology (Vol. 3, pp. 30–33). Science, Research, Development: monografia pokonferencyjna, Warszawa: Diamond trading tour.
32. Mykhailo, K., Olha, R., & Tetiana, M. (2021). Rheological properties of biscuits dough with spelt flour. In the international scientific-practical journal “commodities and markets” (Vol. 38, Issue 2, pp. 94–102). Kyiv National University of Trade and Economics. https://doi.org/10.31617/tr.knute.2021(38)09
33. Iorgachova, K., Makarova, O., & Kotuzaki, E. (2016). The influence of gluten-free flours on the quality indicators of biscuit semi-finished products. In Grain Products and Mixed Fodder’s (Vol. 64, Issue 4, pp. 16–21). Odesa National University of Technology.
34. Borges, M. M., da Costa, D. V., Trombete, F. M., & Câmara, A. K. F. I. (2022). Edible insects as a sustainable alternative to food products: an insight into quality aspects of reformulated bakery and meat products. In Current Opinion in Food Science (Vol. 46, p. 100864). Elsevier BV. https://doi.org/10.1016/j.cofs.2022.100864
35. Ahmadinia, F., Mohtarami, F., Esmaiili, M., & Pirsa, S. (2023). Investigation of physicochemical and sensory characteristics of low calorie sponge cake made from flaxseed mucilage and flaxseed flour. In Scientific Reports (Vol. 13, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-023-47589-5
36. Marzec, A., Kowalska, J., Domian, E., Galus, S., Ciurzyńska, A., & Kowalska, H. (2021). Characteristics of Dough Rheology and the Structural, Mechanical, and Sensory Properties of Sponge Cakes with Sweeteners. In Molecules (Vol. 26, Issue 21, p. 6638). MDPI AG. https://doi.org/10.3390/molecules26216638
37. Kryvoruchko, M. (2018) Elasto-viscous characteristics of dough compositions with coconut fiber. In International Cientific-Ractical Journal Ommodities and Markets (Vol. 23, Issue 7, p. 99–108). State University of Trade and Economics.
38. Stepankova, G. V., Shidakova-Kamenyuka, O. G., Bolkhovitina, O. I., & Horobets, I. S. (2022). Prospects of using pea flour in donuts technology. In Herald of Lviv University of Trade and Economics Technical sciences (Issue 28, pp. 52–58). Publishing House Helvetica (Publications). https://doi.org/10.36477/2522-1221-2021-28-08
39. Barabolia, O. V., Tatarko, Yu. V., & Antonovskiy, O. V. (2020). The influence of variety features of winter wheat grain on the quality of bakery properties. Bulletin of Poltava State Agrarian Academy, (4), 21–27. https://doi.org/10.31210/visnyk2020.04.02
40. Dorohovych, V. (2020). Flour confectionery products for people with diabetes with the use of products from processing carrots. In Scientific Works of National University of Food Technologies (Vol. 26, Issue 1, pp. 238–244). National University of Food Technologies. https://doi.org/10.24263/2225-2924-2020-26-1-28
41. Aleman, R. S., Marcia, J., Pournaki, S. K., Borrás-Linares, I., Lozano-Sanchez, J., & Fernandez, I. M. (2022). Formulation of Protein-Rich Chocolate Chip Cookies Using Cricket (Acheta domesticus) Powder. In Foods (Vol. 11, Issue 20, p. 3275). MDPI AG. https://doi.org/10.3390/foods11203275
42. Mazurek, A., Palka, A., Skotnicka, M., & Kowalski, S. (2022). Consumer Attitudes and Acceptability of Wheat Pancakes with the Addition of Edible Insects: Mealworm (Tenebrio molitor), Buffalo Worm (Alphitobius diaperinus), and Cricket (Acheta domesticus). In Foods (Vol. 12, Issue 1, p. 1). MDPI AG. https://doi.org/10.3390/foods12010001
43. Simeon, E. I. D., Basiru Danjuma, A., & Momoh, A. (2022). Effects of Cricket Addition on the Chemical, Functional, and Sensory Properties of Complementary Formulation from Millet Flour. In Turkish Journal of Agriculture - Food Science and Technology (Vol. 10, Issue 4, pp. 508–516). Turkish Science and Technology Publishing (TURSTEP). https://doi.org/10.24925/turjaf.v10i4.508-516.4414

44. Sereda O., & Melnyk O. (2024). Microbiological indicator of crockets flour. In The Scientific Heritage (Vol. 131, pp. 56–59). Global science center LP.

Funds:

This research received no external funding.

Acknowledgments:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Olha Sereda**, Sumy National Agrarian University, The Faculty of Food Technologies, Technologies of Nutrition Department, Gerasima Kondratieva st., 160, 40021, Ukraine,
Tel.: +380997145294
E-mail: seaol@ukr.net
ORCID: http://orcid.org/0000-0002-2614-725X

Oxana Melnyk, Sumy National Agrarian University, The Faculty of Food Technologies, Technologies of Nutrition Department, Gerasima Kondratieva st., 160, 40021, Ukraine,
Tel.: +3809993671418
E-mail: oxana7@i.ua
ORCID: https://orcid.org/0000-0002-9201-7955

Tetiana Marenkova, Sumy National Agrarian University, The Faculty of Food Technologies, Technologies of Nutrition Department, Gerasima Kondratieva st., 160, 40021, Ukraine,
Tel.: +380503073830
E-mail: tanya_201@ukr.net
ORCID: https://orcid.org/0000-0001-7481-0848

Olena Koshel, Sumy National Agrarian University, The Faculty of Food Technologies, Technologies of Nutrition Department, Gerasima Kondratieva st., 160, 40021, Ukraine,
Tel.: +380501314350
E-mail: koshelolena85@ukr.net
ORCID: https://orcid.org/0000-0002-2184-2106

Sergii Bokovets, Sumy National Agrarian University, The Faculty of Food Technologies, Technologies of Nutrition Department, Gerasima Kondratieva st., 160, 40021, Ukraine,
Tel.: +380671878061
E-mail: sergiy_bokovec@ukr.net
ORCID: https://orcid.org/0000-0003-0466-2426

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 17.2.2024
Revised: 13.3.2024
Accepted: 20.5.2024
Published: 10.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 480-496
https://doi.org/10.5219/1965
ISSN: 1337-0960 online
www.potravinarstvo.com

Spectroscopic assessment and quantitative analysis of the trace element composition of vegetable additives to meat products

Igor Palamarchuk, Fu Yuanxia, Dmytro Zhuravel, Ievgenii Petrychenko, Roksolana Blishch, Anatoliy Holovatyuk, Olexander Domin, Tatiana Kostiuk

ABSTRACT

In this scientific work, using the method of laser-induced breakdown spectroscopy (LIBS), the spectra of beef samples and impurities in meat products, namely, banana, pineapple, kiwi, bergamot, poria coconut, Chinese angelica, chicken blood vine, were measured by using developed experimental devices. The purpose of the research was to evaluate the qualitative characteristics of additives to meat semi-finished products for the potential formation of the desired properties of the products due to the analysis of the received spectrograms of trace elements of the samples when applying the LIBS method, quantitative analysis for processing the received information. The determined values of the electron temperature of the plasma, the electron density of the used raw material samples, and the assessment of the local heat balance were used as evaluation criteria. When processing the obtained data, the characteristics of the laser-induced plasma surface of the presented samples were analyzed; the electron temperature and electron density were determined, and a quantitative analysis of trace elements was carried out. LIBS technology allows rapid real-time monitoring and qualitative analysis of trace elements online and over long distances. During the research, it turned out that quantitative analysis requires further study and optimisation of experimental conditions, such as pre-treatment of samples. These conditions optimise defocusing, double laser pulse, and sample temperature, which increases the signal/noise ratio of all spectral lines. The combination of fluorescence spectroscopy and Raman technology enables higher detection sensitivity and better molecule control, creating a quantitative analysis method model that can reduce matrix effects and overcome the self-absorption effect. Among the difficulties of using LIBS technology, several elements can be noted online simultaneously, compared to Raman. The combination of spectroscopy and fluorescence spectroscopy can obtain more comprehensive information about the composition of materials, which can become a potential platform for monitoring trace elements in food products.

Keywords: laser-induced spectroscopy, radiation spectrum, impurities in meat products, detection limit, quantitative analysis of spectrograms

INTRODUCTION

Trends in improving the quality characteristics of food products promote the development of new food technologies and products using plant and animal-based additives. When developing new, highly effective varieties of meat products, particularly sausages, sausages, anchovies, and other products, it is practically impossible to bypass the use of these components in modern technologies. Elements of cereal and vegetable raw materials are becoming more and more widespread as a source of vitamins and useful microelements that affect the digestion process and the release of harmful toxic substances from the body due to their relatively low-calorie content **[1]**, **[2]**. Additions of wheat fiber with pumpkin pectin have revealed unique hydrophilic properties and

the ability to form emulsions, increasing sausages' functional-technological and structural-mechanical properties **[3]**, **[4]**. In general, fruits, vegetables, and cereals are characterized by a sufficiently high content of vitamin C, iron, magnesium, phosphorus, and other elements, which allows, when added to meat semi-finished products, to create food products with improved organoleptic indicators and increased biological value **[5]**, **[6]**.

The evaluation of the elemental composition of beef and the used impurities of banana, pineapple, angelica, bergamot, leek, and chicken blood vine carried out in this work was based on the use of the method of laser-induced breakdown spectroscopy (LIBS) **[7]**, **[8]**. The essence of this method is that the sample's surface is irradiated with a sufficiently high-density laser beam. As a result, it evaporates, and neutral and ionic substances are formed in excited states. Excited particle emission spectra confirm sample composition and trace concentration quantitatively. Solid, gaseous, and liquid substances can be used as research objects. The main advantages of this technique include minimal destruction of samples, the possibility of online analysis, remote processing of research results, and determination of data in conditions of high temperatures and aggressive environments, which is often impossible to implement with traditional methods of chemical analysis. Such potential opportunities make it possible to successfully use LIBS technologies in many fields of industrial implementation and theoretical research, in particular, in materials science, chemistry, biochemistry, medicine, archeology, metallurgy, ecology, evaluation of food products, etc. **[9]**, **[10]**.

The purpose of the research was to determine the trace element composition of admixtures to meat semi-finished products for the potential formation of the required product properties by using laser-induced breakdown spectroscopy, theoretical methods of processing the obtained results when determining the electron temperature of the plasma, the electron density of the used samples of raw materials and assessing the local heat balance.

Scientific Hypothesis

The main scientific hypothesis of this scientific work is the adequacy of assessment of the technological, biological, and ecological value of edible meat products based on the results of spectrographic and quantitative analysis of samples of plant impurities; that is, with the help of the technology of laser-induced plasma spectroscopy and according to the results of research, the formed plasma on the surface of the sample can be fairly quickly and qualitatively assessed the trace elemental state of solid, liquid and gaseous substances, regardless of the shape of the object, which shows the effectiveness of the impurities used in the preparation of recipes, meat semi-finished products.

MATERIAL AND METHODOLOGY

Samples

The following samples of food products were used for experimental research: coconut poria (Figure 1a), Chinese angelica (Figure 1b), bergamot (Figure 1c), chicken blood vine (Figure 1d), banana, pineapple, kiwi, and beef. These materials were produced as flakes by preliminary washing, cleaning, grinding, and subsequent drying. The presented components are finely ground to powder to prepare sausage fillers.

Chemicals

Ethylenediaminetetraacetate (EDTA), (country of manufacture China).

Potassium permanganate ($KMnO_4$), (country of manufacture China).

Sodium sulfide (Na_2S), (country of manufacture China).

Water (H_2O), (country of manufacture China).

Animals, Plants, and Biological Materials

The following were used for research: banana (*Musa*), pineapple (*Ananas comosus*), and kiwi (*Actinidia deliciosa*).

Medicinal plants: bergamot (*Citrus bergamia*), coco poria (*Poria cocos*), Chinese angelica (*Angélica*), chicken blood vine (*Spatholobi caulis*).

Beef (boneless beef of the first grade, in which muscle tissue with a mass fraction of connective and fatty tissue did not exceed 7%) (bought in the supermarket Kaoshan Road, Anhui, China).

Instruments

The main elements of LIBS experimental equipment include lasers, digital delay pulse generators, ICCD spectrometers, light collectors, focusing lenses, sample holders, and computers (Figure 2).

Slicer (HURAKAN HKN-HM250M), (country of manufacture China).

Laboratory dryer (DZF PK), (country of manufacture China).

Colloid mill (Vektor-FDM-Z-150), (country of manufacture China).

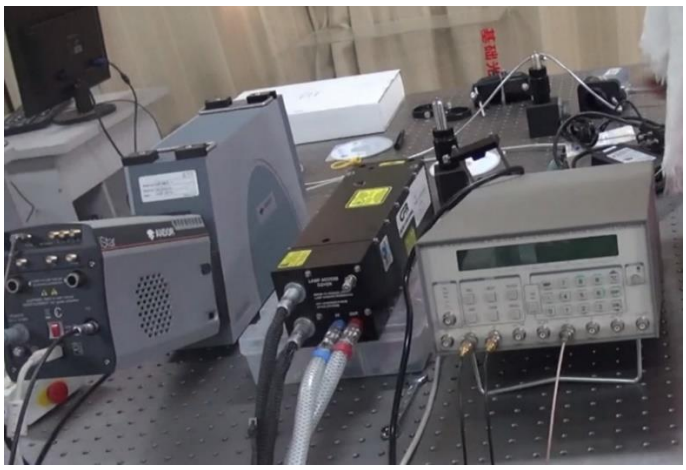
a

b

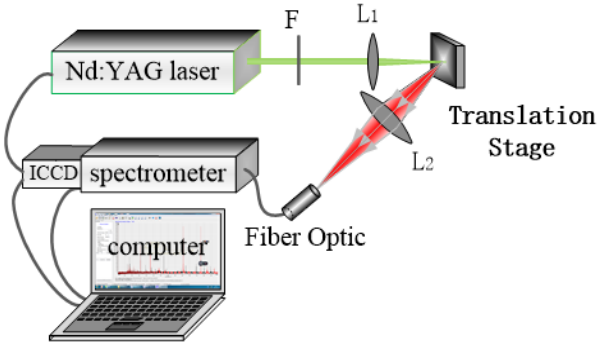
c

d

Figure 1 Form of prepared samples. Note: a – samples of poria coco; b – samples of Chinese angelica; c – samples of bergamot; d – samples of chicken blood vine.



a



b

Figure 2 Experimental equipment for the implementation of the method of laser-induced plasma spectroscopy. Notes: a – experimental spectroscope model; b – structural diagram of the equipment.

Laboratory Methods

The research is based on the method of laser-induced breakdown spectroscopy (LIBS), which allows elemental analysis of the substance **[11]**, **[12]**.

The inclined Boltzmann line method was used to calculate the plasma's electron temperature **[13]**, **[14]**. The current evaluation methods are the standard sample calibration method, the internal standard method, the standard addition method, and the free calibration method (CFLIBS) **[15]**.

When performing a quantitative analysis of the experiment's results, the following were used: the Stark expansion method for determining the electron density of the plasma and the free calibration method **[16]**.

Description of the Experiment

Sample preparation: Before starting the research, the used samples were subjected to the following preliminary treatment: washing and cleaning, cutting into thin slices 3-4 mm thick, and drying was carried out on a laboratory drying unit (Figure 3), which allows maintaining a constant temperature in the working drying area.

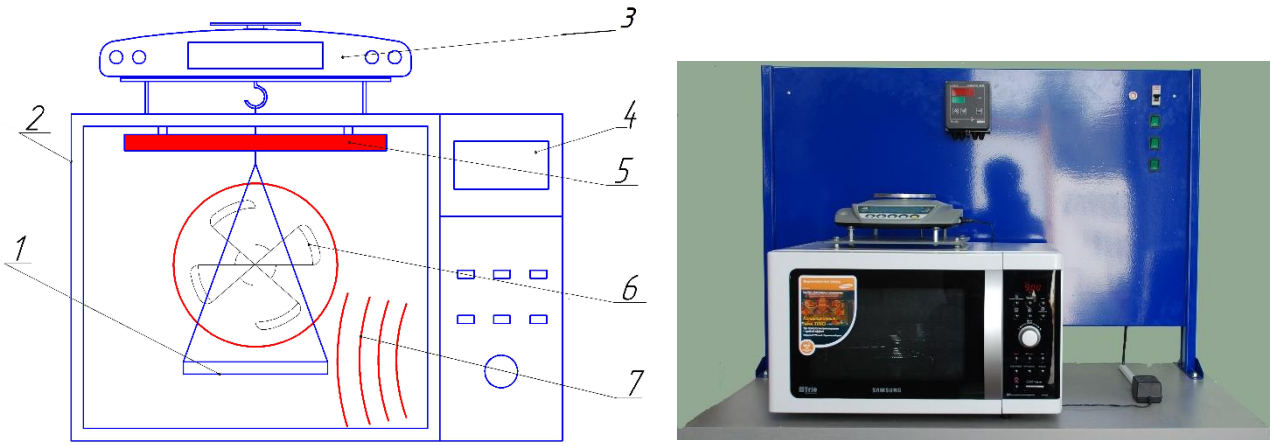


Figure 3 The scheme of the laboratory installation: 1 – bowl with dried material, 2 – camera, 3 – electronic scales, 4 – temperature indicator, 5 – IR heater, 6 – convection heating, 7 – microwave heating.

The drying process was carried out at 70, 80, 90 °C for circles and 90, 100, 110 °C for pieces. This temperature range was chosen because it is impractical to dry samples below 70 °C due to the significant duration of the process and high energy costs. For samples cut into pieces, temperatures above 110 °C lead to a sharp change in color. The beef samples were to be washed, surface cleaned, and cut into slices of 9-10 mm.

Number of samples analyzed: Five samples of beef and six samples of each vegetable additive were used during the experiment.

Number of repeated analyses: 5 repetitions of the analysis were carried out for beef and 6 repetitions for each vegetable additive.

Number of experiment replications: The experiment was repeated five times.

Design of the experiment: During the preparation of the samples, washing, and cleaning of the surface coating, cutting into thin slices, and drying (for plant impurities) were carried out sequentially. The beam generated by the Nd: YAG laser is focused on the samples through a lens. The sample in the focus zone undergoes multiphoton ionization to generate free electrons, followed by avalanche ionization to produce a certain amount of plasma. The plasma radiation spectrum is received by an optical receiver and transmitted to the spectrometer through an optical fiber. The spectrometer separates the collected spectrum by performing ICCD spectroscopy. Next, the collected optical signal is converted into amplified electrical signals, which are already perceived by the computer and displayed as a spectrum diagram of the components.

An ND:YAG laser initiated the ablation process, which enabled second harmonic generation. The radiation parameters were: wavelength up to 532 nm, frequency up to 10 Hz, pulse duration of 8 ns. The monochromator had a 1200 grooves/mm grating and an inverse linear dispersion of 0.1 nm. The resulting time-resolved LIB radiation was fed to an SRS250 model integrator for signal processing.

When applying the Boltzmann slant line method **[17]**, **[18]**, the following steps were performed to calculate the electron temperature. The analysis lines were chosen to belong to the same element, had the same ionization order, had better isolation, a higher signal-to-noise ratio, no self-absorption, and took the largest possible differences in excitation energy levels for evaluation.

When performing a quantitative analysis of the experimental results, the following were used: the double-line Boltzmann method for calculating the electron temperature of the plasma, the Stark expansion method for determining the electron density of the plasma, and the free calibration method. In the absence of elements of

standard samples with a known content, which was ineffective for fruits, the CFLIBS-free calibration method was used. In this way, the conditions of LTE local thermal equilibrium were achieved.

Statistical Analysis

The results were evaluated using statistical software Statgraphics Centurion XVII (StatPoint, USA) – multifactor analysis of variance (MANOVA), LSD test. Statistical processing was performed in Microsoft Excel 2016 in combination with XLSTAT. Values were estimated using mean and standard deviations. The reliability of the research results was assessed according to the Student's test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Discussion of the subject and object of research

Impurities of substances containing pectins create a sufficiently pronounced radioprotective effect **[19]**, protecting the population from the accumulation of radionuclides in the body due to the formation of gels in the presence of organic acids and sugars **[20]**. Ingredients from fucus seaweed and wheat germ, spirulina seaweed with pumpkin oil, nutmeg, or cardamom in the technology of cooked sausages allow to enrich the food product with such biologically active substances as iodine, alginic acids, various vitamins in the studies of Pogorelova and Yakymenko **[21]**. High functional and organoleptic properties of cooked sausages were obtained by the introduction of a multifunctional additive based on animal protein, alginate, carrageenan, guar gum, and xanthine gum, which is substantiated in the scientific works of Vinnikova **[22]**. A composite supplement with elements of essential oils of cornflowers, rosemary, laurel, marjoram oil, lactose, and ascorbic acid provided high organoleptic indicators according to research by Yeresko **[23]**.

In the works of Bal-Prylypko, it was proved that the use of a bacterial preparation based on the denitrifying microorganisms *Staphylococcus carnosus*, *S. carnosus subsp. utilis* in the brine composition made it possible to form high organoleptic properties in sausage products and increase their nutritional value **[24]**, **[25]**. It is with the use of plant additives that the production of meat products of increased biological value with increased protein content, normalization of acidity in the human body, increased digestibility of products, ensuring the optimal ratio of protein and fat, vitamins and mineral elements is ensured, which forms the basis for the implementation of the theory of balanced nutrition according to the formulation of Maksimov **[26]**.

Laser-induced plasma spectroscopy uses laser pulses as energy sources and lenses to focus the laser on the sample surface. According to the research results, this technology can detect the microelemental state of solid, liquid, and gaseous substances, regardless of the object's shape **[27]**, **[28]**.

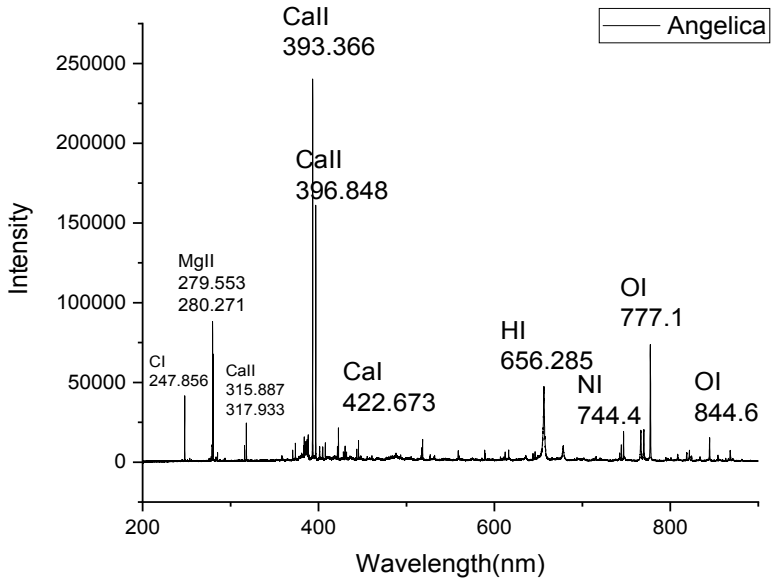
Many researchers used laser-induced plasma spectroscopy not only to analyze the composition of trace elements but also to carry out qualitative and quantitative analyses of elements of heavy metals, research various processing algorithms, plan multifunctional experiments, and constantly improve experimental devices **[29]**. Li from Jiangxi Agricultural University analyzed the metal elements contained in pericarps and pulp of tangerines and navel oranges in this way **[30]**. Abdul Jabbar and his scientific colleagues from Milpur University of Science and Technology used laser-induced plasma spectroscopy to study the elemental composition of roots, stems, seeds, and other parts of rice **[31]**. The studies proved the broad prospects of using laser-induced plasma spectroscopy to assess the quality of raw materials, semi-finished products, and finished products in food and processing industries **[32]**.

Evaluation of the microelement composition of the studied beef samples and plant impurities

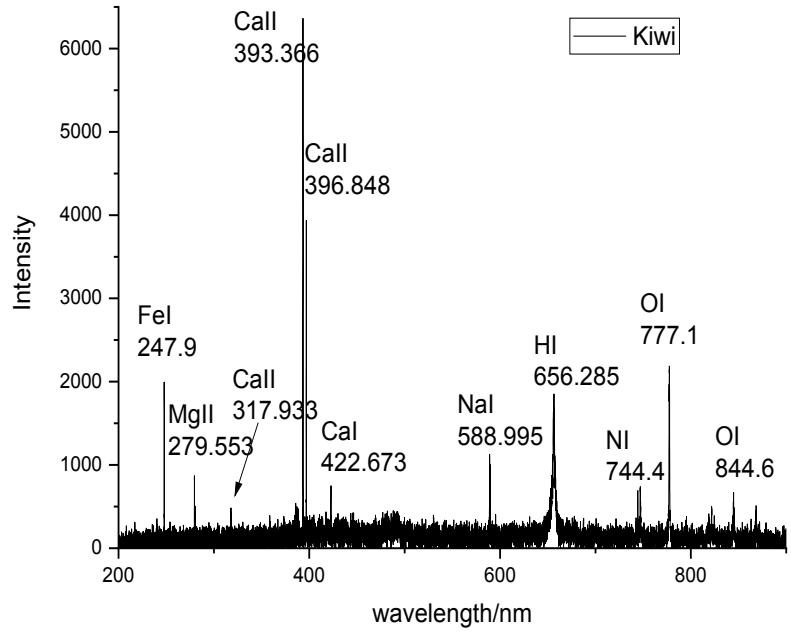
In assessing the microelement composition of the samples of the products presented above, spectrograms were obtained using the method of laser-induced breakdown spectroscopy (Figures 4, 5, 6, and 7). The "intensity" parameter, which shows the intensity of the plasma radiation spectrum of the experimental test sample, was plotted along the ordinate axis. Figures 4, 5, 6, and 7 show the different wavelengths that calibrate different elements, and the characteristic wavelengths of the elements can be requested according to the "NIST: Atomic Spectra Database Row Form". The peak intensity corresponding to different wavelengths is related to many factors, such as the possibility of the transition of energy levels of atoms or ions, the structure of the energy levels of the excitation energy, the composition of the sample (the content of elements), the experimental conditions (laser energy, laser focus on surface defocus of the sample, spread spectrum of the ICCD data, delay, slit width or spectrometer dip, etc.

The LIBS spectrogram for Chinese angelica (Figure 4a) revealed the presence of such elements as calcium, magnesium, hydrogen, oxygen, and nitrogen; the calcium content was almost 2.5 times higher than that of other components. Calcium provides the necessary functional properties of bones, joints, and teeth, mainly in the composition of calcium hydroxy phosphate. Regarding the trace element composition of kiwi (Figure 4b),

compared to angelica, additional iron and sodium were observed, albeit in small amounts; the calcium content exceeds the content of other components by almost 3 times. Trace elements of iron in the human body are mainly involved in forming hemoglobin, which contributes to blood formation. Sodium is a macroelement in the human body, practically irreplaceable in the extracellular fluid.



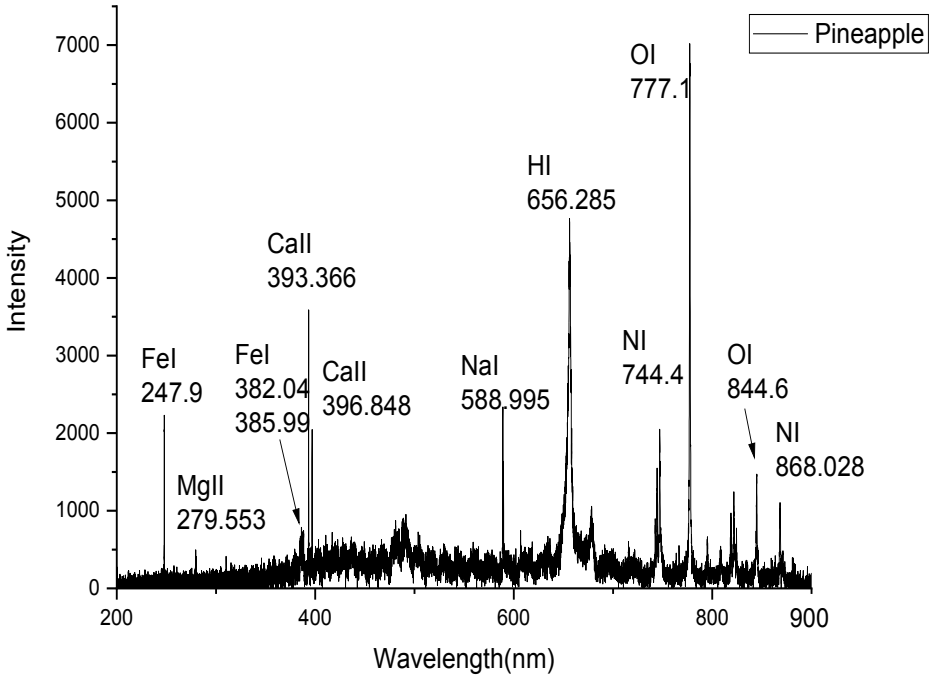
a)



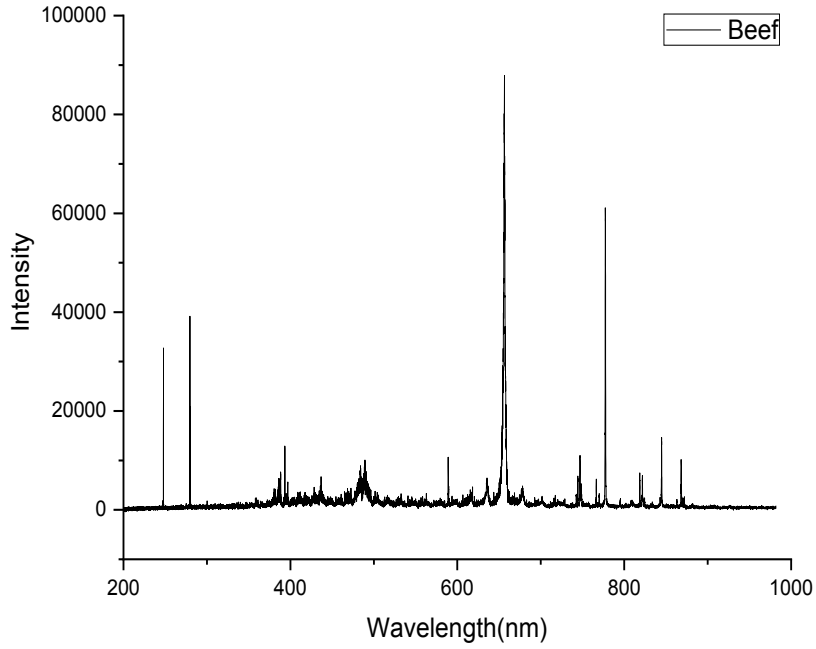
b)

Figure 4 LIBS spectrum of trace elements of vegetable additives to beef in the range of 200-900 nm: a – for the admixture of Chinese angelica; b – for kiwi admixture.

According to LIBS results for pineapple (Figure 5a), the content of calcium, magnesium, iron, and sodium is almost at the same level; the content of hydrogen and oxygen gases turned out to be somewhat higher than that of metals, and nitrogen was almost 2.5-3 times less. The trace element composition of beef practically does not contain metals (Figure 5b), which proves the need to introduce useful metal components into this product using the studied plant impurities.



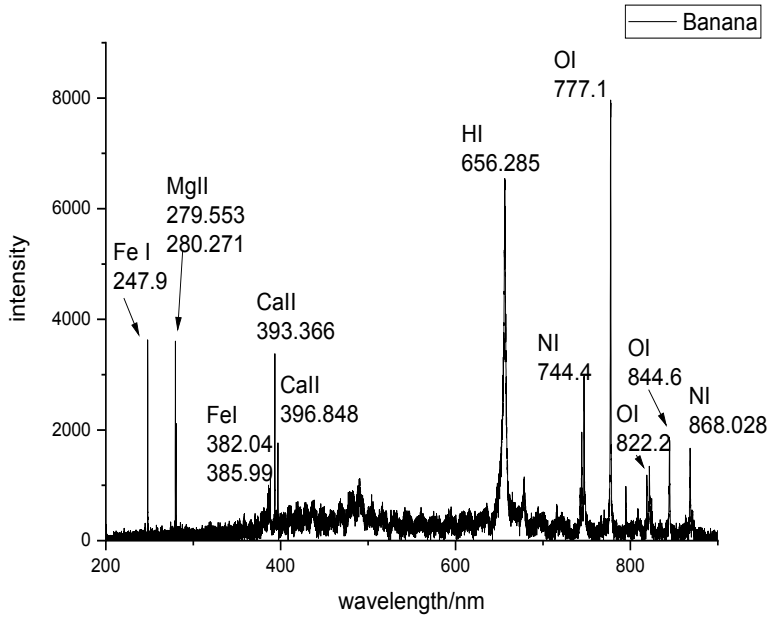
a)



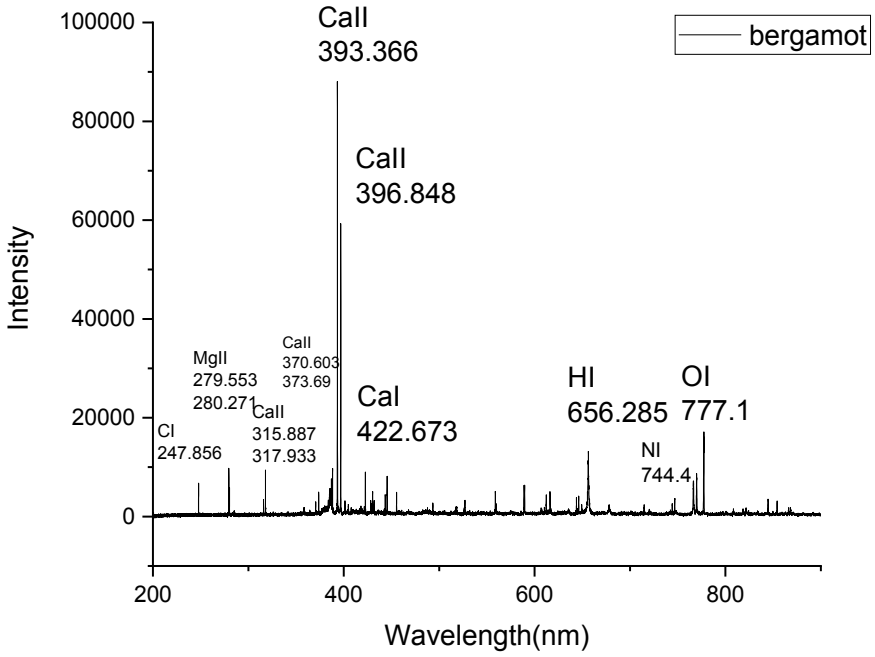
b)

Figure 5 LIBS spectrum of microelements of plant impurities and beef in the range of 200-900 nm: a – for pineapple admixture; b – for beef.

Evaluation of the LIBS spectrogram for a banana sample (Figure 6a) revealed a relatively high magnesium and iron content among metals and oxygen and hydrogen among gases. Magnesium contained in the human body is one of the biologically irreplaceable nutrients. In the bergamot sample, calcium is predominant in terms of content, sodium is much smaller, other metals were not observed (Figure 6b), and a small amount of oxygen, hydrogen, and nitrogen was found among the gases.



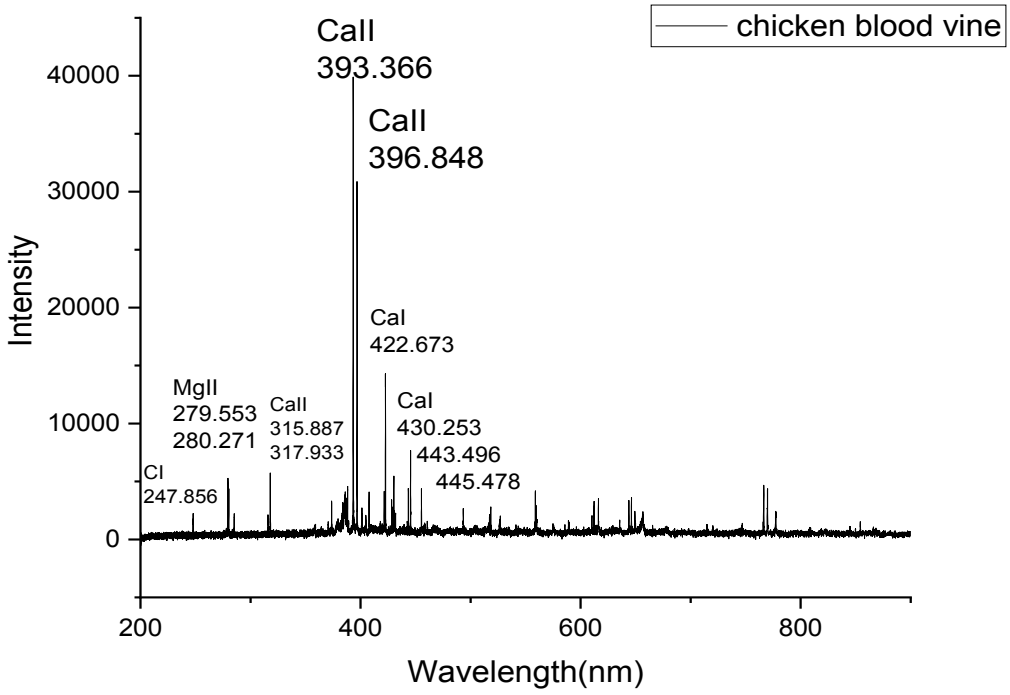
a)



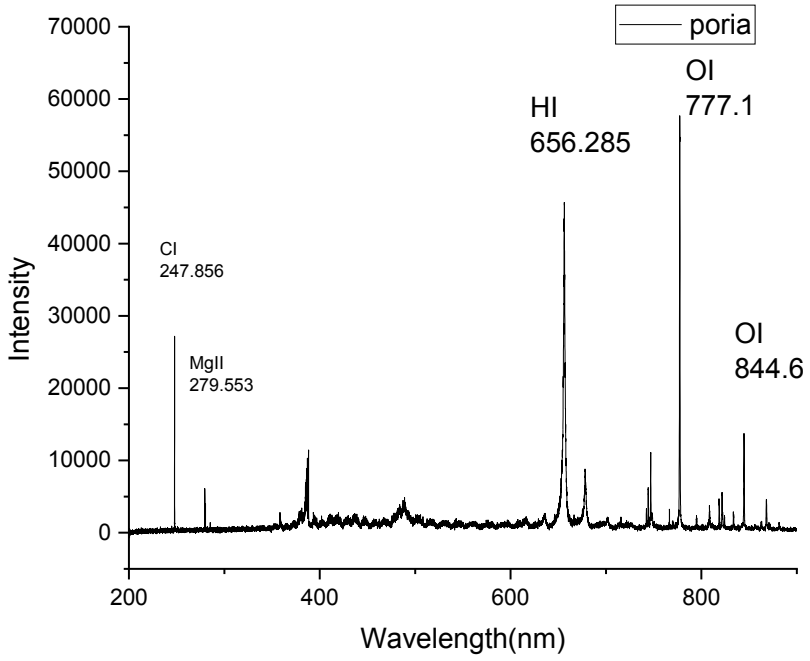
b)

Figure 6 LIBS spectrum of trace elements of vegetable additives to beef in the range of 200-900 nm: a – for banana admixture; b – for the admixture of bergamot.

The LIBS spectrogram for a sample of chicken blood vine (Figure 7a) showed the presence of calcium and magnesium in it; the calcium content was more than 2.5 times higher. Regarding the trace element composition of the coconut poria sample (Figure 7b), the presence of carbon and a small amount of magnesium was observed. Among the gases, oxygen and hydrogen were found in almost equal proportions.



a)



b)

Figure 7 LIBS spectrum of trace elements of vegetable additives to beef in the range of 200-900 nm: a – for the admixture of chicken blood vine; b – for the admixture of poria coconut.

Determination of the main characteristics of plasma

The electron temperature and electron density were calculated to analyse the plasma's characteristics. When calculating the electron temperature of the plasma, you can use the double line method and the inclined Boltzmann method **[13]**, **[14]**, the Sach-Boltzmann method **[33]**, the multi-element Sach-Boltzmann curve method, the

characteristic spectral line, and the continuum ratio method **[34]**. Under the condition of local thermal equilibrium (LTE), the formula of the Boltzmann slope method has the form:

$$\ln\left(\frac{I\lambda}{g_m A}\right) = -\frac{E_m}{k_B T} + C \quad (1)$$

Where:

I – the intensity of the spectral line of the relative intensity obtained by Lorentz fitting, Em is the excitation energy, A – is the transition probability of the energy level, g_m is – weighting factor of the upper energy level, k_B – Boltzmann constant, T – electronic temperature, C – coefficient that takes into account the content individual components and their total sum corresponding to the spectral line.

When plotting along the abscissa axis, the excitation energy Em was plotted, and along the ordinate axis – the value $\ln\left(\frac{I_m \lambda_m}{g_m A_m}\right)$

As a result, we obtained the corresponding inclination angle, using which the electron temperature T was determined.

Table 1 shows the wavelength, excitation energy, the weighting factor of the upper energy level, the product of the transition probability of the four spectral lines of CaII, the spectral line's precision, and the transition's energy level. The weaker spectral lines at 317.933 nm and 373.69 nm are selected based on the experimental intensity and the fitted linear correlation coefficient. Therefore, the error in the electron temperature obtained by fitting two different spectral lines of the excitation energy is sufficiently large. The electronic surface plasma temperatures of banana, pineapple, and kiwi were calculated from the slope at 11606 K, 10811 K, and 10685 K, respectively. The electronic temperatures of these fruits have a small difference. When calculating the plasma electron density, the most common method is the Stark expansion for the "H" spectral line and the "non-H" spectral line.

Table 1 Corresponding parameters of spectral lines.

Wavelength (nm)	Excitation energy (eV)	$g_m A(10^8 s^{-1})$	Precision	Transition energy level
393.366	3.150984	5.88	C	$3p^6 4s\ (^2S)$ -$3p^6 4p\ (^2P°)$
396.848	3.123349	2.80	C	$3p^6 4s\ (^2S)$ -$3p^6 4p\ (^2P°)$
373.69	6.467875	3.40	C	$3p^6 4p\ (^2P°)$ -$3p^6 5s\ (^2S)$
317.933	7.049551	22.00	C	$3p^6 4p(^2P°)$ -$3p^6 4d\ (^2D)$

The method of calculating the H-spectral Stark line using the Sach-Boltzmann equation is presented in the following formulas (2), (3), and (4):

$$n_e(H_\alpha) = 8.02 \times 10^{12} \left(\frac{\Delta\lambda}{\alpha}\right)^{3/2} cm^3 \quad (2)$$

Where:

$\Delta\lambda$ – full width at half maximum line H_α, n_e – electron density of plasma, α – ionic expansion parameter **[14]**:

$$\Delta\lambda = \left[1 + 1.75 \cdot 10^{-4} n_e^{1/4} \alpha (1 - 0.068 n_e^{1/6} T_e^{-1/2})\right] \cdot 10^{-16} w n_e \quad (3)$$

Where:

T_e – temperature of electrons in plasma, w – electron collision coefficient, α - parameter of ionic expansion, which can be found in the paper **[38]**:

$$\frac{I_1}{I_2} = \frac{A_1 g_1 \lambda_2}{A_2 g_2 \lambda_1} \frac{2(2\pi m_e k)^{3/2}}{h^3} \frac{1}{n_e} T^{3/2} \exp\left(-\frac{E_1 - E_2 + E_{1P} - \Delta E}{kT}\right) \quad (4)$$

Where:

subscripts 1 and 2, respectively, represent high and low orders in adjacent ionization orders, m_e – electron rest mass, h – Planck's constant, E_{IP} – Ionization energy; ΔE – correction value of ionization energy; n_e – electron density.

By fitting the starting point to obtain the half-maximum width of the spectrum and the electron temperature Te, the electron density ne can be obtained. The effect of ion broadening is much smaller than that of electronic broadening. Therefore, the method of broadening the Stark spectral lines "not H" is simplified to the formula 5:

$$\Delta\lambda = 2w\left(\frac{n_e}{10^{16}}\right) \quad (5)$$

Table 2 shows the results determined using the H-spectral Stark line-broadening method, the H-spectral Stark line-broadening method, and the method of the Sach-Boltzmann equation.

Table 2 Results of electron density calculation.

Method	Select the spectral line and wavelength (nm)	Electron density of banana ($\times10^{16}$ cm^3)	Electron density of Pineapple ($\times10^{16}$ cm^3)	Electron density of Kiwi ($\times10^{16}$ cm^3)
Stark expansion "H" - spectral line	656.285	7.2	7.8	6.8
"not H" - spectral line Stark broadening	CaII393.366	2.937	3.312	3.197
	CaII396.848	2.964	3.273	2.934
	MgII279.553	0.603	0.778	0.529
	MgII280.271	0.584	0.711	0.4972
	NaI588.9	--	7.366	9.749
Saha-Boltzmann equation	CaII393.366 CaII396.848 CaI422.673	6.41	2.35	1.50

In the process of calculating the electron density, it was found that for samples of banana, pineapple, poria coconut, and beef with a high content of oxygen and hydrogen, more significant self-absorption is observed, which is mainly determined by the influence of atmospheric air. The electron density obtained from the CaII and MgII lines in the “H” spectral line broadening method differs by an order of magnitude, which may be due to the low signal-to-noise ratio for the magnesium spectral line, as well as a large fitting error.

The calculation results of different lines of analysis turned out to be quite different, which may be because the plasma does not correspond to LTE during the spectrum measurement. When using the multi-element Sach-Boltzmann equation to determine the electron density, it was not possible to ensure the correct determination of the electron temperature; the content of each element in the fruit is unknown. Therefore, the use of this method is not appropriate.

Quantitative analysis of the studied samples

Calculation formulas for electron temperature and plasma electron density were formulated under conditions of local thermal equilibrium, and calculation data were obtained in optically thin conditions. Under local thermal equilibrium (LTE), the particle velocity satisfies the Maxwell distribution, obeys the Boltzmann distribution at different energy levels, satisfies the Sach equation at different ionisation states, and Planck's law for the radiation density **[35]**. In carrying out this assessment, it was considered that the plasma is infinitely close to the local thermal equilibrium for a certain delay time. The necessary condition for ensuring such a state can be expressed by the following dependence **[36]**:

$$N_e \geq 1.4\times10^{12}T^{1/2}(E_m - E_n)^3 cm^{-3} \quad (6)$$

The maximum difference in energy levels in this study is 7.05 eV; Electronic Temperature T_e – about 1 eV, and the density of electrons that can be obtained by formula (6) turned out to be greater than the value of $4.9\cdot10^{14}$ cm^{-3}. Thus, the calculated electron density corresponds to the condition of local thermal equilibrium.

The assessment of optical fineness was carried out using the following formula:

$$\frac{I_1}{I_2} = \frac{\lambda_2 A_{K1} g_1}{\lambda_1 A_{k2} g_2}\exp\left[\frac{E_{K1} - E_{K2}}{K_B T}\right] \quad (7)$$

Where: λ_1 and λ_2 are the wavelengths of two spectral lines of the same ionization order for the same element under study

Formula (7) used the parameter values 393.366 nm and 396.848 nm, which have approximately the same excitation energies as those of the studied banana, pineapple, and kiwi samples. The calculation results found that for the studied conditions, the plasmon satisfies the characteristics of an optically thin state at the time of measurement.

For the quantitative analysis of the studied impurities in meat products, it was quite difficult to make standard samples that correspond to the matrix. To comply with this condition, as a rule, elements with known concentrations are added to create a calibration curve that corresponds to intensity and concentration. However, the preliminary processing was quite cumbersome, and the matrix effect was insurmountable. Quantitative analysis was carried out using special data processing methods. In the absence of elements of standard samples with known content, the CFLIBS-free calibration method was used. Then, under the conditions of local thermal equilibrium of LTE and the absence of self-absorption, the dependence was us:

$$\overline{I_{\lambda}^{ki}}\lambda = FC_s A_{ki} \frac{g_k e^{-(E_i/K_B T)}}{U_s(T)} \tag{8}$$

Where: $\overline{I_{\lambda}^{ki}}$ – analysis line intensity, C_s – atomic content corresponding to the line of analysis, F – experimental parameter.

The parameters A_{ki}, E_k, g_k, and the compatible function $U_s(T)$ were obtained from the NIST database, and the parameters F, C_s, and T – were through experimental data processing. The corresponding particle content was obtained by transforming the point of intersection of the Boltzmann slope in formula (1). Then, by summing up the content of atoms and ions, which were determined by the Boltzmann slope of different orders of ionization, the content of the element was obtained. The experimental parameter F was determined by normalizing the content of all elements.

During research, it was observed that when measuring organic samples, such as fruits, it is difficult to detect the spectral lines of all elements that can correspond to the Boltzmann slope.

In scientific works **[37]**, **[38]** the results of the analysis of the microelement composition of meat products to which various kinds of impurities of plant origin were added are given. According to the results of the research, the content of metals in the composition of beef is practically absent. However, the authors emphasize the need to introduce such components into finished products.

The authors of scientific works **[39]**, **[40]** performed a series of experimental studies on developing new recipes for meat products with improved properties due to introducing various components into their composition, particularly vegetables, fruit, and API products. According to the results of the above studies, only an organoleptic assessment was carried out, in our opinion, for such products, it is worth conducting a spectroscopic assessment and quantitative analysis of the trace element composition, taking into account the content of plant impurities, which will allow to improve the quality indicators of the finished products.

Among the sufficiently effective methods of non-destructive control, we can note the technology of hyperspectral detection of material characteristics, which is widely used in food product testing **[41]**, in particular, it expands the possibilities for improving the quality and efficiency of the production of vegetable sausages. This method makes it possible to identify the studied material's main components and predict changes in chemical and microbial indicators.

Thus, the manuscript by Mahdinia et al. **[42]** developed a model of microbial growth of cold fresh beef. Regression prediction and visualization of the structure of meat raw materials are described in detail in the manuscript **[43]** based on hyperspectral imaging to conduct a quantitative analysis of the total number of colonies in sausages.

Shi et al. **[44]** evaluated sausage quality using the hyperspectral near-infrared band, extracted characteristic bands using a continuous projection algorithm and created an effective model for characterizing and identifying sausage varieties.

Fu et al. **[45]**, using the methods of hyperspectral analysis and wavelength screening, found three undesirable plant impurities in sausage products, and developed modeling algorithms to improve the predictive performance of the constructed model.

The proposed research topic is of great importance for ensuring the quality and safety of food products. The studied methods allow the detection of the presence of various chemical elements in products and the accurate determination of their amount. Further research may contribute to improving analytical techniques, developing

quality and safety standards, and formulating effective quality control strategies in the food industry. This is important from the point of view of consumer health and the competitiveness of products on the market.

The study of spectroscopic methods in combination with LIBS to assess the quality of food products is an auspicious direction. From the perspective of further research, the following directions may be engaging in this area:

Expanding the range of products examined, including different food products such as meat, fish, fruits, vegetables, grains, dairy products, etc. To get a more complete idea of the potential application of the method in various branches of the food industry.

Development of standardized methods of analysis that could be used in the food industry for quality control.

Analysis of the interaction of various chemical components in products during storage and processing will help develop optimal storage and processing conditions.

Applying machine learning and data analysis methods automates the processing of spectroscopic measurement results, quickly and efficiently analyzes large volumes of data, and identifies important dependencies between spectra and product quality indicators.

Study of the influence of various internal factors, such as humidity, temperature, pH, etc., on the spectroscopic characteristics of products. Development of portable LIBS devices that can be used at the production site or even at home for rapid analysis of product quality. Identify new spectroscopic markers of product quality that would be sensitive to certain types of contamination or degradation.

All the above-mentioned areas of research can help improve the quality and safety of food products and make the process of their control more efficient and automated.

CONCLUSION

According to the results of the evaluation of the studied product samples by laser-induced LIBS spectroscopy, the trace element composition of beef practically does not contain metals, which proves the need to introduce useful metal components into this product using the studied plant impurities. Since some of the studied samples, particularly fruits, are somewhat different from solid bodies, the noise coefficient of some spectral lines was low, and the electron temperature and electron density calculation error were relatively large. In addition, the heterogeneity of fruits increases the matrix effect. Some microelements are characterized by sufficiently low detection limits, which makes it difficult to create standard samples. Experimental lines were used in the LIBS spectra of the studied fruits, which fully satisfy the selection rules. The chosen wavelengths of 317.933 nm and 373.69 nm turned out to be quite weak, and the excitation energies of 3.15 and 3.12 eV, respectively, for the wavelengths of 393.366 nm and 396.848 nm turned out to be quite close. The projection of which is superimposed on one point. The electronic three-point fitting temperature results for banana, pineapple, and kiwi were 14507 K, 13485 K, and 12173 K, respectively.

REFERENCES

1. Tkach, G., Omelian, A., Kushnir, Y., & Altanova, O. (2023). The effect of a diet based on semifinished products from plant and animal raw materials on reproductive capacity, growth, and development of the organism. In Animal Science and Food Technology (Vol. 14, Issue 4, pp. 87–98). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.4.2023.87
2. Balji, Y. (2023). Preliminary assessment of the safety of genetically modified food products. In Animal Science and Food Technology (Vol. 14, Issue 3, pp. 9–19). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.3.2023.9
3. Palamarchuk, I., Mushtruk, M., Sukhenko, V., Dudchenko, V., Korets, L., Litvinenko, A., Deviatko, O., Ulianko, S., & Slobodyanyuk, N. (2020). Modelling of the process of vybromechanical activation of plant raw material hydrolysis for pectin extraction. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 239–246). HACCP Consulting. https://doi.org/10.5219/1305
4. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/1711
5. Dias-Faceto, L. S., Salvador, A., & Conti-Silva, A. C. (2019). Acoustic settings combination as a sensory crispness indicator of dry crispy food. In Journal of Texture Studies (Vol. 51, Issue 2, pp. 232–241). Wiley. https://doi.org/10.1111/jtxs.1248
6. Lan, G., Li, C., He, L., Zeng, X., & Zhu, Q. (2020). Effects of different strains and fermentation methods on nattokinase activity, biogenic amines, and sensory characteristics of natto. In Journal of Food Science and

Technology (Vol. 57, Issue 12, pp. 4414–4423). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-020-04478-3

7. Fernandes Andrade, D., Pereira-Filho, E. R., & Amarasiriwardena, D. (2020). Current trends in laser-induced breakdown spectroscopy: a tutorial review. In Applied Spectroscopy Reviews (Vol. 56, Issue 2, pp. 98–114). Informa UK Limited. https://doi.org/10.1080/05704928.2020.1739063
8. Velásquez-Ferrín, A., Babos, D. V., Marina-Montes, C., & Anzano, J. (2020). Rapidly growing trends in laser-induced breakdown spectroscopy for food analysis. In Applied Spectroscopy Reviews (pp. 1–21). Informa UK Limited. https://doi.org/10.1080/05704928.2020.1810060
9. Zheplinska, M., Mushtruk, M., Vasyliv, V., Slobodyanyuk, N., & Boyko, Y. (2021). The Main Parameters of the Physalis Convection Drying Process. In Lecture Notes in Mechanical Engineering (pp. 306–315). Springer International Publishing. https://doi.org/10.1007/978-3-030-77823-1_31
10. Thomas, J., & Chandra Joshi, H. (2023). Review on laser-induced breakdown spectroscopy: methodology and technical developments. In Applied Spectroscopy Reviews (Vol. 59, Issue 1, pp. 124–155). Informa UK Limited. https://doi.org/10.1080/05704928.2023.2187817
11. Naozuka, J., & Oliveira, A. P. (2023). <scp>Laser-Induced</scp> Breakdown Spectroscopy in Food Sciences. In Laser-Induced Breakdown Spectroscopy (LIBS) (pp. 781–806). Wiley. https://doi.org/10.1002/9781119758396.ch40
12. Larios, G. S., Nicolodelli, G., Senesi, G. S., Ribeiro, M. C. S., Xavier, A. A. P., Milori, D. M. B. P., Alves, C. Z., Marangoni, B. S., & Cena, C. (2020). Laser-Induced Breakdown Spectroscopy as a Powerful Tool for Distinguishing High- and Low-Vigor Soybean Seed Lots. In Food Analytical Methods (Vol. 13, Issue 9, pp. 1691–1698). Springer Science and Business Media LLC. https://doi.org/10.1007/s12161-020-01790-8
13. Benhamou, J., Channouf, S., Lahmer, E. B., Jami, M., & Mezrhab, A. (2023). Hybrid-lattice Boltzmann Method for the Simulation of Magnetohydrodynamic Conjugate Heat Transfer and Entropy Generation in Three Dimensions. In Arabian Journal for Science and Engineering (Vol. 49, Issue 1, pp. 1181–1206). Springer Science and Business Media LLC. https://doi.org/10.1007/s13369-023-08273-y
14. Benhamou, J., Lahmer, E. B., & Jami, M. (2022). Three-dimensional simulation of conjugate heat transfer using the hybrid lattice Boltzmann-finite difference method. In International Communications in Heat and Mass Transfer (Vol. 139, p. 106486). Elsevier BV. https://doi.org/10.1016/j.icheatmasstransfer.2022.106486
15. Marriam, I., Tebyetekerwa, M., Xu, Z., Chathuranga, H., Chen, S., Chen, H., Zheng, J.-C., Du, A., & Yan, C. (2021). Techniques enabling inorganic materials into wearable fiber/yarn and flexible lithium-ion batteries. In Energy Storage Materials (Vol. 43, pp. 62–84). Elsevier BV. https://doi.org/10.1016/j.ensm.2021.08.039
16. Wang, L., Zhou, Y., Fu, Y., Xu, L., Gong, H., & Cheng, R. (2019). Effect of sample temperature on radiation characteristics of nanosecond laser-induced soil plasma. In Chinese Journal of Chemical Physics (Vol. 32, Issue 6, pp. 760–764). AIP Publishing. https://doi.org/10.1063/1674-0068/cjcp1901015
17. Fu, Y.-X., Wang, L., & Xu, L. (2020). Electronic temperature characteristics of laser-induced Fe plasma in fruits. In Open Physics (Vol. 18, Issue 1, pp. 40–47). Walter de Gruyter GmbH. https://doi.org/10.1515/phys-2020-0005
18. Zheplinska, M., Mushtruk, M., Vasyliv, V., Kuts, A., Slobodyanyuk, N., Bal-Prylypko, L., Nikolaenko, M., Kokhan, O., Reznichenko, Y., & Salavor, O. (2021). The micronutrient profile of medicinal plant extracts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 528–535). HACCP Consulting. https://doi.org/10.5219/1553
19. Mushtruk, N., & Mushtruk, M. (2023). Analysis of the raw material base for pectin production. In Animal Science and Food Technology (Vol. 14, Issue 2). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.2.2023.57
20. Vasyliv, V., Mushtruk, M., Zheplinska, M., Mukoid, R., & Tkachenko, S. (2021). Method of Electrohydraulic Activation of Water-Lime Suspension in Sugar Production. In Lecture Notes in Mechanical Engineering (pp. 664–673). Springer International Publishing. https://doi.org/10.1007/978-3-030-91327-4_64
21. Tang, X., Yu, H., Bui, B., Wang, L., Xing, C., Wang, S., Chen, M., Hu, Z., & Chen, W. (2021). Nitrogen-doped fluorescence carbon dots as multi-mechanism detection for iodide and curcumin in biological and food samples. In Bioactive Materials (Vol. 6, Issue 6, pp. 1541–1554). Elsevier BV. https://doi.org/10.1016/j.bioactmat.2020.11.006
22. Zheplinska, M., Mushtruk, M., Vasyliv, V., Sarana, V., Gudzenko, M., Slobodyanyuk, N., Kuts, A., Tkachenko, S., & Mukoid, R. (2021). The influence of cavitation effects on the purification processes of beet sugar production juices. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 18–25). HACCP Consulting. https://doi.org/10.5219/1494

23. Stadnyk, I., Bodnarchuk, O., Kopylova, K., Petrov, P., Bal-Prylypko, L., & Narizhnyy, S. (2021). Modification of the properties of milk-fat emulsions with the phase structure of "oil in water" in the dependence on the mass part of the lipoid and the stabilizing systems. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 741–748). HACCP Consulting. https://doi.org/10.5219/1389
24. Bal-Prylypko, L., Yancheva, M., Paska, M., Ryabovol, M., Nikolaenko, M., Israelian, V., Pylypchuk, O., Tverezovska, N., Kushnir, Y., & Nazarenko, M. (2022). The study of the intensification of technological parameters of the sausage production process. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 27–41). HACCP Consulting. https://doi.org/10.5219/1712
25. Bober, A., Liashenko, M., Protsenko, L., Slobodyanyuk, N., Matseiko, L., Yashchuk, N., Gunko, S., & Mushtruk, M. (2020). Biochemical composition of the hops and quality of the finished beer. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 307–317). HACCP Consulting. https://doi.org/10.5219/1311
26. Zhang, G., Zhao, X., Li, X., Du, G., Zhou, J., & Chen, J. (2020). Challenges and possibilities for bio-manufacturing cultured meat. In Trends in Food Science & Technology (Vol. 97, pp. 443–450). Elsevier BV. https://doi.org/10.1016/j.tifs.2020.01.026
27. Sun, H., Song, C., Lin, X., & Gao, X. (2022). Identification of meat species by combined laser-induced breakdown and Raman spectroscopies. In Spectrochimica Acta Part B: Atomic Spectroscopy (Vol. 194, p. 106456). Elsevier BV. https://doi.org/10.1016/j.sab.2022.106456
28. Gibbons, E., Léveillé, R., & Berlo, K. (2020). Data fusion of laser-induced breakdown and Raman spectroscopies: Enhancing clay mineral identification. In Spectrochimica Acta Part B: Atomic Spectroscopy (Vol. 170, p. 105905). Elsevier BV. https://doi.org/10.1016/j.sab.2020.105905
29. Korish, M. A., & Attia, Y. A. (2020). Evaluation of Heavy Metal Content in Feed, Litter, Meat, Meat Products, Liver, and Table Eggs of Chickens. In Animals (Vol. 10, Issue 4, p. 727). MDPI AG. https://doi.org/10.3390/ani10040727
30. Roosen, M., Mys, N., Kusenberg, M., Billen, P., Dumoulin, A., Dewulf, J., Van Geem, K. M., Ragaert, K., & De Meester, S. (2020). Detailed Analysis of the Composition of Selected Plastic Packaging Waste Products and Its Implications for Mechanical and Thermochemical Recycling. In Environmental Science & Technology (Vol. 54, Issue 20, pp. 13282–13293). American Chemical Society (ACS). https://doi.org/10.1021/acs.est.0c03371
31. Peng, J., Liu, Y., Ye, L., Jiang, J., Zhou, F., Liu, F., & Huang, J. (2023). Fast detection of minerals in rice leaves under chromium stress based on laser-induced breakdown spectroscopy. In Science of The Total Environment (Vol. 860, p. 160545). Elsevier BV. https://doi.org/10.1016/j.scitotenv.2022.160545
32. Peng, J., Liu, Y., Ye, L., Jiang, J., Zhou, F., Liu, F., & Huang, J. (2022). Fast Detection of Minerals in Rice Leaves Under Chromium Stress Based on Laser-Induced Breakdown Spectroscopy. In SSRN Electronic Journal. Elsevier BV. https://doi.org/10.2139/ssrn.4203274
33. Li, L., Wan, Y., Lu, J., Fang, H., Yin, Z., Wang, T., Wang, R., Fan, X., Zhao, L., & Tan, D. (2020). Lattice Boltzmann Method for Fluid-Thermal Systems: Status, Hotspots, Trends and Outlook. In IEEE Access (Vol. 8, pp. 27649–27675). Institute of Electrical and Electronics Engineers (IEEE). https://doi.org/10.1109/access.2020.2971546
34. Numerical simulation of separated flows using entropic lattice Boltzmann method. (2023). American Institute of Aeronautics and Astronautics (AIAA). https://doi.org/10.2514/6.2023-1569.vid
35. Guo, S., Feng, Y., Jacob, J., Renard, F., & Sagaut, P. (2020). An efficient lattice Boltzmann method for compressible aerodynamics on D3Q19 lattice. In Journal of Computational Physics (Vol. 418, p. 109570). Elsevier BV. https://doi.org/10.1016/j.jcp.2020.109570
36. Ma, J., Wang, Z., Young, J., Lai, J. C. S., Sui, Y., & Tian, F.-B. (2020). An immersed boundary-lattice Boltzmann method for fluid-structure interaction problems involving viscoelastic fluids and complex geometries. In Journal of Computational Physics (Vol. 415, p. 109487). Elsevier BV. https://doi.org/10.1016/j.jcp.2020.109487
37. Mushtruk, M., Palamarchuk, I., Palamarchuk, V., Gudzenko, M., Slobodyanyuk, N., Zhuravel, D., Petrychenko, I., & Pylypchuk, O. (2023). Mathematical modelling of quality assessment of cooked sausages with the addition of vegetable additives. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 242–255). HACCP Consulting. https://doi.org/10.5219/1845
38. Pylypchuk, O., Tyshchenko, L., Israelian, V., & Mushtruk, N. (2022). Influence of parameters of marinating meat semi-finished products on the quality of the finished product. In Animal Science and Food Technology (Vol. 13, Issue 2). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(2).2022.44-52
39. Wang, M., Zhou, J., Tavares, J., Pinto, C. A., Saraiva, J. A., Prieto, M. A., Cao, H., Xiao, J., Simal-Gandara, J., & Barba, F. J. (2022). Applications of algae to obtain healthier meat products: A critical review on

nutrients, acceptability, and quality. In Critical Reviews in Food Science and Nutrition (Vol. 63, Issue 26, pp. 8357–8374). Informa UK Limited. https://doi.org/10.1080/10408398.2022.2054939

40. Ivaniuta, A., Menchynska, A., Ochkolias, O., Qu, X., & Nesterenko, N. (2022). Effectiveness of using beer groats in the technology of semifinished fish products. In Animal Science and Food Technology (Vol. 13, Issue 1, pp. 16–23). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.13(1).2022.16-23
41. Zhu, M., Huang, D., Hu, X., Tong, W., Han, B., Tian, J., & Luo, H. (2020). Application of hyperspectral technology in detection of agricultural products and food: A Review. In Food Science & Nutrition (Vol. 8, Issue 10, pp. 5206–5214). Wiley. https://doi.org/10.1002/fsn3.1852
42. Mahdinia, E., Liu, S., Demirci, A., & Puri, V. M. (2020). Microbial Growth Models. In Food Engineering Series (pp. 357–398). Springer International Publishing. https://doi.org/10.1007/978-3-030-42660-6_14
43. Zhu, Y., He, H., Jiang, S., Ma, H., Chen, F., Xu, B., Liu, H., Zhu, M., Zhao, S., & Kang, Z. (2021). Mining hyperspectral data for non-destructive and rapid prediction of nitrite content in ham sausages. In International Journal of Agricultural and Biological Engineering (Vol. 14, Issue 2, pp. 182–187). International Journal of Agricultural and Biological Engineering (IJABE). https://doi.org/10.25165/j.ijabe.20211402.5407
44. Shi, S., Feng, J., Ma, Y., Cao, C., Li, L., & Jiang, Y. (2023). Rapid determination of two illegal additives in wheat flour by near-infrared spectroscopy and different key wavelength selection algorithms. In LWT (Vol. 189, p. 115437). Elsevier BV. https://doi.org/10.1016/j.lwt.2023.115437
45. Fu, H., Zhang, A., Sun, G., Ren, J., Jia, X., Pan, Z., & Ma, H. (2022). A Novel Band Selection and Spatial Noise Reduction Method for Hyperspectral Image Classification. In IEEE Transactions on Geoscience and Remote Sensing (Vol. 60, pp. 1–13). Institute of Electrical and Electronics Engineers (IEEE). https://doi.org/10.1109/tgrs.2022.3189015

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Dr. for I. Palamdrchuk

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Igor Palamarchuk**, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Processes and Equipment for Processing of Agricultural Production, Heroes of Defense Str., 12 B, 03040, Kyiv, Ukraine,
E-mail: vibroprocessing@gmail.com
ORCID: https://orcid.org/0000-0002-0441-6586

Fu Yuanxia, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Processes and Equipment for Processing of Agricultural Production, Heroes of Defense Str., 12 B, 03040, Kyiv, Ukraine/Bengbu University, Bengbu, 233030, Anhui, China,
E-mail: fyx@bbc.edu.cn
ORCID: https://orcid.org/0000-0002-4238-1847

Dmytro Zhuravel, Dmytro Motornyi Tavria State Agrotechnological University, Department of Technical Service and Systems in the Agro-Industrial Complex, B. Khmelnytskoho Av. 18, Melitopol, 72312, Ukraine,
E-mail: dmytro.zhuravel@tsatu.edu.ua
ORCID: https://orcid.org/0000-0002-6100-895X

Ievgenii Petrychenko, Uman National University of Horticulture, Department of Agroengineering, Institutska St., 1, 20300 Uman, Ukraine,
E-mail: 22102210g@ukr.net
ORCID: https://orcid.org/0000-0003-1037-077X

Roksolana Blishch, Lviv Polytechnic National University, Faculty name, Department of Organic Products Technology, St. George's Square, 2, 79013 Lviv, Ukraine,
E-mail: roksolanaalex1976@gmail.com
ORCID: https://orcid.org/0000-0003-1143-5264

Anatoliy Holovatyuk, Uman National University of Horticulture, Department of Agroengineering, Institutska St., 1, 20300 Uman, Ukraine,
E-mail: golowatyk@ukr.net
ORCID: https://orcid.org/0000-0002-4366-171X

Olexander Domin, National University of Life and Environmental Sciences of Ukraine, Mechanical and Technological Faculty, Department of Transport Technologies and Means of Agro-Industrial Complex, Heroes of Defense Str., 12 B, Kyiv, 03040, Ukraine,
Email: domin31@gmail.com
ORCID: https://orcid.org/0000-0002-3907-0992

Tatiana Kostiuk, National University of Life and Environmental Sciences of Ukraine, Faculty of Economics, Department of Economics, Heroiv Oborony Str.15, 03041, Kyiv, Ukraine,
E-mail: economy_chair@nubip.edu.ua
ORCID: https://orcid.org/0000-0003-1334-2731

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 10.4.2024
Revised: 28.5.2024
Accepted: 5.6.2024
Published: 10.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 497-511
https://doi.org/10.5219/1991
ISSN: 1337-0960 online
www.potravinarstvo.com

Changes in the microbiota of Bryndza cheese after frozen storage

Simona Kunová, Peter Zajác, Jozef Čapla, Jozef Čurlej, Ivana Timoracká, Natália Čmiková, Miroslava Kačániová

ABSTRACT

Bryndza cheese is a traditional Slovakian product. In this research, we have investigated whether it would be possible to freeze bryndza, store it at a temperature of -18°C, and then thaw and place it on the market during the off-production season. The current legislation in Slovakia does not allow this procedure. The freezing process was chosen based on the request of several small food business operators who would like to replace the process of preserving the primary raw material, matured salted ewe's lump cheese, in barrels due to acrid-sour taste. Bryndza cheese is preferred by consumers due to its unique microbial composition, which is beneficial for their health. Many microorganisms present in bryndza are probiotics. For this reason, we wanted to determine how the microflora in the bryndza cheese changes after freezing. These findings have practical implications for the food industry, particularly for small food business operators, who can potentially adopt freezing to preserve bryndza, thereby extending its shelf life and availability to consumers. Additionally, in many households, people store bryndza in their freezers after purchasing and use it to prepare dishes. Understanding the role of microorganisms in the ripening process and during storage can provide valuable data on Brynzda quality and safety. The present study aimed to analyse the representation of microorganisms in "Bryndza" samples at the beginning of storage and after 6 months of storage at a temperature of -18 °C. A total of 10 samples of "Bryndza" cheese made from pasteurised milk were analysed. Analysis of total viable counts of viable bacteria (TVC), coliform bacteria (CB), lactic acid bacteria (LAB), and microscopic filamentous fungi (MFF) was performed using the plate dilution method. Isolated strains of microorganisms were identified with mass spectrometry MALDI-TOF MS Biotyper. A total of 295 isolates from Bryndza cheese were identified at the start of storage and 220 isolates at the end of storage of samples. The dominant species of microorganisms found in Bryndza cheese were lactic acid bacteria, especially *Lactococcus lactis*, with 68 isolates and *Lactobacillus fermentum*, with 41 isolates at the start of storage. The most frequently isolated species were *Lactococcus lactis*, with 62 isolates, and *Limosilactobacillus fermentum*, with 33 isolates. Our results show that important lactic acid bacteria were present in the bryndza even after 6 months of freezing, but coliform bacteria were absent. Experimental outputs: TVC: showed no significant decrease (p-value = 0.0137); LAB: No significant decrease in lactic acid bacteria counts post-storage; MFF: Significant decrease in microscopic filamentous fungi post-storage; CB: Qualitative analysis indicates a significant reduction to undetectable levels after storage. Long-term storage of bryndza at -18°C is safe from a microbiological point of view.

Keywords: lactic acid bacteria, coliform bacteria, mass spectrometry, identification of microorganisms, Bryndza cheese

INTRODUCTION

Bryndza is a natural, white, mature and spreadable cheese manufactured traditionally in specified mountainous areas of the Slovak Republic and dairy factories. Slovenská bryndza (Slovak bryndza) cheese has been protected by Protected Geographical Indication since 2008 **[1]**. It is manufactured using traditional techniques using either 100 % matured ewe's lump cheese or a mix with a maximum of 50 % cow's lump cheese in dry matter **[2]**. The primary compound used in the production of bryndza is ewes' lump cheese, made by a two-stage ripening process that takes eight to fourteen days **[3]**. Bryndza cheese contains natural microflora in raw ewe's milk and cows's lump cheese, as well as the distinctive production process. The raw raw material for making Slovak Bryndza is either matured ewe's lump cheese or a mix of matured ewe's and cow's **[4]**. Bryndza is usually made from unpasteurised milk with unique microflora in traditional farms. In dairy companies, pasteurised milk is inoculated by starter bacteria. The production process includes renneting, curd cutting, lump cheese forming, fermentation and ripening of the lump cheese. In the first fermentation step, the temperature in the fermentation room is 21 – 25 °C for 2 – 3 days until the pH drops to 5.2. In the second step, the temperature is 8 – 20 °C for 4 – 6 days until the pH is 4.2 – 4.8 **[5]**. The quality of "Bryndza" cheese, including its composition, properties and microbial diversity, depends on the quality of the ewe milk **[6]** and the production process **[5]**, **[7]**.

The manufacturing season of fresh ewe's Bryndza ends in the autumn; animals are stable, and milk production is stopped. To manage the overproduction of ewe's lump cheese during the season, it is preserved by salting. The process involves layering the sliced sheep's lump cheese in foil-lined barrels. Each layer is salted and pressed. The filled barrel is then closed and kept in cold storage. We called it barreled ewe's lump cheese. This barreled cheese is mixed with fresh cow's lump cheese in winter to soften its sharp taste.

Traditional producers have asked us to verify whether this traditional technological phase can be replaced by the freezing process. For this purpose, we examined how this cheese's microflora would change due to freezing and storage.

Numerous research was conducted using Bryndza cheese since it is thought that the composition and activity of microflora give different types of cheese their flavour and aroma. Data from older studies, which identified *Lactobacillus* spp., *Lactococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *Kluyveromyces marxianus* and *Geotrichum candidum* as main components of the microflora of Bryndza cheese **[3]**. Some authors **[8]**, **[9]** revealed the presence *of Lactobacillus delbrueckii*, *Lactobacillus brevis, Lactobacillus lactis, Lactobacillus raffinolactis, Streptococcus macedonicus, Streptococcus thermophilus, Leuconostoc pseudomesenteroides, Debaromyces hansenii, Mucor fragilis, Yarrowia lipolytica and Galactomyces geotrichum / Geotrichum candidum* (now called *Galactomyces candidus / Geotrichum candidum*) in Bryndza cheese.

This work aims to determine whether the process of preserving Bryndza by salting and storing it in barrels can be replaced by freezing and how the freezing process may affect the variability of microorganisms in Bryndza cheese during storage at -18°C.

Figure 1 Ewe's lump cheese, barrelled and salted ewe's lump cheese, Bryndza.

Scientific Hypothesis

Microflora of Bryndza cheese will change after the freezing and storage at -18°C.
Ewes´cheese Bryndza is a valuable source of lactic acid bacteria.

MATERIAL AND METHODOLOGY

Samples

Ten samples of Bryndza cheese from various regions of Slovakia were evaluated for microbiological quality in the present study. Table 1 shows the region of production and ewes´ milk content.

Table 1 Ewe's Bryndza cheese sample

Sample	Producer and region of production	Ewe's and Cow's lump cheese content
1	Veľký Krtíš	100 %
2	Humenné	100 %
3	Liptovský Mikuláš	100 %
4	Turčianske Teplice	50 %
5	Považská Bystrica	50 %
6	Ilava	50 %
7	Dolný Kubín	50 %
8	Zvolen	50 %
9	Detva	50 %
10	Gelnica	50 %

Chemicals

Distilled water (Sigma-Aldrich, St. Louis, MO, USA), absolute ethanol (Bruker Daltonik, Bremen, Germany), 70 % formic acid (v/v) (Sigma-Aldrich, USA), acetonitrile (Sigma-Aldrich, USA), trifluoroacetic acid (Sigma-Aldrich, USA).

Plate count agar (PCA, Oxoid, Basingstoke, UK), Violet Red Bile lactose agar (VRBL, Oxoid, Basingstoke, UK), Rogosa and Sharpe agar (MRS, Oxoid, Basingstoke, UK), Dichloran-rose Bengal chloramphenicol agar (DRBC, Oxoid, Basingstoke, UK), Tryptone Soya Agar (TSA agar, Oxoid, UK).

Instruments

Shaker (GFL 3031, Burgwedel,Germany), centrifuge (ROTOFIX 32A, Ites, Vranov, Slovakia), MALDI-TOF-MS Biotyper (Bruker Daltonics, Bremen,Germany).

Laboratory Methods

The plate dilution method was used to analyze microorganisms from Bryndza cheese. The TVC was determined according to ISO 4833-2:2013 **[10]**, the number of CB was determined according to ISO 4832:2006 **[11]**, the number of LAB was performed according to ISO 15214:1998 **[12]**, and the number of MFF was performed according to ISO 21527-1:2008 **[13]**. A MALDI-TOF MS Biotyper mass spectrometer (Bruker, Daltonics, Bremen, Germany) was used to identify isolated microorganisms from samples.

Description of the Experiment

Sample preparation:

The samples were obtained in May 2023. The samples were placed in sterile sample containers and transported to the microbiological laboratory at 5 °C. Microbiological analyses were performed immediately at the same sampling day and then after 6 months of storage of samples at -18 °C.

Number of samples analyzed: 10
Number of repeated analyses: 3
Number of experiment replication: 3

Design of the experiment:

Microbioloogical analysis:

The primary dilution of the samples was made to prepare them for testing as follows: 5 mL of sample was added to 45 mL of 0.89 % saline solution. The samples were homogenized for 30 minutes using a shaker (GFL 3031, Burgwedel, Germany). Then, the serial dilutions (10^{-2} to 10^{-4}) were done, and 0.1 mL of an aliquot from the appropriate dilution was pipetted and spread on plate count agar media.

Total viable counts (TVC) were determined using plate count agar (PCA, Oxoid, Basingstoke, UK), and inoculated Petri dishes were incubated at 30 °C for 48 – 72 h. Coliform bacteria (CB) were determined using

Violet Red Bile lactose agar (VRBL, Oxoid, Basingstoke, UK) and inoculated Petri dishes were incubated at 37 °C for 24 – 48 h. Lactic acid bacteria (LAB) were determined using Rogosa and Sharpe agar (MRS, Oxoid, Basingstoke, UK), and inoculated Petri dishes were incubated with 5% CO_2 at 30 °C for 48 – 72 h.

Microscopic filamentous fungi (MFF) were determined using Dichloran-rose Bengal chloramphenicol agar (DRBC, Oxoid, Basingstoke, UK). Inoculated Petri dishes were incubated at 25 °C for 5 – 7 days. All measurement analyses were conducted in triplicate.

Preparation of MALDI matrix solution:

The stock solution consisted of acetonitrile (50 %) (Sigma-Aldrich, USA), water (47.5 %) (Sigma-Aldrich, USA), and trifluoroacetic acid (2.5 %) (Sigma-Aldrich, USA). 500 µL of 100 % acetonitrile, 475 µL of distilled water, and 25 µL of 100 % trifluoroacetic acid were pipetted into an Eppendorf tube.

Sample Preparation and MALDI-TOF MS Measurement:

The identification of microorganisms isolated from „Bryndza“ ewes' cheese samples was performed using MALDI-TOF (matrix-assisted laser desorption/ionization time of flight) MS Biotyper (Daltonics, Bremen, Germany). Before identification, bacterial and yeast colonies were subcultured on Tryptone Soya Agar (TSA agar, Oxoid, UK) for 18 – 24 hours. Out of the eight bacterial isolates, one colony was chosen. Colonies of bacteria and yeast were suspended in a solution containing 900 µL of absolute ethanol (Bruker Daltonik, Bremen, Germany) and 300 µL of distilled water (Sigma-Aldrich, St. Louis, MO, USA). The mixture was centrifuged at 13,000 rpm for 2 min. After draining the supernatant, the pellet was combined with 50 µL of 70 % formic acid (v/v) (Sigma-Aldrich, USA) and 50 µL of acetonitrile (Sigma-Aldrich, USA). Following another centrifugation, 1 µL of the supernatant was applied to a steel plate and air-dried at 20 °C. Subsequently, 1 µL of MALDI matrix was applied to the samples. The MALDI Biotyper 3.0 program evaluated the mass spectra data (Bruker Daltonik, Germany). A score between 2.000 and 2.299 indicated a secure genus identification with probable species identification, a score between 1.700 and 1.999 suggested probable identification at the genus level, and a score below 1700 was considered unreliable for identification. These scores were used to determine the identification criteria.

Statistical Analysis

All experiments were carried out in triplicate. The means and standard deviations were calculated for microbial counts, lactic acid bacteria counts, coliform bacteria counts, and microscopic filamentous fungi counts. Krona charts were used to visualise the relatedness of the identified microbial isolates. We have used XLSTAT 2024.1 (Lumivero).

Firstly, we performed the Wilcoxon signed-rank test to analyse if there was a statistically significant decrease in Total Viable Counts (TVC), Coliform Bacteria (CB), Lactic Acid Bacteria (LAB), and Microscopic Filamentous Fungi (MFF) in Brynzda cheese stored at -18 °C during 6 months. Null Hypothesis (H0): There is no decrease in the TVC, CB, LAB and MFF after freezing and storage at -18 °C. Alternative Hypothesis (H1): Freezing and storage at -18 °C for 6 months decreases the TVC, CB, LAB and MFF.

Thereafter, we performed the chi-squared test to assess whether the proportions of microorganism species significantly changed from the beginning of storage to after 6 months.

RESULTS AND DISCUSSION

Freezing is a popular food preservation method that applies low temperatures to the product, which converts liquid water into ice crystals. The low water activity (a_w) and decreased molecular mobility resulting from freezing significantly slow down the kinetics of chemical and enzymatic reactions like proteolysis and oxidation, as well as physical changes such as mass transfers, including recrystallisation or phase separations **[14]**, **[15]**. Generally, lower freezing temperatures slow the kinetics of these deteriorative reactions **[16]**. Moreover, during frozen storage, the growth of microorganisms is stopped or delayed. The freezing process and the storage at subzero temperatures may increase the mortality of microorganisms because of the mechanical damage caused by the intracellular and extracellular ice crystal formation to the microbial membranes, dehydration of the cells caused by water pressure differences and the presence of osmotic gradients **[17]** and **[18]**. A significant challenge in the ewes' milk and goats' milk cheese manufacturing industry is the seasonal variation in milk production, leading to considerable differences in cheese output between summer and winter. To address this issue and ensure a consistent cheese supply, curds and fresh, brined, unripened cheeses produced during peak milk production periods are frozen and stored. These can then be thawed and ripened during times of lower milk production. This approach maintains a steady market supply throughout the year **[19]**.

In our research, we have found that freezing impacted the viability of the microbiota in the cheese. Although there was a slight recovery, reaching normal growth levels during the subsequent ripening period was insufficient.

The average number of total viable count (TVC) was in the range from 4.26 ±0.01 log CFU.g^{-1} in sample no. 8 to 5.03 ±0.02 log CFU.g^{-1} in sample no. 4. In the study of Kačániová et al. **[20]**, the total viable counts in the samples of Bryndza cheese ranged from 3.87 to 4.32 log CFU.g^{-1}. Coliforms exhibit a high mortality rate during freezing **[21]**, **[22]** and **[23]**. In our study, coliform bacteria (CB) were not present in sample no. 1. The highest number of CB was 2.87 ±0.02 log CFU.g^{-1} in sample no. 3. Other authors **[6]** reported the numbers of coliform bacteria in spring Bryndza at the level of 3.87 log CFU.g^{-1}, which represents higher numbers compared to our results. The average value of lactic acid bacteria (LAB) ranged from 4.00 ±0.03 log CFU.g^{-1} in sample no. 7 to 4.90 ±0.02 log CFU.g^{-1} in sample no. 3. Other authors **[6]** and **[24]** detected higher numbers of lactic acid bacteria (from 10^8 to 10^9 CFU.g^{-1}, respectively) in Bryndza cheese made from unpasteurised ewe milk. Yeasts belong to the natural microbiota of Bryndza cheese and contribute to the ripening of the cheese **[25]**. The average number of microscopic filamentous fungi (MFF) was in the range from 1.00 ±0.01 log CFU.g^{-1} in sample no. 8 to 2.30 ±0.02 log CFU.g^{-1} in sample no. 4. Yeasts and moulds significantly contribute to the deterioration of dairy products, which can lead to changes in the taste, texture and colour of the products. In addition, the spread of moulds in dairy products poses health risks to consumers **[26]**. Table 2 shows the results of the microbial analysis of "Bryndza" cheese at the beginning of storage.

Table 2 Microbiota of Bryndza cheese at the beginning of storage (average ± SD log CFU.g^{-1}).

	TVC	CB	LAB	MFF
1	4.71 ±0.04	< 1	4.79 ±0.02	2.18 ±0.02
2	5.01 ±0.02	2.44 ±0.02	4.58 ±0.02	1.68 ±0.02
3	4.89 ±0.01	2.87 ±0.02	4.90 ±0.02	2.02 ±0.02
4	5.03 ±0.02	2.73 ±0.02	4.71 ±0.01	2.30 ±0.02
5	4.67 ±0.01	2.65 ±0.02	4.76 ±0.02	1.99 ±0.01
6	5.02 ±0.03	2.00 ±0.01	4.76 ±0.01	1.68 ±0.02
7	4.64 ±0.02	1.46 ±0.01	4.00 ±0.03	1.93 ±0.01
8	4.26 ±0.01	1.11 ±0.01	4.77 ±0.02	1.00 ±0.01
9	4.41 ±0.01	1.86 ±0.04	4.83 ±0.02	1.47 ±0.02
10	4.80 ±0.01	2.42 ±0.02	4.78 ±0.02	1.02 ±0.01

Note: TVC – total viable counts, CB – coliforms bacteria, LAB – lactic acid bacteria, MFF – microscopic filamentous fungi, SD – standard deviation.

The number of individual groups of microorganisms was determined after 6 months of storage at a temperature of -18 °C. The average number of TVCs ranged from 4.86 ±0.01 (sample no. 1) to 5.31 ±0.01 log CFU.g^{-1} (sample no. 9). Coliform bacteria were not present in any of the analyzed samples after 6 months of storage. The average number of LAB were in the range from 4.03 ±0.02 (sample no. 7) to 5.44 ±0.01 (sample no. 3). The average number of MFF ranged from 1.51 ±0.02 (sample no. 10) to 2.69 ±0.01 log CFU.g^{-1} (sample no. 1).

Food cultures produced by microorganisms include yeasts, fungi, and bacteria. These microorganisms determine the fermented food's flavour, texture, and acidity and provide health advantages beyond basic nourishment **[27]**. Table 3 shows the results of the microbial analysis of "Bryndza" cheese at the end of storage.

Table 3 Microbiota of Bryndza cheese at the end of storage (average ± SD log CFU.g^{-1}).

	TVC	CB	LAB	MFF
1	4.86 ±0.01	< 1	4.45 ±0.01	2.69 ±0.01
2	4.90 ±0.01	< 1	4.67 ±0.01	1.89 ±0.02
3	5.09 ±0.02	< 1	5.44 ±0.01	2.02 ±0.02
4	5.09 ±0.01	< 1	5.03 ±0.01	2.17 ±0.02
5	4.97 ±0.01	< 1	4.66 ±0.01	2.13 ±0.02
6	4.86 ±0.01	< 1	4.10 ±0.01	1.76 ±0.03
7	4.96 ±0.01	< 1	4.03 ±0.02	2.03 ±0.02
8	5.00 ±0.01	< 1	4.82 ±0.01	1.72 ±0.03
9	5.31 ±0.01	< 1	4.93 ±0.02	1.66 ±0.02
10	5.10 ±0.01	< 1	4.90 ±0.02	1.51 ±0.02

Note: TVC – total viable counts, CB – coliforms bacteria, LAB – lactic acid bacteria, MFF – microscopic filamentous fungi, SD – standard deviation.

The Wilcoxon signed-rank test was conducted to evaluate whether there was a decrease in the TVC, CB, LAB and MFF after 6 months of storage at -18 °C. The test results yielded p-values of 0.0137 (TVC), 0.3125 (LAB), and 0.0104 (MFF), not calculated for (CB). This result indicates a statistically significant reduction in TVC, not a statistically significant decrease in LAB, but a statistically significant reduction in MFF. Since the CB counts are reported as "<1" for all samples after storage, this indicates a reduction to below the detection limit in all cases. This qualitative result strongly suggests a significant decrease in CB due to storage, although a specific p-value cannot be provided without exact counts.

A total of 295 isolates from cheese "Bryndza" were identified using MALDI-TOF Biotyper at the beginning of storage. The dominant species of microorganisms found in Bryndza cheese were lactic acid bacteria, especially, *Lactococcus lactis* (68 isolates), *Lactobacillus fermentum* (41 isolates), *Lactobacillus delbrueckii* (33 isolates), *Lactiplantibacillus plantarum* (32 isolates), *Lacticaseibacillus rhamnosus* (30 isolates) and *Lactiplantibacillus paraplantarum* (28 isolates). Other authors **[24]** determined that during one production season, the genus *Lactobacillus* was present in every sample of bryndza cheese manufactured in seven specialised factories in various Slovakia locations. The bacteria *Lactococcus lactis* was isolated and detected in every sample of bryndza cheese. The absence of pathogenic and spoilage bacteria in ewes cheese could depend on the quality of raw milk utilised **[28]**; moreover, the thermal treatment applied during stretching **[29]** and the microbial biofilms of the wooden vats **[30]** also contribute to the safety of the resulting cheeses. Isolated species of microorganisms from Bryndza cheese at the beginning of storage are shown in Table 4.

Table 4 Number of isolates of microorganisms from Bryndza cheese at the beginning of storage.

Species	**Sample**										
	1	2	3	4	5	6	7	8	9	10	Total
Geotrichum silvicola	2										2
Lactococcus lactis	5	9	8	7	8	7	6	4	6	8	68
Yarrowia lipolytica		1	3	2			2	1			9
Escherichia coli				1		8		2			11
Raoultella ornithinolytica							1				1
Pichia cactophila							1				1
Enterococcus faecium									4		4
Enterobacter cloacae										2	2
Enterobacter xiangfangensis										1	1
Lactiplantibacillus plantarum	4	3	2	6	2	8	3	1	1	2	32
Limosilactobacillus fermentum	2	5	4	3	4	3	6	3	5	6	41
Lactobacillus delbrueckii	2	3	3	3	4	5	4	5	2	2	33
Lactiplantibacillus paraplantarum	2	1	3	2	3	4	2	3	5	3	28
Lacticaseibacillus rhamnosus	2	2	3	3	4	3	2	3	5	3	30
Hafnia alvei									1		1
Latilactobacillus curvatus	2	1	1	3	2	4	2	3		1	19
Leuconostoc lactis	3		1		1		3				8
Leuconostoc pseudomesenteroides				1			1	2			4
Total											**295**

Diverse microbiota comprises the *Lactococcus, Streptococcus, Lactobacillus,* and *Enterococcus genera. Lactococcus lactis* subsp. *lactis and Lactococcus lactis* subsp. *cremoris* were present in all tested Bryndza cheese samples **[31]**. In another study, *Lactococcus, Pediococcus, Enterococcus*, and *Streptococcus* were abundant in Bryndza from different Slovak regions **[32]**.

Our research identified 220 isolates from Bryndza cheese after 6 months of storage at -18 °C (Table 5). The most frequently isolated species included *Lactococcus lactis* (62 isolates), *Limosilactobacillus fermentum* (33 isolates), *Lactiplantibacillus plantarum* (30 isolates), and *Levilactobacillus brevis* (18 isolates).

Table 5 Number of isolates of microorganisms from Bryndza cheese after 6 months of storage at -18 °C.

Species	Sample										
	1	2	3	4	5	6	7	8	9	10	Total
Limosilactobacillus fermentum	1	4	4	1	3	6	5	2	4	3	33
Pediococcus pentosaceus	1					1			2	1	5
Levilactobacillus brevis	2		3	1		2		4	2	4	18
Lactiplantibacillus plantarum	3	3	5	4	6	3	2	2	1	1	30
Leuconostoc lactis	2	1	1	3	1			2	1	1	12
Candida krusei		3									3
Candida rugosa		1									1
Enterococcus faecalis	5	1							1		7
Lactococcus lactis		9	10	8	4	1	2	7	10	11	62
Yarrowia lipolytica		1	2	4	2			2	1		12
Geotrichum silvicola					2						2
Enterococcus faecium	6					7		3	1		17
Streptococcus salivarius ssp. *thermophilus*				1							1
Enterococcus durans					2						2
Geotrichum candidum	4	2			3	2					11
Kluyveromyces lactis						1					1
Candida intermedia									2	1	3
Total											**220**

Table 6 The classification of microorganisms into families.

Bacteria	Family
Lactobacillus plantarum	*Lactobacillaceae*
Limosilactobacillus fermentum	
Lactobacillus delbrueckii	
Lactiplantibacillus paraplantarum	
Lacticaseibacillus rhamnosus	
Latilactobacillus curvatus	
Leuconostoc lactis	
Leuconostoc pseudomesenteroides	
Pediococcus pentosaceus	
Levilactobacillus brevis	
Lactococcus lactis	*Streptococcaceae*
Streptococcus salivarius ssp. thermophilus	
Escherichia coli	*Enterobacteriaceae*
Raoultella ornithinolytica	
Enterobacter cloacae	
Enterobacter xiangfangensis	
Hafnia alvei	
Enterococcus faecium	*Enterococcaceae*
Enterococcus faecalis	
Enterococcus durans	
Yeasts	
Yarrowia lipolytica	*Dipodascaceae*
Geotrichum candidum	
Geotrichum silvicola	
Pichia cactophila	*Saccharomycetaceae*
Candida krusei	
Candida rugosa	
Candida intermedia	
Kluyveromyces lactis	

The key findings of the chi-squared test were:

- *Lactococcus lactis*: Minor decrease, not statistically significant (P-value = 0.205061).
- *Enterococcus faecium* showed a significant change in its count, increasing from 4 to 17 (p-value = 0.000654). This indicates a significant increase in proportion relative to other microorganisms over the storage period.
- Other species, like *Limosilactobacillus fermentum and Yarrowia lipolytica,* did not show significant changes, although their p-values were relatively low (P-value = 0.205061 and 0.247978). This suggests some change, but not statistically significant.

These results suggest that while some species exhibit notable changes in their population dynamics, only *Enterococcus faecium* showed a statistically significant increase. This could be due to adaptive advantages or changes in the cheese environment favouring its growth over time.

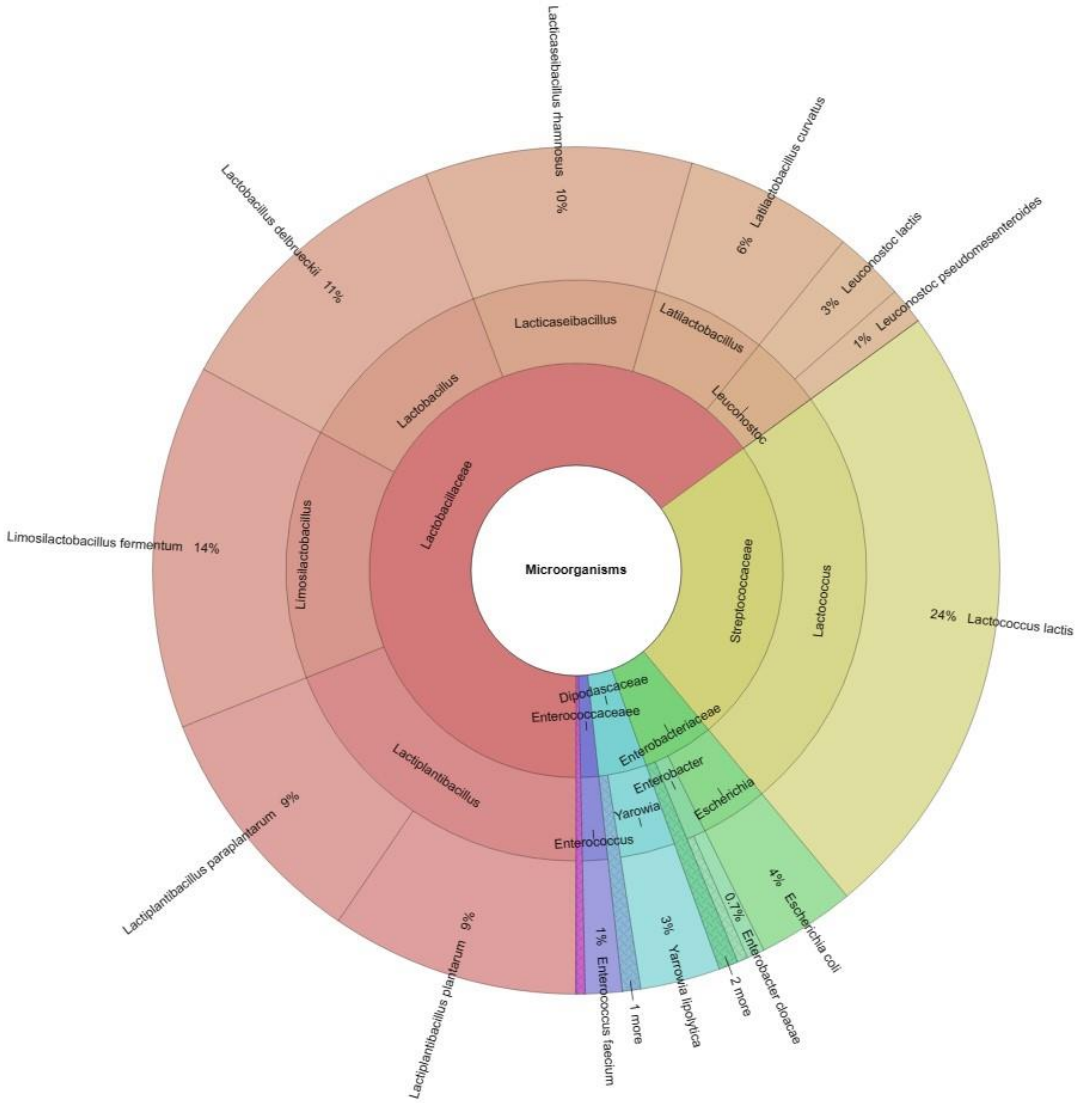


Figure 2 Krona chart for microorganisms isolated from "Bryndza" cheese at the begining of storage.
Note: outermost ring: species, middle ring: genus, innermost ring: family.

Lactic acid bacteria produce organic acids (lactic and acetic acid), hydrogen peroxide, ethanol, diacetyl, acetaldehyde, acetoin, carbon dioxide, and bacteriocins—metabolites categorised as antimicrobial agents. Additionally, the low pH caused by organic acid synthesis limits pathogenic microorganisms' action **[33]**.

In the study of **[34]**, the genus *Enterococcus*, belonging to the family *Enterococcacae*, was detected in 3.18 % of tested cheeses. While enterococci are sometimes thought of as pathogens in cheeses, they can also operate as probiotic microbiota, producing antimicrobial substances known as bacteriocins **[35]**, **[36]**.

The classification of microorganisms into families is shown in Table 6.
Lactic acid bacteria, mainly *Lactobacillus* species, *Lactococcus, Pediococcus, Enterococcus*, and *Streptococcus,* were the dominant group of bacteria found in Bryndza from several locations in Slovakia **[37]**.

Our results show that 9 species of bacteria belonging to the *Lactobacillaceae* family (63 %) were isolated from Bryndza cheese samples immediately after opening. Bacteria belonging to the *Streptococcaceae* family represented 24 % of the isolated microorganisms. Microorganisms belonging to *Enterobacteriaceae* (6 %) were also represented, where 4 bacterial species were isolated. The family *Dipodascaceae* (4 %) with 2 species, *Enterococcaceae* (1 %) with 1 species, and the family *Saccharomycetaceae* (0.34 %) represented by 1 species (Figure 2).

Lactic acid bacteria belong to the helpful microbiota in milk. Representatives of the genera *Streptococcus, Leuconostoc*, or *Lactobacillus* can utilize lactose, broken down in the lactic acid **[38]**.

Lactic acid bacteria (i. e. *Lactococcus* spp., *Streptococcus* spp., *Lactobacillus* spp. and *Leuconostoc* spp.) dominated, forming a share of 57.8–99.6 % of all identified bacteria. After 6 months of sample storage, 5 species belonging to the family Lactobacillaceae (45 %) were isolated, followed by microorganisms belonging to the family Streptococcaceae (28 %) with 1 species, the family *Enterococcaceae* (12 %) with 3 species, the family *Dipodascaceae* (11 %) with 3 species and the family *Saccharomycetaceae* (4 %) with 4 species (Figure 3).

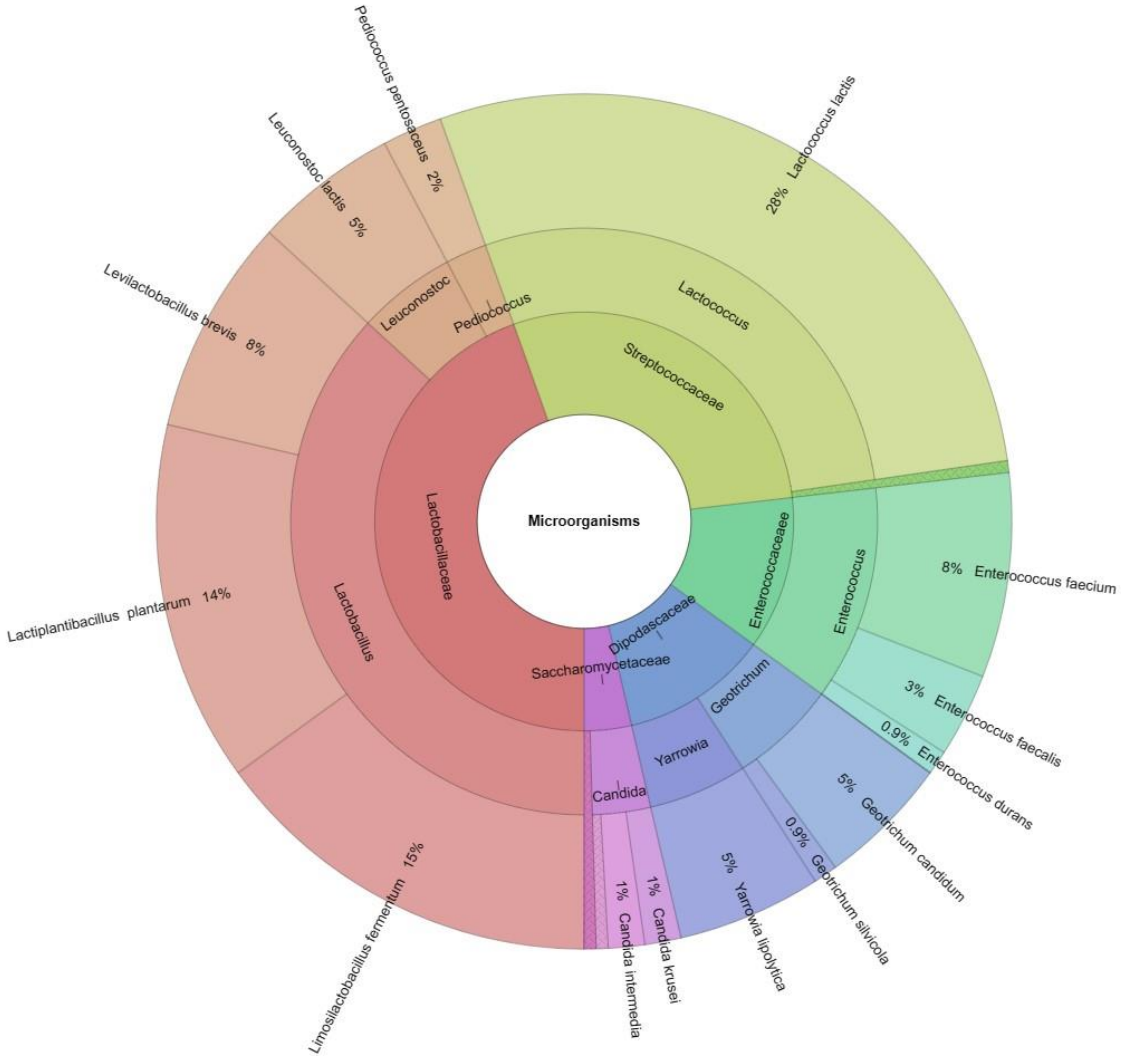


Figure 3 Krona chart for microorganisms isolated from "Bryndza" cheese at the end of storage.
Note: outermost ring: species, middle ring: genus, innermost ring: family.

Compared with other cheeses, Gouda cheese-associated microbial population analyzed by the sequencing approach represents the genera *Lactococcus*, *Streptococcus*, *Staphylococcus,* and *Lactobacillus.* These microorganisms were predominant in cheese **[39]**. In comparison with Bryndza cheese. We and other authors identified lactococci and staphylococci as dominant microflora in Bryndza cheese **[37]**. *Lactobacillus casei, Enterococcus* spp., and *Lactobacillus delbrueckii* ssp. *bulgaricus* were the dominant lactic acid bacteria in

Brazilian mozzarella cheese obtained from whole raw milk and natural whey culture **[40]**. In the study of **[41]**, *Streptococcus* spp. and *Lactobacillus* spp. were the dominant bacteria of sheep's cheeses, while *Phyllobacterium* spp. and *Staphylococcus* spp. were the most common genera in milk. Genus *Enterococcus* **[42]**, *Staphylococcus* **[43]** and fungal species **[44]** were identified in Bryndza cheese in several studies.

When we compare the representation of microorganisms at the beginning of storage and after 6 months of storage, we can conclude that lactic acid bacteria were represented by 10 species at the beginning of storage and 6 species at the end of storage. Four species of bacteria belonging to the family *Enterobacteriaceae* were initially represented in bryndza cheese samples, but no bacteria belonging to this family were identified at the end of storage.

From the point of view of food safety, the increase in the number of bacteria from the *Enterocaccaceae* family appears to be a problem; specifically, the *Enterococcus faecalis* species was detected only in frozen samples in the number of 4 isolates. The number of identified *Enterococcus faecium* isolates increased from 4 to 17. Dairy products must fulfil the legislation requirements **[45]**, **[46]**.

Our findings align with Chebeňová-Turcovská et al. **[9]**, where *Lactococcus lactis* was prevalent in Bryndza, contributing to the cheese's typical sensory properties by producing lactic acid and other aromatic compounds. Similarly, *Enterococcus* spp. Our study has noted their role in probiotic formulations, owing to their resilience and beneficial interactions within the human gut, despite concerns about their safety **[9]**. Even after frozen storage, these probiotics' survival and functional activity in Bryndza cheese indicate robust adaptability crucial for maintaining viable probiotic populations in dairy products. Our results corroborate findings from Pangallo et al. **[6]**, who noted that the microbiota dynamics in Bryndza adapt well to storage conditions without significant loss of viable probiotic bacteria.

Our study extends the understanding of Bryndza's microbiota by highlighting these microorganisms' survival and functional stability under frozen storage conditions. This aspect is crucial for extending the shelf life of Bryndza while maintaining its health benefits, which aligns with the findings from **[6]**, where they emphasise the role of microbial communities in influencing the sensory and textural profiles of Bryndza during and after storage. Furthermore, the microbial profile changes noted in our study during the storage period reflect adaptive responses that could potentially enhance the probiotic efficacy of Bryndza cheese. This is supported by Pangallo et al. **[6]**, who discuss how microbial dynamics are not merely about survival but also the interaction between microbial species that can lead to enhanced probiotic functions.

The resilience of probiotic strains in Bryndza cheese, such as *Lactococcus lactis* and *Limosilactobacillus fermentum*, during frozen storage, underscores their potential to maintain gut health and modulate the immune system. These strains have been widely recognised for their abilities to survive gastrointestinal conditions and adhere to intestinal cells, thus making them effective at maintaining intestinal barrier integrity and modulating the host's immune response. *Lactococcus lactis*, known for its immunomodulatory effects, can enhance mucosal immunity and has been used in vaccine delivery. Its presence in Bryndza, even post-freezing, suggests that consuming frozen Bryndza might confer these immunological benefits to consumers. *Limosilactobacillus fermentum*, another significant strain found in Bryndza, contributes to gut health by improving the balance of gut microbiota and enhancing the intestinal barrier. It also plays a role in fermentation, contributing to the cheese's flavour and texture over time.

The stability of these microorganisms during frozen storage, as indicated by the minimal changes in viable counts, suggests that Bryndza cheese can serve as an effective carrier of live probiotics. This could be particularly beneficial in dietary applications where probiotics are recommended for maintaining or restoring gut health.

Preserving Bryndza cheese through freezing maintains its microbial integrity and ensures the continuance of its probiotic benefits, making it a valuable functional food product. The study's findings can be applied to improve the storage and distribution of Bryndza cheese, helping it reach a broader market without losing its health-promoting properties.

This enhanced discussion integrates the beneficial properties of probiotic microorganisms found in Bryndza cheese and highlights the potential health benefits these microorganisms can confer, even after extended frozen storage periods. By focusing on these specific probiotic properties and linking them to the health benefits they can confer, the discussion provides valuable insights for readers, highlighting the significance of these findings in both food science and nutritional health contexts.

CONCLUSION

Freezing impact on the microbiological composition of Bryndza cheese highlights several crucial findings that could reshape storage practices for seasonal cheese products. Detailed analysis over six months determined that key microbial communities, particularly lactic acid bacteria, remain viable and largely unaffected by extended periods of freezing at -18 °C during 6 months. This preservation of microbial integrity ensures that the cheese retains its beneficial probiotic qualities even beyond typical production seasons. Interestingly, while total viable counts and lactic acid bacteria showed no significant decline, specific groups, such as microscopic filamentous fungi, experienced a marked reduction. Additionally, the complete suppression of coliform bacteria to undetectable levels underscores the effectiveness of freezing as a storage method, maintaining quality and enhancing product safety. These outcomes support the potential for freezing as a viable alternative to traditional salting and barrel storage, managing seasonal overproduction while maintaining the cheese's safety and nutritional value. This study extends our understanding of Bryndza cheese microbiological dynamics under freezing conditions. It supports similar applications in other dairy products, potentially leading to more flexible and economically viable practices for cheese producers. This research underscores the need for updated regulatory perspectives and legislation to accommodate new scientific insights into food preservation, ensuring that traditional foods can meet modern standards of safety, quality, and accessibility.

The average number of microorganisms in Bryndza stored at -18 °C during 6 months were: TVCs ranged from 4.86 ±0.01 to 5.31 ±0.01 log CFU.g^{-1}. Coliform bacteria were absent in any of the analysed samples after 6 months of storage. The average number of LAB ranged from 4.03 ±0.02 to 5.44 ±0.01. The average number of MFF ranged from 1.51 ±0.02 to 2.69 ±0.01 log CFU.g^{-1}.

REFERENCES

1. Semjon, B., Reitznerová, A., Poláková, Z., Výrostková, J., Maľová, J., Koréneková, B., Dudriková, E., & Lovayová, V. (2018). The effect of traditional production methods on microbial, physico-chemical and sensory properties of 'Slovenská bryndza' Protected Geographical Indication cheese. In International Journal of Dairy Technology (Vol. 71, Issue 3, pp. 709–716). Wiley. https://doi.org/10.1111/1471-0307.12522
2. Kawęcka, A., & Sosin-Bzducha, E. (2014). Seasonal changes of the chemical composition of cheese obtained from the milk of indigenous Polish breeds of sheep. In Journal of Animal and Feed Sciences (Vol. 23, Issue 2, pp. 131–138). The Kielanowski Institute of Animal Physiology and Nutrition, PAS. https://doi.org/10.22358/jafs/65701/2014
3. Palo, V., Kaláb, M. (1984). Slovak sheep cheeses. In Milchwissenschaft, (Vol. 39, pp. 518-521).
4. Commission Regulation (EC) No 676/2008 of 16 July 2008 registering certain names in the Register of protected designations of origin and protected geographical indications (Ail de la Drôme (PGI), Všestarská cibule (PDO), Slovenská bryndza (PGI), Ajo Morado de Las Pedroñeras (PGI), Gamoneu or Gamonedo (PDO), Alheira de Vinhais (PGI), Presunto de Vinhais or Presunto Bísaro de Vinhais (PGI)). Official Journal of the European Union, L 189.
5. Zaják, P., Beňová, E., Židek, R., Čapla, J., Benešová, L., Čurlej, J., & Golian, J. (2021). Detection of adulteration of traditional Slovak bryndza ewe's cheese with cow's lump cheese by isoelectric focusing of gamma caseins. In International Journal of Food Properties (Vol. 24, Issue 1, pp. 1034–1060). Informa UK Limited. https://doi.org/10.1080/10942912.2021.1953066
6. Pangallo, D., Šaková, N., Koreňová, J., Puškárová, A., Kraková, L., Valík, L., & Kuchta, T. (2014). Microbial diversity and dynamics during the production of May bryndza cheese. In International Journal of Food Microbiology (Vol. 170, pp. 38–43). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2013.10.015
7. Zaják, P., Čurlej, J., Čapla, J., Benešová, L., Jakabová, S., Partika, A., & Zubrická, S. (2023). Fatty acid profile of traditional Slovak ewe's and cow's lump cheese. In International Journal of Food Properties (Vol. 26, Issue 1, pp. 1426–1444). Informa UK Limited. https://doi.org/10.1080/10942912.2023.2219860
8. Kačániová, M., Nagyová, Ľ., Štefániková, J., Felsöciová, S., Godočíková, L., Haščík, P., Horská, E., & Kunová, S. (2020). The characteristic of sheep cheese "Bryndza" from different regions of Slovakia based on microbiological quality. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 69–75). HACCP Consulting. https://doi.org/10.5219/1239
9. Chebeňová-Turcovská, V., Ženišová, K., Kuchta, T., Pangallo, D., & Brežná, B. (2011). Culture-independent detection of microorganisms in traditional Slovakian bryndza cheese. In International Journal of Food Microbiology (Vol. 150, Issue 1, pp. 73–78). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2011.07.020
10. ISO 4833-2:2013; Microbiology of the Food Chain. Horizontal Method for the Enumeration of Microorganisms: Colony Count at 30 °C by the Pour Plate Technique. ISO, Geneva, CH, 2013.
11. ISO 4832:2006; Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coliforms. Colony count technique. ISO: Geneva, CH, 2006.

12. ISO 15214:1998; Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Enumeration of Mesophilic Lactic Acid Bacteria. Colony-Count Technique at 30 °C. ISO, Geneva, CH, 1998.
13. ISO 21527-1:2008; Microbiology of Food and Animal Feeding Stuffs. Horizontal Method for the Enumeration of Yeasts and Moulds. ISO, Geneva, CH, 2008.
14. Sun, D.-W. (Ed.). (2016). Handbook of Frozen Food Processing and Packaging. CRC Press. https://doi.org/10.1201/b11204
15. Verdini, R. A., & Rubiolo, A. C. (2002). Effect of Frozen Storage Time on the Proteolysis of Soft Cheeses Studied by Principal Component Analysis of Proteolytic Profiles. In Journal of Food Science (Vol. 67, Issue 3, pp. 963–967). Wiley. https://doi.org/10.1111/j.1365-2621.2002.tb09436.x
16. El-Kest, S. E., & Marth, E. H. (1992). Freezing of Listeria monocytogenes and Other Microorganisms: A Review. In Journal of Food Protection (Vol. 55, Issue 8, pp. 639–648). Elsevier BV. https://doi.org/10.4315/0362-028x-55.8.639
17. Smith, E. M., Monaghan, E. M., Huntley, S. J., & Green, L. E. (2011). Short communication: Preliminary investigation into the effect of freezing and a cryopreservant on the recovery of mastitis pathogens from ewe milk. In Journal of Dairy Science (Vol. 94, Issue 10, pp. 4850–4855). American Dairy Science Association. https://doi.org/10.3168/jds.2010-4076
18. Katsiari, M. C., Voutsinas, L. P., & Kondyli, E. (2002). Manufacture of yoghurt from stored frozen sheep's milk. In Food Chemistry (Vol. 77, Issue 4, pp. 413–420). Elsevier BV. https://doi.org/10.1016/s0308-8146(01)00367-3
19. Fontecha, J., Peláez, C., Juárez, M., & Martín-Hernández, M. C. (1994). Effect of freezing and frozen storage on the physicochemical, organoleptic and microbiological characteristics of a semi-hard ewes' milk cheese. In Journal of Dairy Research (Vol. 61, Issue 1, pp. 133–142). Cambridge University Press (CUP). https://doi.org/10.1017/s0022029900028120
20. Šaková, N., Sádecká, J., Lejková, J., Puškárová, A., Koreňová, J., Kolek, E., Valík, Ľ., Kuchta, T., & Pangallo, D. (2015). Characterization of May bryndza cheese from various regions in Slovakia based on microbiological, molecular and principal volatile odorants examination. In Journal of Food and Nutrition Research (Vol. 54, pp. 239–251).
21. Katsiari, M. C., Voutsinas, L. P., & Kondyli, E. (2002). Manufacture of yoghurt from stored frozen sheep's milk. In Food Chemistry (Vol. 77, Issue 4, pp. 413–420). Elsevier BV. https://doi.org/10.1016/s0308-8146(01)00367-3
22. Voutsinas, L. P., Katsiari, M. C., Pappas, C. P., & Mallatou, H. (1995). Production of brined soft cheese from frozen ultrafiltered sheep's milk. Part 1. Physicochemical, microbiological and physical stability properties of concentrates. In Food Chemistry (Vol. 52, Issue 3, pp. 227–233). Elsevier BV. https://doi.org/10.1016/0308-8146(95)92817-4
23. Cruz, A. G., Antunes, A. E. C., Sousa, A. L. O. P., Faria, J. A. F., & Saad, S. M. I. (2009). Ice-cream as a probiotic food carrier. In Food Research International (Vol. 42, Issue 9, pp. 1233–1239). Elsevier BV. https://doi.org/10.1016/j.foodres.2009.03.020
24. Jaster, H., Judacewski, P., Ribeiro, J. C. B., Zielinski, A. A. F., Demiate, I. M., Los, P. R., Alberti, A., & Nogueira, A. (2019). Quality assessment of the manufacture of new ripened soft cheese by Geotrichum candidum: physico-chemical and technological properties. In Food Science and Technology (Vol. 39, Issue 1, pp. 50–58). FapUNIFESP (SciELO). https://doi.org/10.1590/fst.25717
25. Sengun, I., Yaman, D., & Gonul, S. (2008). Mycotoxins and mould contamination in cheese: a review. In World Mycotoxin Journal (Vol. 1, Issue 3, pp. 291–298). Brill. https://doi.org/10.3920/wmj2008.x041
26. Vogel, R. F., Hammes, W. P., Habermeyer, M., Engel, K., Knorr, D., & Eisenbrand, G. (2011). Microbial food cultures – opinion of the Senate Commission on Food Safety (SKLM) of the German Research Foundation (DFG). In Molecular Nutrition & Food Research (Vol. 55, Issue 4, pp. 654–662). Wiley. https://doi.org/10.1002/mnfr.201100010
27. Oliver, S. P., Jayarao, B. M., & Almeida, R. A. (2005). Foodborne Pathogens in Milk and the Dairy Farm Environment: Food Safety and Public Health Implications. In Foodborne Pathogens and Disease (Vol. 2, Issue 2, pp. 115–129). Mary Ann Liebert Inc. https://doi.org/10.1089/fpd.2005.2.115
28. Gaglio, R., Francesca, N., Di Gerlando, R., Cruciata, M., Guarcello, R., Portolano, B., Moschetti, G., & Settanni, L. (2013). Identification, typing and investigation of the dairy characteristics of lactic acid bacteria isolated from "Vastedda della valle del Belice" cheeses. In Dairy Science & Technology (Vol. 94, Issue 2, pp. 157–180). Springer Science and Business Media LLC. https://doi.org/10.1007/s13594-013-0150-5
29. Scatassa, M. L., Gaglio, R., Macaluso, G., Francesca, N., Randazzo, W., Cardamone, C., Di Grigoli, A., Moschetti, G., & Settanni, L. (2015). Transfer, composition and technological characterization of the lactic

acid bacterial populations of the wooden vats used to produce traditional stretched cheeses. In Food Microbiology (Vol. 52, pp. 31–41). Elsevier BV. https://doi.org/10.1016/j.fm.2015.06.008

30. Planý, M., Kuchta, T., Šoltýs, K., Szemes, T., Pangallo, D., & Siekel, P. (2016). Metagenomic Analysis of Slovak Bryndza Cheese Using Next-Generation 16S rDNA Amplicon Sequencing. In Nova Biotechnologica et Chimica (Vol. 15, Issue 1, pp. 23–34). Walter de Gruyter GmbH. https://doi.org/10.1515/nbec-2016-0003
31. Bertaová, G., Chebeňová, V., Brežná, B., Pangallo, D., Valík, Ľ., & Kuchta, T. (2009). Identification of lactic acid bacteria in Slovakian bryndza cheese. In Journal of Food and Nutrition Research (Vol. 48, Issue 2, pp. 65-71).
32. Mojgani, N., Amimia, C. (2006). Kinetics of Growth and Bacteriocin Production in L. casei RN 78 Isolated from a Dairy Sample in IR Iran. In International Journal of Dairy Science (Vol. 2, Issue 1, pp. 1–12). Science Alert. https://doi.org/10.3923/ijds.2007.1.12
33. Lauková, A., Micenková, L., Pogány Simonová, M., Focková, V., Ščerbová, J., Tomáška, M., Dvorožňáková, E., & Kološta, M. (2021). Microbiome Associated with Slovak Traditional Ewe's Milk Lump Cheese. In Processes (Vol. 9, Issue 9, p. 1603). MDPI AG. https://doi.org/10.3390/pr9091603
34. Tofalo, R., Perpetuini, G., Battistelli, N., Pepe, A., Ianni, A., Martino, G., & Suzzi, G. (2019). Accumulation γ-Aminobutyric Acid and Biogenic Amines in a Traditional Raw Milk Ewe's Cheese. In Foods (Vol. 8, Issue 9, p. 401). MDPI AG. https://doi.org/10.3390/foods8090401
35. Lauková, A., Focková, V., & Pogány Simonová, M. (2020). Enterococcus mundtii Isolated from Slovak Raw Goat Milk and Its Bacteriocinogenic Potential. In International Journal of Environmental Research and Public Health (Vol. 17, Issue 24, p. 9504). MDPI AG. https://doi.org/10.3390/ijerph17249504
36. Kačániová, M., Kunová, S., Štefánikova, J., Felšociová, S., Godočíková, L., Horská, E., Nagyová, L'., Haščík, P., & Terentjeva, M. (2019). Microbiota Of The Traditional Slovak Sheep Cheese "Bryndza". In Journal of Microbiology, Biotechnology and Food Sciences (Vol. 9, Issue Special issue, pp. 482–486). Slovak University of Agriculture in Nitra. https://doi.org/10.15414/jmbfs.2019.9.special.482-486
37. Uhrín, V., Lauková, A., Jančová, A., & Plintovič, V. Mlieko a Mliečna Žl'aza. Milk and Mammary Gland. (2002). Faculty of Natural Sciences of the University Constantinus Philosophus: Nitra, Slovakia, 2002; pp. 5–167, ISBN 80-8050-511-X. (in Slovak)
38. Sádecká, J., Čaplová, Z., Tomáška, M., Šoltys, K., Kopuncová, M., Budiš, J., Drončovský, M., Kolek, E., Koreňová, J., & Kuchta, T. (2019). Microorganisms and volatile aroma-active compounds in bryndza cheese produced and marketed in Slovakia. In Journal of Food and Nutrition Research (Vol. 58, Issue 4, pp.382–392).
39. Salazar, J. K., Carstens, C. K., Ramachandran, P., Shazer, A. G., Narula, S. S., Reed, E., Ottesen, A., & Schill, K. M. (2018). Metagenomics of pasteurized and unpasteurized gouda cheese using targeted 16S rDNA sequencing. In BMC Microbiology (Vol. 18, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12866-018-1323-4
40. Silva, L. F., De Dea Lindner, J., Sunakozawa, T. N., Amaral, D. M. F., Casella, T., Nogueira, M. C. L., & Penna, A. L. B. (2021). Biodiversity and succession of lactic microbiota involved in Brazilian buffalo mozzarella cheese production. In Brazilian Journal of Microbiology (Vol. 53, Issue 1, pp. 303–316). Springer Science and Business Media LLC. https://doi.org/10.1007/s42770-021-00629-6
41. Endres, C. M., Castro, Í. M. S., Trevisol, L. D., Severo, J. M., Mann, M. B., Varela, A. P. M., Frazzon, A. P. G., Mayer, F. Q., & Frazzon, J. (2021). Molecular characterization of the bacterial communities present in sheep's milk and cheese produced in South Brazilian Region via 16S rRNA gene metabarcoding sequencing. In LWT (Vol. 147, p. 111579). Elsevier BV. https://doi.org/10.1016/j.lwt.2021.111579
42. Jurkovic, D., Krizková, L., Sojka, M., Belicová, A., Dušinský, R., Krajcovic, J., Snauwaert, C., Naser, S., Vandamme, P., & Vancanneyt, M. (2006). Molecular identification and diversity of enterococci isolated from Slovak Bryndza cheese. In The Journal of General and Applied Microbiology (Vol. 52, Issue 6, pp. 329–337). Microbiology Research Foundation. https://doi.org/10.2323/jgam.52.329
43. Mikulášová, M., Valáriková, J., Dušinský, R., Chovanová, R., & Belicová, A. (2013). Multiresistance of Staphylococcus xylosus and Staphylococcus equorum from Slovak Bryndza cheese. In Folia Microbiologica (Vol. 59, Issue 3, pp. 223–227). Springer Science and Business Media LLC. https://doi.org/10.1007/s12223-013-0286-y
44. Laurencik, M., Sulo, P., Slavikova, E., Pieckova, E., Seman, M., & Ebringer, L. (2008). The diversity of eukaryotic microbiota in the traditional Slovak sheep cheese — Bryndza. In International Journal of Food Microbiology (Vol. 127, Issues 1–2, pp. 176–179). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2008.06.016

45. Čapla, J., Zajác, P., Ševcová, K., Čurlej, J., & Fikselová, M. (2022). Milk and diary products – summary of European legislation, hygiene manuals, ISO standards and Codex Alimentarius standards. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 431–462). HACCP Consulting. https://doi.org/10.5219/1744
46. Ševcová, K., Zajác, P., Čapla, J., & Čurlej, J. (2021). Development of the food act of the Slovak Republic from 1995 to 2021. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 982–994). HACCP Consulting. https://doi.org/10.5219/1689

Funds: This work was supported by grant APVV-19-0180 „Application of molecular-biological methods in the development of reference materials intended for detection of adulteration and evaluation of food quality“.
Acknowledgments: -
Conflict of Interest: The authors declare no conflict of interest.
Ethical Statement: This article does not contain any studies that would require an ethical statement.

Contact Address:

Simona Kunová, The Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia,
Tel.: +421 37 641 5807,
E-mail: simona.kunova@uniag.sk
ORCID: https://orcid.org/0000-0003-2240-1756

***Peter Zajác**, The Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia,
Tel.: +421 37 6414371,
E-mail: peter.zajac@uniag.sk
ORCID: https://orcid.org/0000-0002-4425-4374

Jozef Čapla, The Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia,
Tel.: +421 37 6414371,
E-mail: jozef.capla@uniag.sk
ORCID: https://orcid.org/0000-0001-9475-6359

Jozef Čurlej, The Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia,
Tel.: +421 37 6415825,
E-mail: jozef.curlej@uniag.sk
ORCID: https://orcid.org/0000-0003-0039-5332

Ivana Timorácká, The Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Institute of Food Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia,
E-mail: xtimoracka@uniag.sk
ORCID: https://orcid.org/0009-0001-2387-7652

Natália Čmiková, The Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Institute of Horticulture,Tr. A. Hlinku 2, 949 76, Nitra, Slovakia,
E-mail: xcmikova@uniag.sk
ORCID: https://orcid.org/0000-0003-4419-0590

Miroslava Kačániová, The Slovak University of Agriculture in Nitra, Faculty of Horticulture and Landscape Engineering, Institute of Horticulture,Tr. A. Hlinku 2, 949 76, Nitra, Slovakia,University of Economics and Human Sciences in Warsaw,Schoolof Medical and Health Sciences,Okopowa 59, Warszawa, 01 043, Poland, Tel.: +421 37 641 4715,
E-mail: miroslava.kacaniova@uniag.sk
ORCID: https://orcid.org/0000-0002-4460-0222

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 12.3.2024
Revised: 30.3.2024
Accepted: 23.5.2024
Published: 12.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 512-522
https://doi.org/10.5219/1970
ISSN: 1337-0960 online
www.potravinarstvo.com

Effect of light intensity on the photo-oxidation stability of red and yellow palm olein mixture

Dewi Fortuna Ayu, Andarini Diharmi, Netti Herawati, Rahmadini Payla Juarsa

ABSTRACT

Palm oil is an edible oil derived from the mesocarp of oil palm fruit (*Elaeis guineensis*), which has a high content of carotenoids and tocopherol components. This research aimed to study the effects of light intensity on the photo-oxidation stability of a red and yellow palm olein mixture. The red and yellow palm oleins were mixed into 100, 200, and 350 ppm carotene content. The photo-oxidation stability of the palm olein mixture was investigated under fluorescent light intensities of 5,000 and 10,000 lux at 31 ±2 °C for 7 days. Changes in the content of chlorophyll, carotene, tocopherols, and peroxide value (PV) were evaluated daily. The results showed that an increase in carotene and tocopherol contents effectively improved the photo-oxidative stability of the palm olein mixture. Degradation of chlorophyll, tocopherols, and increased PV were proportional to light intensity during photo-oxidation. There were no significant changes in carotene content at 5,000 lux light intensity exposure. The degradation rates of chlorophyll and tocopherols can be described as first-order reaction kinetics. In contrast, the increase rate of PV can be described as a zero-order kinetics model with *k*-values of 6.6×10^{-2}, 4.9×10^{-2}, 3.7×10^{-2} mequiv.kg^{-1}.h^{-1}, and 8.3×10^{-2}, 6.8×10^{-2}, and 5.6×10^{-2} mequiv.kg^{-1}.h^{-1} in palm olein mixture, which contains 100, 200, and 350 ppm carotene at 5,000 and 10,000 lux light intensity exposure, respectively. These results suggested that carotene protected tocopherol in palm olein and that tocopherol and carotene synergistically acted as singlet oxygen quenchers during photo-oxidation.

Keywords: carotenoids, intensity, palm olein, photo-oxidation, tocopherol

INTRODUCTION

Indonesia is one of the world's largest palm oil (PO) producing countries besides Malaysia **[1]**. As one of the world's most produced and consumable edible oils, PO has a high content of carotenoids and tocopherols. Generally, PO in Indonesia is consumed in the form of cooking oil, which is packaged in transparent plastic. Packaging palm cooking oil in transparent plastic allows photo-oxidation reactions during storage and distribution.

The palm oil used as cooking oil has undergone a refining and bleaching process so that it contains limited carotene and tocopherol, indicated by its transparent yellow colour. However, palm oil can also be processed into red palm oil (RPO) to retain β-carotene and vitamin E. Red palm oil is processed crude palm oil from the fruit mesocarp using pre-treatment of deacidification and deodorization via short-path distillation and without bleaching process **[2]**. In refining without blanching, RPO also maintains chlorophyll content. The amount of chlorophyll in refined palm oil is 582.9-579.4 μg.kg^{-1}, the highest among cottonseed, rapeseed, safflower, sunflower, corn, and soybean oils **[3]**. Studies have shown that the amount of retained carotenoids after refining ranges from 500 to 786 parts per million, depending on the condition of crude (red) palm oil before refining. About 80% of the carotenoids retained were found to consist of 0.2% phytoene, 0.6% phytofluene, 41.3% α-carotene, 10.2% cis-α-carotene, 41.0% β-carotene, cis-β-carotene, 0.6% ζ-carotene, 0.8% γ-carotene, 0.8% δ-

carotene, 0.2% neurosporene, 0.5% α-zeacarotene, 1.3% β-zeacarotene and 1.0% lycopene **[4]**. Among the 13 carotenoids retained, only α-carotene, β-carotene, and γ-carotene can show provitamin A activity and have 15, 44, and 300 times more retinol than carrots, leafy vegetables, and tomatoes, respectively **[4]**. The meta-analysis study demonstrated that RPO might effectively prevent or alleviate vitamin A deficiency **[5]**.

Other than carotenoids, tocopherols and tocotrienols also contribute to the oxidative stability of red palm oil. About 85% of tocopherols and tocotrienols are retained after refining, ranging from 600 to 1000 parts per million **[2]**. The tocopherols and tocotrienols present are in the form of 19% α-tocopherol, 29% α-tocotrienol, 41% γ-tocotrienol and 10% δ-tocotrienol with 70% are tocotrienols, and 30% are tocopherols. As an antioxidant, it may prolong food products' shelf-life, stabilize oils and fats, and lower free radical damage **[6]**.

Although RPO contains chlorophyll, characterized as a photosensitizer, the high content of β-carotene and tocopherol compounds in RPO has antioxidant activity in photo-oxidation **[7]**. The mechanism of singlet oxygen quenching or another excited sensitizer compound by β-carotene was done via energy transfer **[8]**. Meanwhile, tocopherol acts as a physical quencher of singlet oxygen, for which α-tocopherol has the highest activity, followed by γ- and δ-tocopherol **[8]**. Because of that, red palm oil had the rate of chlorophyll degradation slower than virgin olive oil, and a mixture of olive oil and perilla **[9]**.

Light intensity triggers photo-oxidation reactions in food containing photosensitizers and influences its nutritional quality **[10]**. Meanwhile, the presence of β-carotene has an important role in the photo-oxidation stability of RPO, which also contains chlorophyll and tocopherols **[7]**, **[9]**, **[10]**. Even though palm cooking oil might be exposed to light intensity during storage and distribution, photo-oxidation in palm cooking oil has not been reported yet. This research objective was to study the effect of light intensity on the photo-oxidation stability of red and yellow palm olein mixture. The deterioration rate during photo-oxidation can be used to obtain suitable storage conditions for palm cooking oil.

Scientific hypothesis

The bioactive components in palm oil (carotenoids, tocopherol, and chlorophyll) significantly affected the photo-oxidation stability of palm cooking oil. Information about the influence of light intensity on photo-oxidation stability and the changes of bioactive compounds during photo-oxidation storage can be used to apply suitable storage conditions for palm cooking oil.

MATERIAL AND METHODOLOGY

Samples

Crude palm oil (CPO) was obtained from Salim Ivomas Pratama Ltd., Jakarta. Crude palm oil was processed into red palm olein (RPO) and yellow palm oil (YPO) in March 2022. The RPO and YPO were used as materials in this study.

Chemicals

Beta-carotene and tocopherol standards were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). N-hexane, toluene, isooctane, methylene chloride, ethanol, 2,2 bipyridine, and $FeCl_3.6H_2O$ were obtained from JT Baker (Phillipsburg, NJ, USA). All chemicals were analytical grade.

Instruments

Chlorophyll, β-carotene, and tocopherol changes in mixed oil were analyzed by Shimadzu UV-2450 spectrophotometer (Shimadzu Co., Tokyo, Japan). The photo-oxidation stability of palm oil during storage was carried out using a mirror glass box (60 × 50 × 30 cm) equipped with nine fluorescent cool white lights 18 watts as a light source. The box was placed in a wooden box (70 cm × 60 × 50 cm) equipped with a heater, thermocouple, and 4 blowers relayed at the bottom of the glass box. Light intensity and temperature during storage were controlled using a lux meter (Digital Lightmeter QM1587, China) and thermocouple digital (Digital LED Display DC 12V K-type, China), respectively.

Laboratory Methods

Peroxide value (PV) was evaluated using the AOCS method Cd 8-53 **[11]**. The chlorophyll content was determined using the AOCS method Cc 13i-96 **[12]**. The absorbances of samples were measured at 670, 630, and 710 nm using CH_2Cl_2 as blank. Chlorophyll content was calculated as Equation 1.

$$\text{Chlorophyll content (ppm)} = \frac{34.5 \times (A_{670} - 0.5 \times A_{630} - 0.5 \times A_{710})}{L} \quad (1)$$

Where:
A = absorbance at each wavelength; L = cuvette thickness (mm).

Carotene content in oil was determined using the PORIM method **[13]**. As much as 4 mg of palm oil was dissolved with hexane and diluted into 10 ml. The solution was transferred into a 1 cm quartz cuvette, and the absorbance was measured at 446 nm against hexane. The carotene content in oil was expressed as ppm (molar absorption coefficient was 1.40×10^5 l.mol^{-1}.cm^{-1}).

Tocopherol content was determined based on **[14]**. A total of 40 mg samples were put into a 10 ml volumetric flask, added 5 ml toluene, 3.5 ml 2,2-bipyridine (0.07% w/v in 95% ethanol), 0.5 mL $FeCl_3.6H_2O$ (0.2% w/v in 95% ethanol), and appropriated 10 ml with 95% ethanol. The sample was shaken and allowed to stand for 10 minutes. The absorbance of the sample was measured at 520 nm. Tocopherol content was calculated based on the standard curve α-tocopherol in the range of 250-2000 ppm.

Description of the Experiment

Sample preparation: Red palm oil was processed from CPO at the laboratory of the Pilot Plant of Oil and Fat, Southeast Asian Food and Agricultural Science and Technology Center, Bogor, Indonesia. The RPO contains 4.36 ±0.03 ppm total chlorophyll, 559.39 ±4.26 ppm β-carotene, and 1262.47 ±2.31 ppm tocopherols, with the composition of fatty acids; 40.32% oleic, 35.81% palmitic, 11.00% linoleic, 3.92% stearic, and 0.30% linolenic acids. Yellow palm olein (YPO) was purified from CPO by bleaching and application processes in the chromatographic column to remove chlorophyll, carotene, and tocopherol compounds. Red palm oil and YPO were mixed according to the ratio, so each treatment had a carotene content of 100, 200, and 350 ppm. The carotene, tocopherol, and chlorophyll content were measured before and during photo-oxidation storage.

Number of samples analyzed: Six samples from forty-eight total samples were analyzed daily during photo-oxidation storage. The samples were exposed to light on intensities of 5,000 and 10,000 lux for 7 days at 31 ±2 °C.

Number of repeated analyses: The samples were collected in triplicate, and the changes in chlorophyll contents, carotene, tocopherol, and peroxide values were analyzed daily.

Number of experiment replication: Each study was carried out three times. The samples were eight for seven days of storage. Consequently, twenty-four repeated analyses were carried out for 5,000 and 10,000 lux intensities, respectively.

Design of the experiment: The study was conducted experimentally. Palm oil photo-oxidation was performed according to **[10]** with light intensity modification. Palm oil as much as 30 ml was poured into a 100 ml transparent serum bottle with 77% headspace. The bottle was tightly capped with rubber and sealed with plastic parafilm. The samples were placed in a mirror glass box and exposed to light on intensities of 5,000 and 10,000 lux for 7 days of photo-oxidation storage at 31 ±2 °C. The samples were collected and analyzed every d in triplicate. The change rate of chlorophyll, β-carotene, tocopherol, and peroxide values in the palm olein mixture were determined during photo-oxidation. Evaluation of the kinetics model describes the reaction rate as a function of experimental variables, making it possible to predict changes in photooxidation. The general equation for studying chlorophyll, β-carotene, tocopherol, and PV changes can be given as Equation 2.

$$dQ/dt = -kQ^n \quad (2)$$

Where:
k – represents the reaction rate constant; Q – represents the content of chlorophyll, β-carotene, tocopherol (ppm), or PV mequiv.kg^{-1}) at time t; n – represents the reaction order; t – represents the time (h).

Statistical Analysis

The changes of chlorophyll, β-carotene, and tocopherol contents, and peroxide values in the palm olein mixture were analyzed by linear programming on Microsoft Excel 16 and IBM SPSS Statistics 24. All experiments were carried out in triplicate and the results reported are the results of those replicate determinations with standard deviations. The accuracy of the experimental data was determined using the Student's t-test with a confidence coefficient $p \leq 0.05$.

RESULTS AND DISCUSSION

Chlorophyll, β-carotene and tocopherol changes during photo-oxidation of palm olein mixture

The presence of minor components, especially chlorophyll, contributed to the photo-oxidation reactions that occur in oil. According to **[15]**, light exposure can lead to chlorophyll being excited and acting as a photosensitizer type II that reacts with triplet oxygen and transforms into highly reactive singlet oxygen. Singlet oxygen directly attacks the double bond in chlorophyll-a between the fifth and sixth carbon, resulting in a subsequent shift of

position of the double bond and formation of hydro-peroxides, which are then further cleaved through oxygen-oxygen linkage and form degradation products **[16]**. Figures 1a and 1b showed that the higher light intensity exposure accelerated the degradation rate of chlorophyll in the palm olein mixture, especially on the lowest carotene content (100 ppm). Chlorophyll content in palm oil, which contains 100, 200, and 350 ppm carotene, decreased from 0.41, 1.19, 1.96 to 0.13 (68.29%), 0.57 (52.10%), and 1.19 ppm (39.28%) after 7 days of photo-oxidation storage on light intensity of 5,000 lux.

Meanwhile, on light intensity storage of 10,000 lux, chlorophyll content in palm oil decreased faster than 5,000 lux, starting from 0.33, 1.08, 1.84 to 0.09 (72.73%), 0.47 (65.88%), and 0.92 ppm (50%) after 7 days of photo-oxidation storage which contains 100, 200, and 350 ppm carotene, respectively. The degradation rate of chlorophyll content in PO can be described as a first-order kinetics model. Exposure to the light intensity of 5,000 lux caused degradation of chlorophyll in palm oil, which contains 100, 200, and 350 ppm carotene with a reaction rate constant (k values) of 6 x 10^{-3} (r^2 = 0.77), 3.5 x 10^{-3} (r^2 = 0.81) and 2.7 x $10^{-3}h^{-1}$ (r^2 = 0.86), respectively. Meanwhile, exposure to light intensity of 10,000 lux caused degradation of chlorophyll faster than 5,000 lux in palm oil, which contains 100, 200, and 350 ppm carotene with k values of 6 x 10^{-3} (r^2 = 0.66), 4 x 10^{-3} (r^2 = 0.80) and 3.5 x $10^{-3}h^{-1}$ (r^2 = 0.82), respectively (Figure 1b). This was similar to the photo-degradation of chlorophyll in RPO, which followed the first-order kinetics model **[9]**.

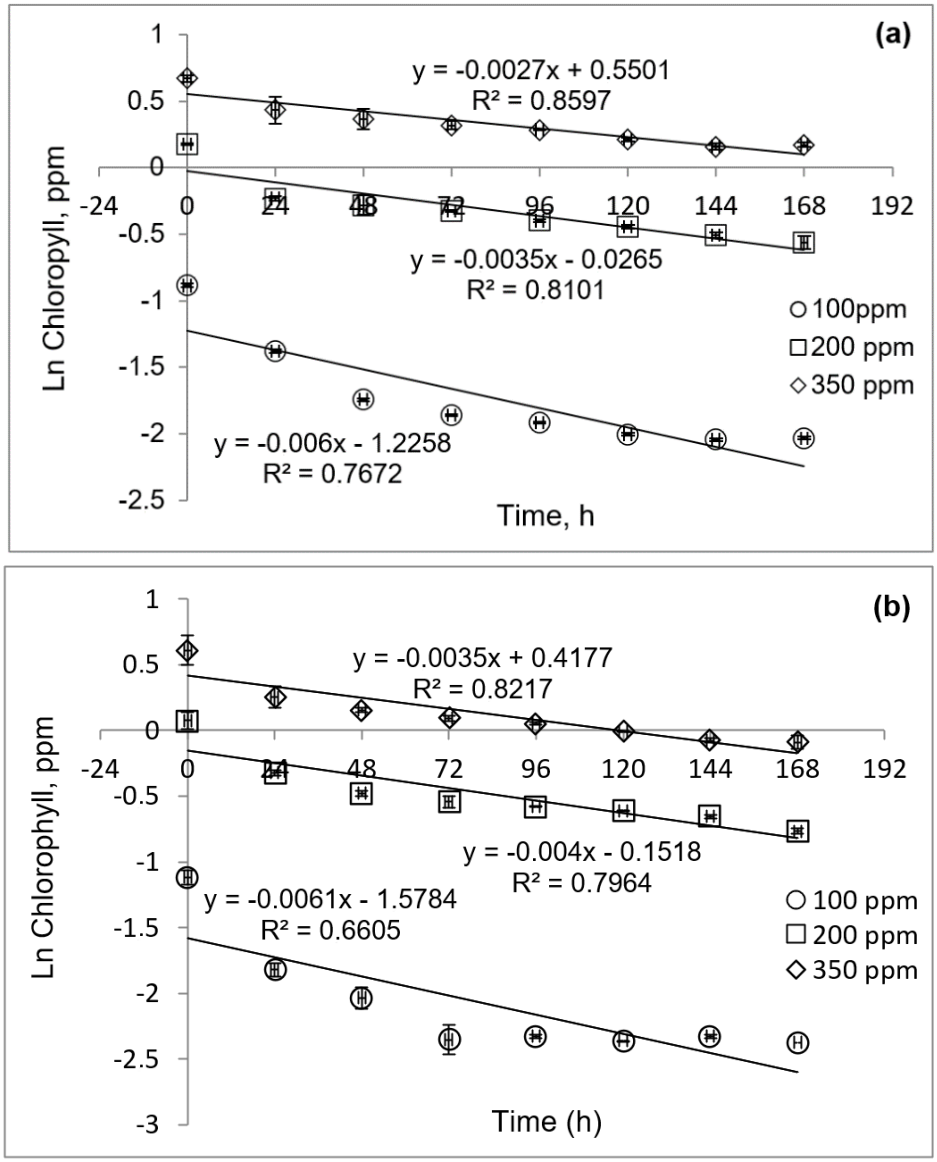


Figure 1 The first-order kinetics of chlorophyll degradation rate in red and yellow palm olein mixture which contain (o) 100 ppm, (□) 200 ppm, and (◊) 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux.

The concentration of carotene showed the role of maintaining chlorophyll content in oil, which indicated the antioxidant activity of carotene. The study conducted by **[17]** stated that carotenoids added to soybean oil could singlet oxygen quencher to protect the soybean oil from chlorophyll photo-sensitized oxidation. The greater the number of double bond conjugations, the greater the effect of protection against oxidation of singlet oxygen. According to **[18]**, 1 molecule of β-carotene can quench 250-1000 molecules of singlet oxygen with a k value of 3×10^{10} $M^{-1}s^{-1}$ via energy transfer. The excitation energy of the electron is transferred from the singlet oxygen to the singlet state carotenoids (CAR), producing carotenoid triplet state (^{3}CAR) and triplet oxygen, called quenching singlet oxygen. Energy is also transferred from the excited triplet sensitizer state (^{3}Sen*) to the carotenoid singlet state (^{1}CAR), called quenching triplet sensitizer.

Changes in carotene contents in palm oil during storage when exposed to light at intensities of 5,000 and 10,000 lux are presented in Figure 2. Figure 2a showed no significant changes in carotene content in palm olein mixture (p >0.05) during 7 days of 5,000 lux light intensity exposure. This indicated that exposure to light at an intensity of 5,000 lux could not cause carotene degradation in the palm olein mixture. According to **[19]**, visible light causes the degradation of carotene, which is slower, as much as 1/100, than UV rays. The photo-degradation rate of β-carotene was slow in this research, similar to β-carotene in pure olive oil up to 22.5 h on exposure to the light intensity of 12100 lux **[20].** An increase in amounts of β-carotene in RPO during photo-oxidation storage was unlikely **[7]**, probably associated with tocopherol was acting as an antioxidant for the protection of β-carotene via a delay in the isomerization process of trans-β-carotene **[21]**. 13,15-di-cis-β-carotene is the main isomer formed due to light exposure with a molar extinction coefficient higher than trans-β-carotene **[16]**, **[22]**.

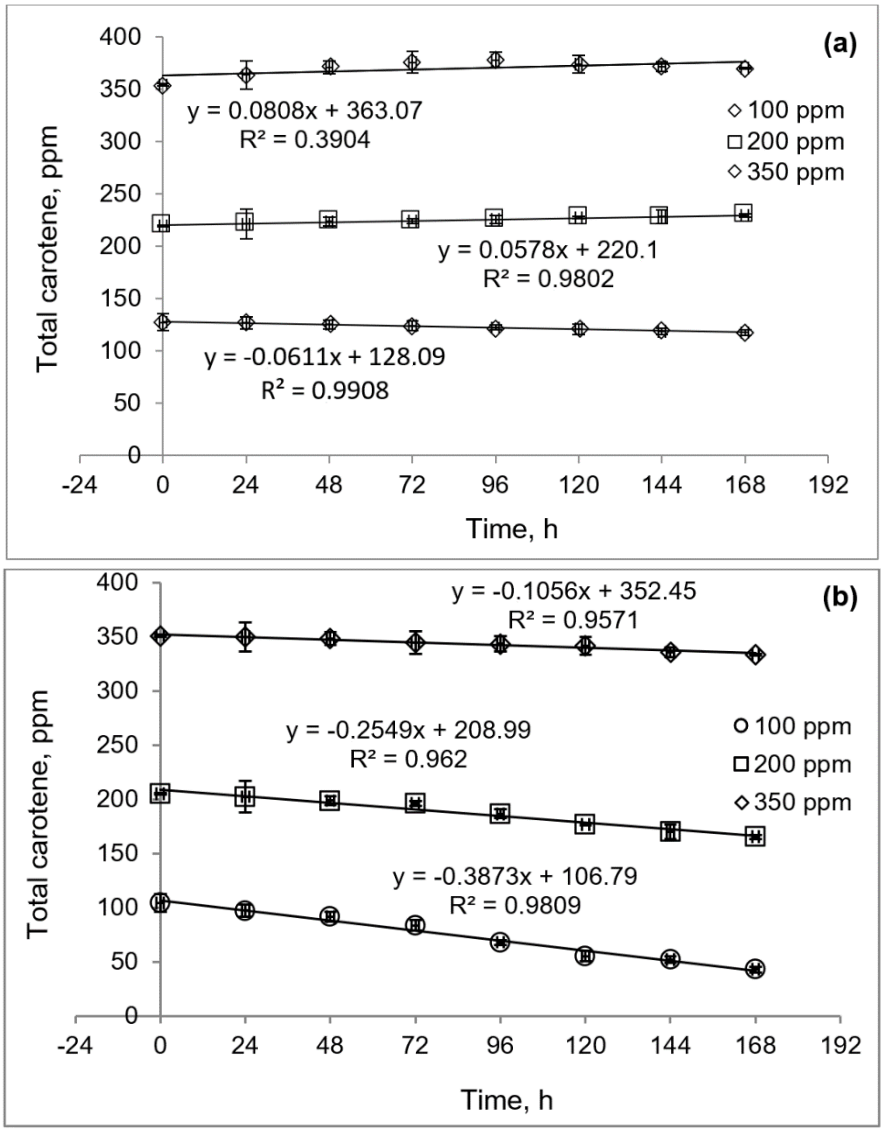


Figure 2 The zero-order kinetics of β-carotene degradation rate in red and yellow palm olein mixture which contain (o) 100 ppm, (□) 200 ppm, and (◊) 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux.

In the case of 10,000 lux light intensity exposure, carotene in the palm olein mixture significantly decreased (p <0.05) during 7 days of photo-oxidation storage (Figure 2b). After 7 days of storage, exposure to the light intensity of 10,000 lux caused degradation of 100, 200, and 350 ppm carotene in palm olein mixture as much as 58.93% (42.81 ppm), 19.35% (165.29 ppm), and 4.98% (333.49 ppm), respectively. Figure 2b also shows that the degradation rate of carotene in palm olein mixture during exposure to 10,000 lux is described as a zero-order kinetics model. Light intensity accelerated degradation of carotene in palm olein mixture (p <0.05), which contains 100, 200, and 350 ppm with k values of 0.39 (r^2 = 0.98), 0.25 (r^2 = 0.96) and 0.11 ppm.h^{-1} (r^2 = 0.96), respectively. The appearance of red and yellow palm olein mixture during exposure to 5,000 and 10,000 lux for 7 days is shown in Figure 3.

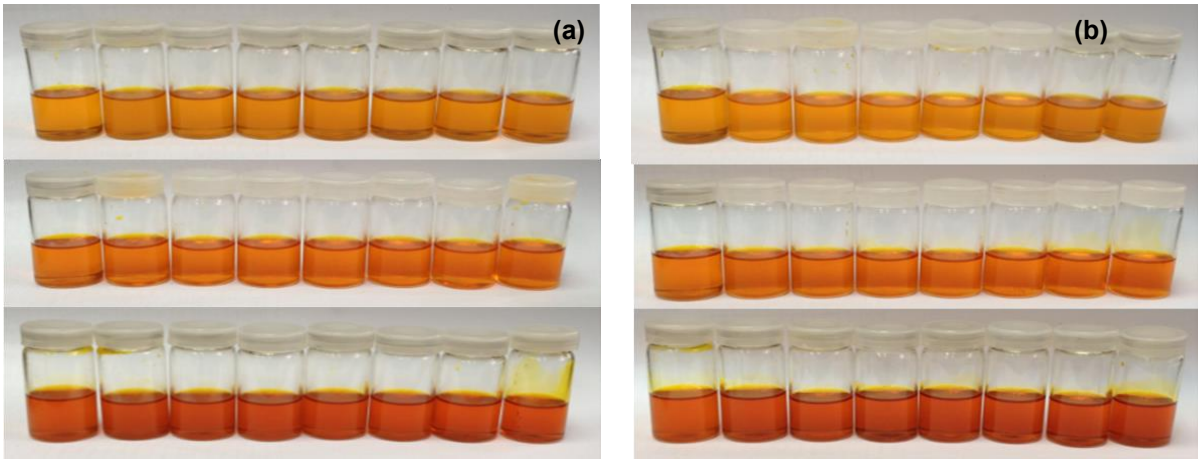


Figure 3 The red and yellow palm olein mixture which contain 100 ppm, 200 ppm, and 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux for seven days.

The slow rate of carotene photo-degradation in RPO was also shown by **[9]**. This study measured carotene degradation after storage for 12, 11, and 10 days at light intensities of 5,000, 10,000, and 15,000 lux, respectively. Exposure to low light intensity (5,000 lux) during 7 days of storage was not strong enough to cause carotene degradation, which can be observed using a spectrophotometer at a wavelength of 446 nm. The isomerization process of trans-β-carotene into 13,15-di-cis-β-carotene increased absorbance in the measurement of total carotene **[22]**. Because of that, carotene was relatively stable during dark storage, and the degradation rate of β-carotene in RPO followed the zero-order kinetics model with $t_{1/2}$ for 12 months **[23]**.

The photo-degradation of carotene in the palm olein mixture had a different pattern from the auto or thermal oxidation reaction in RPO. Temperature treatments of 60, 75, and 90 °C during storage increased the degradation rate of β-carotene in RPO, which followed the first-order reaction kinetics with k values of 9.13 x 10^{-3}, 2.15 x 10^{-2}, and 4.79 x 10^{-2} h^{-1} at 60, 75, and 90 °C storage, respectively **[24]**. The high energy exposure to carotene caused a higher rate of carotene degradation.

In the case of tocopherol, exposure to light and carotene concentration resulted in changes in tocopherol content in the palm olein mixture (Figure 4). Figure 4 showed that light intensity accelerated the degradation of tocopherol in palm olein mixture (p <0.05), especially on the low content of carotene (100 and 200 ppm). Tocopherol content in palm oil, which contains 100, 200, and 350 ppm of carotene, decreased from 670, 911.13, and 1027.47 to 46.82 (93.01%), 201.92 (77.84%), and 288.56 ppm (71.92%) after 7 days of photo-oxidation storage on light intensity of 5,000 lux. Meanwhile, on light intensity storage of 10,000 lux, tocopherol content in palm olein mixture decreased from 746.14, 944.78, 1189.81 to 53.76 (92.79%), 170.68 (81.93%), and 274.49 ppm (69/69%) after 7 days of photo-oxidation storage which contains 100, 200, and 350 ppm carotene, respectively.

The degradation rate of tocopherol during light exposure in red and palm olein mixture is described as a first-order kinetics model, as shown in Figure 4. Exposure to light intensity of 5,000 lux caused degradation of tocopherol in palm oil, which contains 100, 200, and 350 ppm carotene with k values of 1.5 x 10^{-2} (r^2 = 0.99), 9.0x10^{-3} (r^2=0.99), and 7.4x10^{-3} h^{-1} (r^2=0.98), respectively (Figure 4a). Meanwhile, exposure to the light intensity of 10,000 lux caused degradation of tocopherol faster than the light intensity of 5,000 lux, which contains 100, 200, and 350 ppm carotene with k values of 1.9 x 10^{-2} (r^2 = 0.92), 9,7.0 x 10^{-3} (r^2 = 0.90), and 7.4 x $10^{-3}h^{-1}$ (r^2 = 0.99), respectively (Figure 4b). High-light-intensity supplied more energy to result in singlet oxygen for further progression of oxidation. The degradation of tocopherol during photo-oxidation was due to its chemical quenching of singlet oxygen and free-radical scavenging **[8]**. Light also supplies energy to break the O–H bond and ether linkage in the structure of tocopherol, resulting in semiquinone radicals and quinones **[25]**.

The photo-degradation rate of tocopherol in 10,000 lux was higher than 5,000 lux light intensity, especially on the low carotene content (100 and 200 ppm) (Figure 4). This result indicated that carotene had a protective effect on tocopherol in palm oil during photo-oxidation. Although the light intensity accelerated the degradation of tocopherol, the presence of carotene was more protective of tocopherol due to light exposure. At the highest carotene content (300 ppm), exposure to light intensities of 5,000 and 10,000 lux showed the same rate of tocopherol degradation (7.4 x $10^{-3}h^{-1}$). According to **[7]**, changes in tocopherol to the tocopheroxyl radical occurred at a slower rate in the TAG+Toc+Car model system and became measurable due to the antioxidant activity of β-carotene. Beta-carotene is excited by light energy, becomes more active, and donates hydrogen, thus protecting tochoperol against free radicals **[25]**.

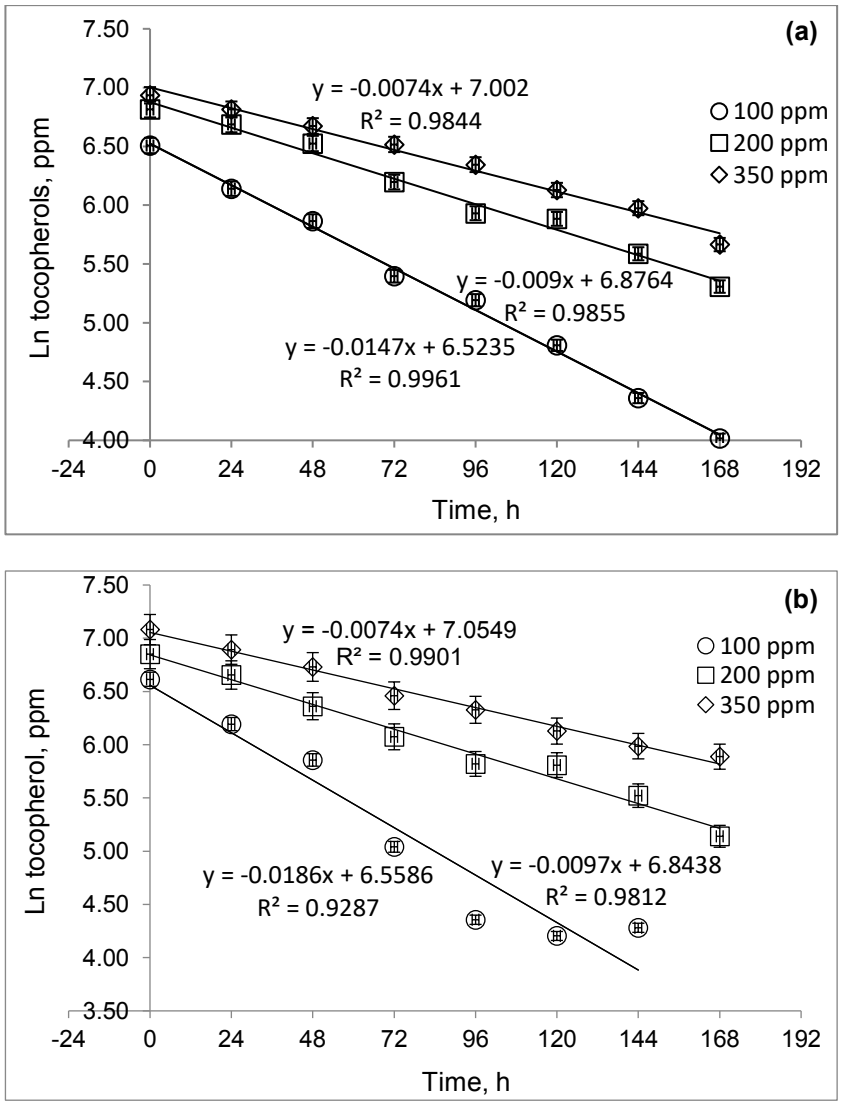


Figure 4 The first-order kinetics of tocopherol degradation rate in red and yellow palm olein mixture which contain (o) 100 ppm, (□) 200 ppm, and (◊) 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux.

Effect of light intensity and β-carotene on photo-oxidation stability of palm oil

The effect of light intensity and carotene during photo-oxidation storage on PV in palm olein mixture is shown in Figure 5. Exposure to 5,000 and 10,000 lux light intensities directly increased PV in the palm olein mixture (p <0.05). Peroxide value in palm oil which contains 100, 200, and 350 ppm carotene, increased from 0.00, 0.69, 1.39 to 11.17, 8.91, and 7.51 mequiv.kg^{-1} after 7 days of photo-oxidation storage on light intensity of 5,000 lux (Figure 5a). Meanwhile, on light intensity storage of 10,000 lux, PV in palm olein mixture increased higher than 5,000 lux, starting from 0.49, 0.99, 1.68 to 14.52, 12.46, and 11.05 mequiv.kg^{-1} after 7 days of photo-oxidation storage which contains 100, 200, and 350 ppm carotene, respectively (Figure 5b). The high PV in the palm olein

mixture after 7 days of light storage made the oil no longer fit for consumption as cooking oil, the quality standard for cooking oil in Indonesia at a maximum PV of 10 mequiv.kg^{-1} **[26]**. This result indicated that light exposure was very effective in initiating photo-oxidation. The higher the light intensity, the faster the PV in palm oil increases. Light accelerates oil oxidation, especially if the oil contains sensitizers such as chlorophylls **[27]**.

As shown in Figure 5, the increased rate of PV can be explained by the zero-order kinetics model. Exposure to light at an intensity of 5,000 lux increased k values of PV to 6.6 x 10^{-2} (r^2 = 0.99), 4.9 x 0^{-2} (r^2 = 0.99), and 3.7 x 10^{-2} (r^2 = 0.99) mequiv.kg^{-1}.h^{-1} in palm olein mixture which contains 100, 200 and 350 ppm carotene, respectively (Figure 5a). Exposure to light at an intensity of 10,000 lux also proportionally increased k values to 8.3 x 10^{-2} (r^2 = 0.99), 6.8 x 10^{-2} (r^2 = 0.99), and 5.6 x 10^{-2} (r^2 = 0.99) mequiv.kg^{-1}.h^{-1} in palm olein mixture which contains 100, 200, and 350 ppm carotene, respectively (Figure 5b). Exposure to high light intensity accelerated the chlorophyll excitation and transferred more energy onto adjacent triplet oxygen to form active singlet oxygen **[15]**. Electrophilic singlet oxygen directly reacts with the double bond's high-electron density, producing conjugate and non-conjugate hydro-peroxides **[15]**, **[27]**, **[28]**. The quantity of hydro-peroxides formed during photo-oxidation was proportional to the amount of light absorbed **[27]**.

In this research, carotene could inhibit the increase of PV in palm olein mixture on either the light intensities of 5,000 or 10,000 lux (Figure 5). The higher carotene content slowed the increase of PV during exposure to light. This was because of carotene activity as a singlet oxygen quencher during photo-oxidation in RPO **[7]**. However, mixing red and yellow palm olein will also increase the tocopherol content in the olein mixture. Tocopherol in the palm olein mixture suggested cooperation with carotene as an antioxidant during photo-oxidation **[7]**, **[21]**. Carotene had a protective effect on tocopherol due to exposure to light intensity, while tocopherol could be an antioxidant in photo- and auto-oxidation **[8]**. The palm olein mixture is used as cooking oil, which has high carotene and tocopherol content and can be stored in dark conditions to maintain its photo-oxidation stability.

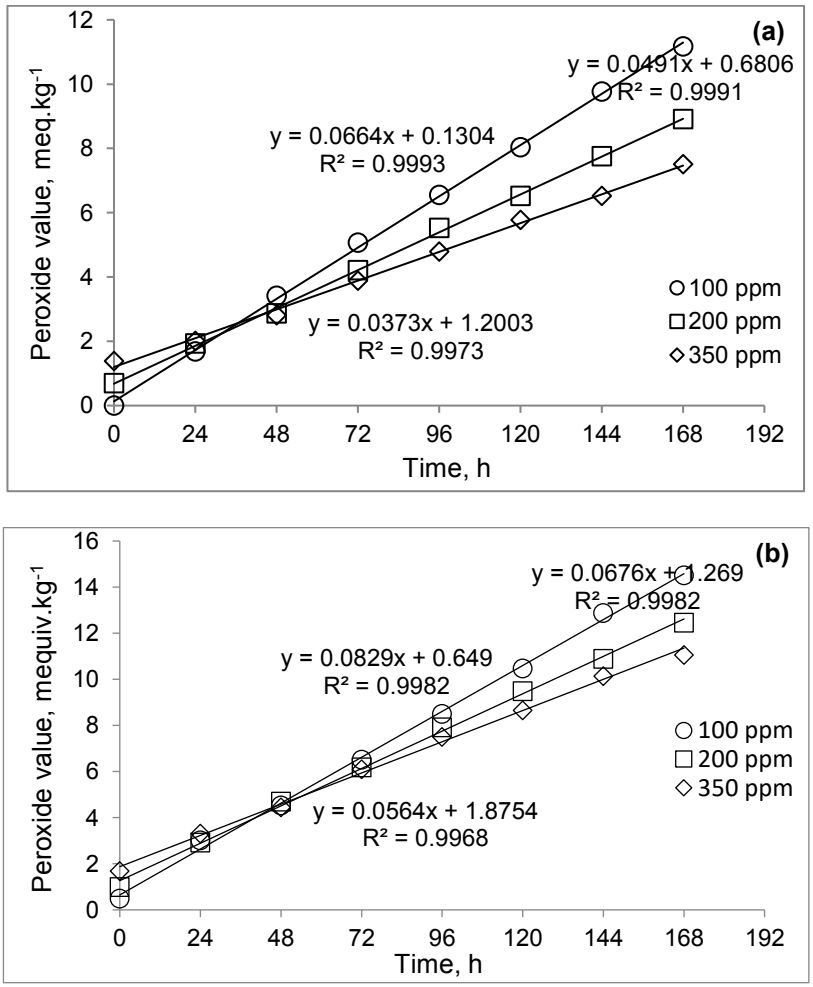


Figure 5 The zero-order kinetics of peroxide value increase rate in red and yellow palm olein mixture which contain (o) 100 ppm, (□) 200 ppm, and (◊) 350 ppm total carotene during photo-oxidation at light intensities of (a) 5,000 and (b) 10,000 lux.

CONCLUSION

The degradation of chlorophyll and tocopherols and an increase in PV were proportional to light intensity during photo-oxidation in red and yellow palm olein mixtures. The degradation rates of chlorophyll contents can be described as first-order reactions with *k*-values of 6 x 10^{-3}, 3.5 x 10^{-3}, 2.7 x $10^{-3}h^{-1}$, and 6 x 10^{-3}, 4 x 10^{-3}, 3.5 x $10^{-3}h^{-1}$, respectively in palm olein mixture, which contains 100, 200, and 350 ppm carotene at 5,000 and 10,000 lux light intensity exposure. The degradation rates of tocopherols contents can be described as first-order reaction with *k*-values of 1.5 x 10^{-2}, 9.0 x 10^{-3}, 7.4 x $10^{-3}h^{-1}$, and 1.9 x 10^{-2}, 9,7.0 x 10^{-3}, and 7.4 x $10^{-3}h^{-1}$, respectively in palm olein mixture, which contains 100, 200, and 350 ppm carotene at 5,000 and 10,000 lux light intensity exposure. Meanwhile, the increase rate of PV is described as a zero-order kinetics model with *k*-values of 6.6 x 10^{-2}, 4.9 x 10^{-2}, 3.7 x 10^{-2} mequiv.$kg^{-1}.h^{-1}$, and 8.3 x 10^{-2}, 6.8 x 10^{-2}, and 5.6 x 10^{-2} mequiv.$kg^{-1}.h^{-1}$ in palm olein mixture, which contains 100, 200, and 350 ppm carotene at 5,000 and 10,000 lux light intensity exposurel. The carotene content in the palm olein mixture showed no decrease at low light-intensity storage conditions. The carotene content at high concentrations might act as a singlet oxygen quencher, decrease tocopherol degradation, and eliminate the effect of differences in light intensity up to 10,000 lux. The palm olein mixture used as cooking oil should be stored in dark packaging to delay the rancidity and degradation of the carotene and tocopherol contents.

REFERENCES

1. Mba, O. I., Dumont, M. -J., & Ngadi, M. (2015). Palm oil: Processing, characterization and utilization in the food industry–A review. In Food Bioscience (Vol. 10, pp. 26–41). Elsevier BV. https://doi.org/10.1016/j.fbio.2015.01.003
2. Tan, C. H., Lee, C. J., Tan, S. N., Poon, D. T. S., Chong, C. Y. E., & Pui, L. P. (2021). Red palm oil: A review on processing, health benefits and its application in food. Journal of Oleo Science, 70(9), 1201–1210. United States National Library of Medicine. https://doi.org/10.5650/jos.ess21108
3. Usuki, R., Suzuki, T., Endo, Y., & Kaneda, T. (1984). Residual amounts of chlorophylls and pheophytins in refined edible oils. In Journal of the American Oil Chemists' Society (Vol. 61, Issue 4, pp. 785–788). Wiley. https://doi.org/10.1007/bf02672136
4. Loganathan, R., Subramaniam, K. M., Radhakrishnan, A. K., Choo, Y.-M., & Teng, K.-T. (2017). Health-promoting effects of red palm oil: evidence from animal and human studies. In Nutrition Reviews (Vol. 75, Issue 2, pp. 98–113). Oxford University Press (OUP). https://doi.org/10.1093/nutrit/nuw054
5. Dong, S., Xia, H., Wang, F., & Sun, G. (2017). The Effect of Red Palm Oil on Vitamin A Deficiency: A Meta-Analysis of Randomized Controlled Trials. In Nutrients (Vol. 9, Issue 12, p. 1281). MDPI AG. https://doi.org/10.3390/nu9121281
6. Al-Saqer, J. M., Sidhu, J. S., Al-Hooti, S. N., Al-Amiri, H. A., Al-Othman, A., Al-Haji, L., Ahmed, N., Mansour, I. B., & Minal, J. (2004). Developing functional foods using red palm olein. IV. Tocopherols and tocotrienols. In Food Chemistry (Vol. 85, Issue 4, pp. 579–583). Elsevier BV. https://doi.org/10.1016/j.foodchem.2003.08.003
7. Ayu, D. F., Andarwulan, N., Hariyadi, P., & Purnomo, E. H. (2016). Effect of tocopherols, tocotrienols, β-carotene, and chlorophyll on the photo-oxidative stability of red palm oil. In Food Science and Biotechnology (Vol. 25, Issue 2, pp. 401–407). Springer Science and Business Media LLC. https://doi.org/10.1007/s10068-016-0055-1
8. Choe, E., & Min, D. B. (2009). Mechanisms of Antioxidants in the Oxidation of Foods. In Comprehensive Reviews in Food Science and Food Safety (Vol. 8, Issue 4, pp. 345–358). Wiley. https://doi.org/10.1111/j.1541-4337.2009.00085.x
9. Ayu, D. F., Andarwulan, N., Hariyadi, P., & Purnomo, E. H. (2016b). Photodegradation kinetics of chlorophyll, tocopherol, and carotenoid in red palm oil. In Jurnal Agritech (Vol. 36, Issue 02, p. 117). Universitas Gadjah Mada. https://doi.org/10.22146/agritech.12855
10. Ayu, D. F., Andarwulan, N., Hariydi, P., & Purnomo, E. H. (2017). Photo-oxidative changes of red palm oil as affected by light intensity. In International Food Research Journal (Vol. 24, Issue 3, pp. 1270–1277). Universiti Putra Malaysia, Malaysia.
11. AOCS. (2003). AOCS Official Method Cd 8-53. Peroxide value acetic acid–chl oroform method. In Official Methods and Recommended Practices of the AOCS. (Issue 2003, pp. 1–2). The American Oil Chemists' Society, USA.
12. AOCS. (2017). AOCS Official Method Cc 13i-96. Determination of Chlorophyll Pigments in Crude Vegetable Oils. In Official Methods and Recommended Practices of the AOCS. The American Oil Chemists' Society, USA.

13. PORIM. (1995). Carotene content: methods of test for palm oil and palm oil products. In PORIM methods, Palm Oil Research Institute of Malaysia (pp. 43–44). Palm Oil Research Institute of Malaysia, Kuala Lumpur, Malaysia.
14. Wong, M. L., Timms, R. E., & Goh, E. M. (1988). Colorimetric determination of total tocopherols in palm oil, olein and stearin. In Journal of the American Oil Chemists' Society (Vol. 65, Issue 2). Wiley. https://doi.org/10.1007/bf02636412
15. Min, D. B., & Boff, J. M. (2002). Chemistry and Reaction of Singlet Oxygen in Foods. In Comprehensive Reviews in Food Science and Food Safety (Vol. 1, Issue 2, pp. 58–72). Wiley. https://doi.org/10.1111/j.1541-4337.2002.tb00007.x
16. Chen, B. H., & Huang, J. H. (1998). Degradation and isomerization of chlorophyll a and β-carotene as affected by various heating and illumination treatments. In Food Chemistry (Vol. 62, Issue 3, pp. 299–307). Elsevier BV. https://doi.org/10.1016/s0308-8146(97)00201-x
17. Jung, M. Y., & Min, D. B. (1991). Effects of quenching mechanisms of carotenoids on the photosensitized oxidation of soybean oil. In Journal of the American Oil Chemists' Society (Vol. 68, Issue 9, pp. 653–658). Wiley. https://doi.org/10.1007/bf02662288
18. Kim, H. J., & Min, D. B (2008). Chemistry of lipid oxidation. In Akoh, C. C., & Min, D. B. (eds.). Food Lipids, Chemistry, Nutrition, and Biotechnology. CRC Press. Inc., pp. 299–320.
19. Jørgensen, K., & Skibsted, L. H. (1990). Light sensitivity of carotenoids used as food colours. In Zeitschrift für Lebensmittel-Untersuchung und -Forschung (Vol. 190, Issue 4, pp. 306–313). Springer Science and Business Media LLC. https://doi.org/10.1007/bf01184498
20. Psomiadou, E., & Tsimidou, M. (2002). Stability of Virgin Olive Oil. 2. Photo-oxidation Studies. In Journal of Agricultural and Food Chemistry (Vol. 50, Issue 4, pp. 722–727). American Chemical Society (ACS). https://doi.org/10.1021/jf010847u
21. Wrona, M., Korytowski, W., Różanowska, M., Sarna, T., & Truscott, T. G. (2003). Cooperation of antioxidants in protection against photosensitized oxidation. In Free Radical Biology and Medicine (Vol. 35, Issue 10, pp. 1319–1329). Elsevier BV. https://doi.org/10.1016/j.freeradbiomed.2003.07.005
22. Zechmeister, L., & Polgár, A. (1944). cis—trans Isomerization and cis-Peak Effect in the α-Carotene Set and in Some Other Stereoisomeric Sets. In Journal of the American Chemical Society (Vol. 66, Issue 1, pp. 137–144). American Chemical Society (ACS). https://doi.org/10.1021/ja01229a039
23. Puspitasari-Nienaber, N. L. (2002). Stability of carotenoids in red palm oil and its effects in their bioavailability, provitamin A activity and toxicity [Doctoral dissertation, OSU, Columbus, Ohio].
24. Ayustaningwarno, F. (2010). Kinetika parameter stabilitas oksidasi minyak sawit merah. [Doctoral dissertation, IPB Graduate School, Bogor, Indonesia].
25. Sabliov, C. M., Fronczek, C., Astete, C. E., Khachaturyan, M., Khachatryan, L., & Leonardi, C. (2009). Effects of Temperature and UV Light on Degradation of α-Tocopherol in Free and Dissolved Form. In Journal of the American Oil Chemists' Society (Vol. 86, Issue 9). Wiley. https://doi.org/10.1007/s11746-009-1411-6
26. Badan Standarisasi Nasional Indonesia. (2013) SNI No 3741:2013. Minyak Goreng. Badan Standardisasi Nasional. Jakarta. Retrieved from http://sispk.bsn.go.id/SNI/DetailSNI/9013.
27. Rukmini, A., & Raharjo, S. (2010). Pattern of Peroxide Value Changes in Virgin Coconut Oil (VCO) Due to Photo-Oxidation Sensitized by Chlorophyll. In Journal of the American Oil Chemists' Society (Vol. 87, Issue 12, pp. 1407–1412). Wiley. https://doi.org/10.1007/s11746-010-1641-7
28. Choe, E., & Min, D. B. (2006). Mechanisms and Factors for Edible Oil Oxidation. In Comprehensive Reviews in Food Science and Food Safety (Vol. 5, Issue 4, pp. 169–186). Wiley. https://doi.org/10.1111/j.1541-4337.2006.00009.x

Funds:

The research was funded by an Innovation Research Grant of Riau University, Indonesia.

Acknowledgments:

We would like to thank Riau University for research funding and Emma Riftyan, S.TP. M.Sc.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Dewi Fortuna Ayu**, Riau University, Department of Agricultural Technology, Kampus Bina Widya Km 12.5 Simpang Baru Panam, Pekanbaru, 28293, Indonesia,
Tel.: +62 81398917607
E-mail: Dewi.fortuna@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0001-8573-1790

Andarini Diharmi, Riau University, Department of Fisheries Product Technology, Pekanbaru, 28293, Indonesia,
Tel.: +62 81288374901
E-mail: rini_abrar@yahoo.com
ORCID: https://orcid.org/000-0001-5765-1400

Netti Herawati, Riau University, Department of Agricultural Technology, Kampus Bina Widya Km 12.5 Simpang Baru Panam, Pekanbaru, 28293, Indonesia,
Tel.: +62 8129999313
E-mail: nettiherawati.paud@gmail.com
ORCID: https://orcid.org/000-0002-6673-8943

Rahmadini Payla Juarsa, Riau University, Department of Agricultural Technology, Kampus Bina Widya Km 12.5 Simpang Baru Panam, Pekanbaru, 28293, Indonesia,
Tel.: +62 81275929407
E-mail: rahmadinipayla@lecturer.unri.ac.id
ORCID: https://orcid.org/0000-0002-0875-2675

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 17.3.2024
Revised: 25.5.2024
Accepted: 3.6.2024
Published: 19.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 523-546
https://doi.org/10.5219/1972
ISSN: 1337-0960 online
www.potravinarstvo.com

Improving meat quality and safety: innovative strategies

Maksim Rebezov, Mars Khayrullin, Bahytkul Assenova, Smolnikova Farida, Dmitry Baydan, Larisa Garipova, Raisa Savkina, Svetlana Rodionova

ABSTRACT

Ensuring meat products' quality and safety is paramount in today's food industry. This extended abstract delves into innovative strategies to enhance meat quality and safety throughout the production, processing, and distribution stages. The paper explores various cutting-edge approaches, technologies, and regulatory frameworks to mitigate risks and improve consumer confidence in meat products. The discussion begins with examining advancements in meat processing techniques, such as high-pressure processing (HPP), modified atmosphere packaging (MAP), and irradiation. These techniques are instrumental in reducing microbial contamination, extending shelf life, and preserving the nutritional integrity of meat products. Furthermore, the paper explores emerging technologies like nanotechnology and blockchain, which offer novel solutions for enhancing traceability, transparency, and accountability in the meat supply chain. Much of the paper discusses the role of quality control measures in ensuring meat safety and compliance with food safety regulations. From carcass inspection and microbiological testing to chemical residue analysis and packaging standards, rigorous quality control protocols are essential for identifying and mitigating potential hazards at every stage of meat production. Moreover, the paper highlights the importance of animal husbandry practices, feed management, and genetics in influencing meat quality attributes such as flavour, texture, and tenderness. Producers can enhance meat products' overall quality and palatability by implementing improved animal husbandry practices, optimizing feed formulations, and selectively breeding animals for desirable traits. In addition to technological advancements and quality control measures, the paper emphasizes the need for regulatory compliance and government oversight to uphold food safety standards. Ensuring adherence to regulations such as Hazard Analysis and Critical Control Points (HACCP) and implementing comprehensive food safety management systems are essential for safeguarding public health and consumer trust. In conclusion, this extended abstract provides a comprehensive overview of innovative strategies for improving meat quality and safety in the food industry. By embracing advancements in processing techniques, leveraging new technologies, implementing stringent quality control measures, and adhering to regulatory requirements, stakeholders can enhance the safety, integrity, and consumer perception of meat products in the marketplace.

Keywords: meat, quality, safety, meat processing, innovative strategies,

INTRODUCTION

Meat quality and safety are crucial to the global food supply chain, impacting human health, economic sustainability, and societal well-being. Achieving superior meat quality involves a complex interplay of factors, from genetic traits of livestock breeds to post-harvest processing techniques **[1]**.

Ensuring meat safety requires vigilance against microbial pathogens, chemical contaminants, and other hazards that could jeopardise food integrity. Innovative strategies play a pivotal role in enhancing meat quality and safety **[2]**. Advancements in technology, genetics, and production methodologies are harnessed to address challenges and optimise meat quality and safety. Research explores various aspects

such as meat processing, quality assessment methods, and food safety regulatory frameworks **[3]**. The relationship between animal husbandry practices, nutrition, genetics, and environmental conditions in shaping the final meat product is also scrutinised. Emerging trends like novel processing techniques and cellular agriculture are under examination for their potential impact on the meat industry. Scientific inquiry aims to foster a comprehensive understanding of these developments, considering their promises and complexities. By embracing a spirit of inquiry and commitment to excellence, researchers strive to propel the field towards unprecedented levels of quality and safety in meat production **[3]**, **[4]**.

Understanding Meat Quality

Meat quality is a complex concept encompassing various sensory characteristics, nutritional composition, and technological properties **[5]**. Recent research has shed light on myosin heavy chain isoforms, significantly impacting meat quality attributes like pH, drip loss, and sensory properties **[5]**. Furthermore, natural antioxidants derived from sources like blackcurrant and honeysuckle extracts have been found to effectively delay lipid oxidation in meat products, preserving their quality without adverse effects on colour or texture. In evaluating meat quality, electronic technologies such as electronic nose (E-nose), eye (E-eye), and tongue (E-tongue) offer efficient and cost-effective methods **[6]**, **[7]**, **[8]**.

These technologies can distinguish between different preservation methods, detect adulteration, monitor processing conditions, and even identify pathogenic microorganisms in meat products. Additionally, studies in fish quality have highlighted the importance of desensitisation methods, where electric shock intensities can affect various quality parameters in fish meat during refrigerated storage. Sensory evaluation is crucial in determining consumer acceptance of meat products **[9]**. For example, research on sausages with varying proportions of fish meat demonstrated that consumer preference decreased with higher fish content, particularly impacting factors like consistency, appearance, and tenderness **[10]**. Comprehending the multifaceted aspects of meat quality is essential for ensuring consumer satisfaction, optimising dietary value, and maintaining consistency across diverse product lines. Ongoing research efforts continue to explore various factors influencing meat quality, aiming to enhance our understanding and appreciation of this crucial component of human nutrition and culinary culture

Factors Affecting Meat Quality

Pre-Harvest Management Practices: Diet composition and management practices significantly impact meat quality attributes, influencing fat deposition, muscle development, and overall carcass composition **[11]**.

Minimising stress during pre-slaughter procedures is essential for preserving meat quality. Post-Harvest Handling and Processing: Humane slaughter methods are imperative for ensuring meat quality, while controlled ageing enhances tenderness and flavour development **[12]**. Proper packaging techniques help preserve meat quality by minimising oxidative reactions and spoilage, and innovative processing techniques offer opportunities to optimise meat quality attributes. Environmental Factors: Environmental conditions, such as temperature and humidity, influence factors like muscle glycogen depletion, microbial growth, and enzymatic activity, thereby affecting meat quality. Seasonal variations also impact animal growth rates, carcass composition, and meat quality attributes. Consumer Preferences and Market Demands: Consumer perceptions of meat quality are influenced by cultural factors, while market demands drive innovation in production practices to meet evolving preferences. Understanding the intricate interplay of genetic, management, environmental, and market-related factors is essential for optimising meat quality to meet consumer expectations in a dynamic marketplace **[12]**.

Importance of Meat Quality for Consumers

Consumers derive immense pleasure from the sensory attributes of high-quality meat. The rich, savoury flavours, often accompanied by subtle hints of sweetness, umami, and aromatic compounds, elevate culinary pleasure **[13]**. Moreover, attributes like tenderness, juiciness, and succulence profoundly impact eating experiences, contributing to consumer satisfaction. Visual cues such as colour, marbling, and surface texture further indicate freshness and quality, reassuring discerning consumers

[13]. Beyond sensory enjoyment, meat also holds substantial nutritional value and contributes to overall health. As a vital source of essential amino acids, vitamins (such as B vitamins), and minerals crucial for optimal nutrition, meat plays a significant role in maintaining health and well-being **[14]**. The fat content and composition of meat are particularly noteworthy, influencing flavour, juiciness, and mouthfeel while balancing flavour enhancement with health considerations. Ensuring the safety and integrity of meat products is another critical aspect that resonates with consumers **[12]**.

They prioritize meat products that adhere to stringent safety standards and are free from contaminants and hazards. Transparency in production practices, animal welfare, and ethical considerations are also highly valued by consumers, highlighting the importance of traceability in the supply chain **[15]**. Moreover, meat holds cultural significance and contributes to culinary satisfaction beyond its nutritional and sensory attributes. High-quality meat offers culinary versatility, allowing consumers to explore various cooking methods and unleash creativity. Additionally, meat plays a central role in cultural traditions and social gatherings across diverse societies, further accentuating its importance in the culinary landscape **[16]**. Understanding and meeting consumer preferences for high-quality meat products are paramount for stakeholders in the food supply chain. By prioritizing meat quality and addressing consumer expectations regarding sensory enjoyment, nutritional value, safety, and cultural significance, stakeholders can establish trust, loyalty, and satisfaction in an increasingly discerning market environment. Factors influencing meat purchasing decisions encompass various aspects, including quality control measures implemented by supermarkets and the accessibility of specific meat types across different locations **[17]**.

Consumers often rely on indicators such as colour and price to gauge meat quality, while freshness remains a critical consideration for consumers and meat handlers. Key indicators of meat safety include expiry dates, colour changes, and aroma, with consumer awareness influenced by educational status. Combining different types of meat or meat alternatives, hybrid meat products are increasingly evaluated by consumers based on ethical considerations, sustainability, taste, ingredients, healthiness, naturalness, innovation, and environmental impact **[18]**. Positive perceptions of hybrid products increase after consumers engage in co-creation activities. Ethical, moral, and social dimensions in farm production practices play a significant role in consumer perceptions of meat quality. For instance, Irish consumers exhibit varying perceptions of meat quality linked to farm-level practices such as organic farming and high animal welfare standards. These perceptions vary across consumer segments, influenced by gender, age, and motivations for meat purchase. Additionally, the choice of frying method can significantly impact the cooking yield, tenderness, and sensory properties of meat, such as chicken breast. Research indicates that air frying methods result in higher cooking yield and tenderness compared to traditional deep fat frying methods, highlighting the importance of cooking techniques in maintaining meat quality **[12]**.

Microbial Contamination in Meat: Risks, Sources, and Mitigation Strategies

The search results provide valuable insights into microbial contamination within the meat industry, highlighting various aspects and implications:

Reduction of Microbial Load in Meat Maturation Rooms: This study investigated the survival of pathogens such as Listeria monocytogenes, Escherichia coli, Salmonella spp., and Staphylococcus aureus on the inner surface of dry ageing chambers. It assessed the efficacy of alkaline electrolyzed water in reducing foodborne pathogens during meat storage, emphasizing the importance of implementing effective sanitation measures in meat processing facilities **[19]**. Microbial Contamination in Meat Industry: Research focused on assessing the prevalence of food infections and microbial contamination in meat products. The study evaluated microbial quality based on Iranian national standards by examining sausages, burgers, kebabs, and cutlets from factories, highlighting the need for stringent hygiene practices and quality control measures in meat production processes **[20]**. Post-slaughter Handling and Meat Quality: A study conducted in South Africa explored the perceptions and knowledge of consumers and meat handlers regarding meat quality and safety throughout the distribution chain. It identified factors influencing perceptions of meat quality, safety indicators, and consumer

preferences, emphasizing the importance of education and training in ensuring proper handling practices to minimize microbial contamination and maintain meat quality **[17]**.

Chemical Hazards in Meat: Sources, Risks, and Management Strategies

The search results provide valuable insights into chemical hazards in the food industry, particularly concerning meat products. Studies have examined various aspects related to food safety and quality:

Research conducted in the Eastern Cape Province, South Africa, investigated consumer and meat handlers' perceptions and knowledge regarding meat quality and safety throughout the distribution chain. The findings highlighted factors influencing perceptions of meat quality, safety indicators, and consumer preferences **[21]**. Similarly, a study in Saudi Arabia aimed to determine microbial contamination in imported fish and chicken products. The research analyzed parameters such as total plate count, Staphylococcus aureus, Vibrio parahaemolyticus, and Pseudomonas in samples, assessing their potential impact on food industry production workers. Moreover, research focused on determining the survival of pathogens like Listeria monocytogenes, Escherichia coli, Salmonella spp., and Staphylococcus aureus on the inner surface of dry ageing chambers **[22]**. In another study, researcher tested the efficacy of alkaline electrolyzed water for reducing foodborne pathogens during meat storage. These studies underscore the importance of addressing microbial and chemical hazards in the meat industry. Effective mitigation strategies are crucial to ensuring food safety, maintaining quality standards, and safeguarding consumer health **[19]**.

Ensuring Compliance with Food Safety Regulations in the Meat Industry

Regulatory compliance is the cornerstone of safety in the meat industry, safeguarding public health and maintaining consumer confidence. Stakeholders must understand and implement national and international standards, such as the Food Safety Modernization Act (FSMA) and the European Union's General Food Law, set by organizations like the Codex Alimentarius Commission. Regulatory authorities like the FDA and EFSA enforce standards, conduct inspections, and sanction non-compliance. Good Manufacturing Practices (GMPs) establish facility, equipment, and processes hygiene standards. Hazard Analysis and Critical Control Points (HACCP) principles identify and mitigate hazards **[23]**, **[24]**, **[25]**. The basic requirements for the HACCP system are based on the legal regulations in force in the country. They are applied in all branches of the food industry **[138]**, **[139]**, as well as the ISO 22000 standard and various private standards for food safety and quality recognized by the Global Food Safety Initiative such as IFS, BRC, PRIMUS GFS, RED MEAT, GLOBAL GAP, SQF, FSSC 22000 etc.

Traceability systems and recall procedures ensure swift responses to safety concerns. Mandatory labelling provides essential information to consumers. Implementation strategies include comprehensive education and training, thorough documentation, and continuous improvement of safety management systems. Collaboration among stakeholders fosters transparency and collective responsibility. Audits, both internal and external, assess compliance and identify areas for improvement. External inspections ensure accountability through enforcement measures. Maintaining compliance with food safety regulations demands commitment and collaboration from all stakeholders. By adhering to standards, implementing robust management systems, and embracing continuous improvement, the meat industry can ensure the safety and quality of its products, thus safeguarding public health and consumer trust **[26]**, **[27]**.

Innovative Technologies Transforming Meat Processing

The search results offer valuable insights into innovative technologies and food safety considerations within the meat industry. One study discusses the safety assessment of cell-cultured meat and seafood, highlighting the importance of a responsible, data-driven approach to ensure consumer acceptance and safe commercialization **[28]**. Another research explores the feasibility of pulsed light technology for surface decontamination of meat and meat contact materials, assessing its effects on microbial inactivation, lipid peroxidation, sensory quality, and colour of meat products. provide the most **[29]**, **[30]**. Additionally, a study investigates the efficacy of alkaline electrolyzed water for reducing foodborne

pathogens during meat storage in dry ageing chambers. These studies collectively underscore ongoing efforts to enhance efficiency, quality, and safety standards in the meat processing industry by leveraging innovative technologies and advanced food safety practices. Incorporating such approaches is crucial for improving food safety, meeting regulatory requirements, and fostering consumer confidence in meat products **[19]**.

High-Pressure Processing (HPP) in Meat Industry: Principles, Applications, and Benefits

High-pressure processing (HPP) is a non-thermal technique involving subjecting packaged food items to pressures between 100 and 600 megapascals within a hydraulic pressurization chamber. This process disrupts the cellular structures and enzymatic activities of microorganisms like pathogenic bacteria, viruses, and yeasts, rendering them inactive and ensuring food safety. Applications of HPP in meat processing are diverse **[31]**. It effectively reduces microbial contamination in raw and processed meat products, mitigating the risk of foodborne illnesses caused by pathogens such as Salmonella, E. coli, Listeria monocytogenes, and Campylobacter **[19]**. Additionally, HPP extends the shelf life of meat products by inhibiting spoilage microorganisms, enzymatic reactions, and oxidative degradation processes, thereby preventing quality deterioration, off-flavours, and discolouration. Furthermore, HPP enables clean label claims by eliminating the need for chemical preservatives or additives, meeting consumer demand for minimally processed natural foods **[32]**. The benefits of HPP adoption in meat processing are substantial. It significantly reduces pathogen levels without compromising product quality, ensuring consumer safety. Additionally, HPP preserves the nutritional quality of meat products by minimizing nutrient degradation and protein denaturation associated with thermal processing methods **[33]**. Moreover, HPP maintains meat products' natural colour, texture, and flavour, enhancing sensory attributes and consumer acceptance. It also extends the refrigerated shelf life of meat products, aiding inventory management and meeting market demand for fresh, high-quality products. However, equipment costs, operational complexity, and consumer education may hinder the widespread adoption of HPP in the meat industry. Continued innovation in HPP technology through advancements in equipment design, process optimization, and packaging solutions is expected to drive integration into meat processing facilities and expand applications. Regulatory agencies play a crucial role in establishing guidelines for HPP implementation to ensure compliance with food safety regulations and address consumer concerns regarding labelling and product claims **[34]**.

Irradiation Techniques in Meat Processing: Principles, Applications, and Considerations

Irradiation techniques are a valuable asset in the meat processing industry, providing effective solutions for bolstering food safety, prolonging shelf life, and diminishing microbial contamination in meat products. These techniques expose food items to ionizing radiation emitted by gamma rays, electron beams, or X-rays **[35]**. This radiation interacts with the molecular structure of microorganisms, rendering them incapable of reproduction or causing foodborne illnesses **[36]**. The irradiation applications in meat processing are diverse, encompassing pathogen reduction, shelf-life extension, and quarantine treatment for imported meat products **[37]**. Additionally, irradiation eradicates quarantine pests like insects and larvae while upholding product quality and safety standards **[38]**. Despite its benefits, irradiation comes with several considerations and challenges, including regulatory approval, consumer acceptance, proper packaging and handling, and cost considerations.

Nanotechnology Applications in Meat Processing: Innovations, Benefits, and Considerations

Nanotechnology presents various applications within the meat processing industry, offering innovative solutions to enhance food safety, quality, and sustainability. Through the integration of nanomaterials, significant advancements have been made in various areas:

Nanomaterials for Packaging: Active nanocomposites, comprising nanoparticles and nanofibers, are utilized to inhibit microbial growth, scavenge oxygen, and extend the shelf life of meat products.

Antimicrobial nanoparticles like silver and zinc oxide embedded in packaging materials prevent spoilage and reduce the risk of foodborne pathogens **[39]**. Additionally, barrier nanocoatings enhance packaging surfaces' properties, improving stability and freshness **[40]**. Nanosensors for Quality Monitoring: Nanobiosensors offer real-time detection of biochemical markers, pathogens, and spoilage indicators in meat products. Gas nanosensors rapidly assess product freshness and safety by detecting gases released during spoilage **[41]**. Nanoelectronic noses equipped with nanomaterial-based sensors aid in quality control by detecting volatile odour compounds emitted by meat products **[42]**.

Nanoparticle-Based Interventions: Antimicrobial nanoparticles such as silver, copper, and zinc oxide exhibit potent properties against foodborne pathogens and spoilage microorganisms. Nanoliposomes enhance the stability and efficacy of antimicrobial agents for targeted delivery in meat products **[43]**. Nanoscale emulsions improve the dispersion and functionality of bioactive compounds in meat formulations **[44]**. Nanostructured Additives for Quality Enhancement: Nanoencapsulated nutrients are incorporated into meat products to enhance nutritional value and sensory attributes **[45]**.

Nanofibrous scaffolds carry bioactive compounds, improving texture, juiciness, and shelf life. Nanocomposite coatings applied to meat surfaces create protective barriers, preventing moisture loss and enhancing texture and appearance **[45]**. Regulatory and Safety Considerations: The safety evaluation of nanomaterials is crucial for assessing their physicochemical properties, toxicity profiles, and potential impacts on human health and the environment. Regulatory compliance by agencies such as the FDA and EFSA ensures safety standards are met for nanotechnology-based food additives in meat processing. Addressing consumer perception through transparency, communication, and education is vital to mitigate concerns regarding the safety and benefits of nanotechnology applications in meat processing **[46]**.

Improving Meat Quality Through Animal Husbandry Practices

The search results provide insights into animal husbandry practices and meat quality. Studies conducted in regions like the north-western Himalayan region of India highlight issues such as open slaughtering, inadequate water provisions, and lack of light in lairage premises, impacting animal welfare and meat quality **[47]**. Research in Mongolia emphasizes the importance of hygienic practices in slaughter establishments to ensure the microbiological quality of meat products and prevent foodborne pathogens **[48]**. Additionally, nanotechnology offers potential benefits in meat processing, including improved bioavailability, antimicrobial effects, enhanced sensory acceptance, and targeted delivery of bioactive compounds **[49]**. However, challenges exist in ingredient production, stability of delivery systems, health risks, public acceptance, economics, and regulation. These findings underscore the significance of implementing proper animal welfare practices hygienic standards in slaughterhouses and exploring innovative technologies like nanotechnology to enhance meat quality and safety in the meat processing industry **[49]**.

Genetics and breeding for desirable meat traits

Various research findings reveal that Genetics and breeding play a crucial role in developing desirable meat traits in livestock. One study examined 26 different meat cuts, meat quality, and carcass traits in pigs, emphasizing the significant impact of genetic factors on the overall quality of meat produced **[50]**. Moreover, advancements in modern technologies such as advanced sequencing and genome editing are reshaping livestock genetic and breeding programs, specifically focusing on enhancing meat quality traits across different animal species **[51]**. Research into growth traits in meat pigeons has identified key mutations associated with muscle mass, offering valuable insights for selective breeding practices to improve the quality of meat produced **[52]**.

Similarly, studies on turkeys have explored genetic parameters for meat quality traits, including white striping, underscoring the importance of integrating considerations of meat quality into poultry breeding strategies to ensure the production of high-quality meat products **[53]**. Additionally, an investigation into Nellore cattle has assessed genomic prediction for carcass and meat quality traits using advanced algorithms, demonstrating promising results in terms of prediction accuracy **[54]**.

Integrating Animal Welfare Considerations into Meat Production Systems

The search results offer valuable insights into the integration of animal welfare considerations within meat production systems:

A study introduces a tool designed to benchmark animal welfare across countries, specifically on pigs and chickens. This tool evaluates parameters related to housing and management practices, aiming to facilitate the monitoring and comparison of animal welfare standards on an international scale **[55]**. In developed countries, animal welfare significantly influences beef production. Researchers explore various indicators for assessing animal welfare and its impact on meat quality. They also discuss potential strategies for improving beef production in developing countries by raising animal welfare standards. Farmers in New Jersey advocate for local food systems to address ethical concerns surrounding farm animal welfare **[56]**, **[57]**, **[58]**, **[59]**.

They emphasize the importance of maintaining happy animals in small-scale farming operations, highlighting local food systems as an organic solution to ensure the well-being of livestock. A review examines the role of reproductive hormones in cattle production and their effects on economically important traits, animal welfare, and human health. The review underscores the importance of considering animal welfare and consumer health when implementing hormone usage in beef and dairy cattle production **[60]**.

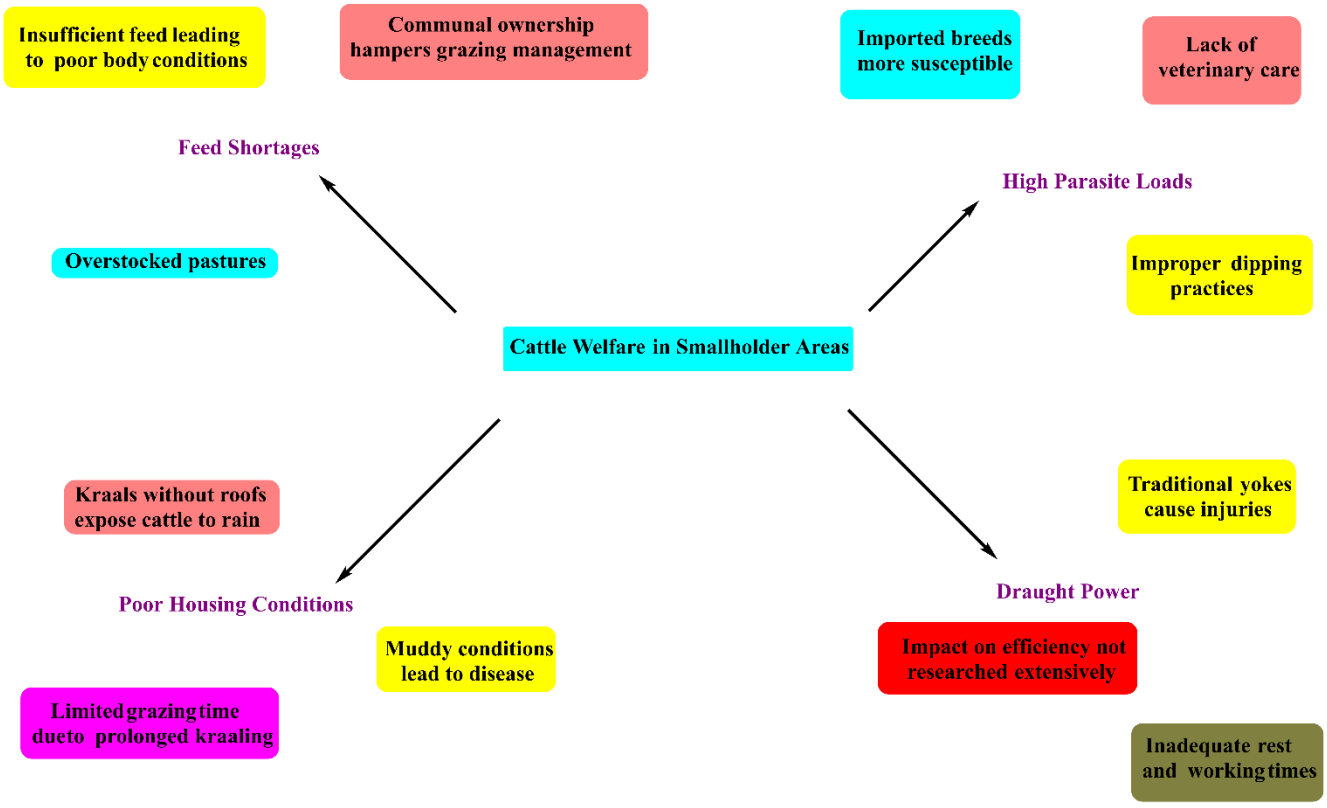


Figure 1. Factors Affecting Cattle Welfare in Smallholder Areas.

Furthermore, cell-cultured meat technology is explored from a bioethical standpoint, discussing its potential benefits for sustainable development, animal welfare improvement, and resource demand reduction. However, ethical risks related to food safety, technology misuse, and regulatory oversight are also addressed. Collectively, these studies emphasize the imperative of incorporating animal welfare considerations into meat production systems. By prioritizing ethical and sustainable practices, the meat industry can ensure the well-being of livestock and promote responsible production processes **[61]**. Figure 1 illustrates key challenges impacting cattle welfare in smallholder farming systems.

Quality Control and Assurance in Meat Production: Ensuring Safety, Consistency, and Compliance

The insights gleaned from various studies shed light on different dimensions of quality control and assurance in meat production:

One study explores the potential benefits of cell-cultured meat technology for the meat production industry, consumer groups, and sustainable development. It outlines how this technology could improve animal welfare, reduce resource demand, enhance nutritional function, and stimulate growth in other industries. However, the study also underscores ethical risks related to food safety, technology misuse, and the need for effective technical supervision **[62]**. Another discussion revolves around a tool designed to benchmark animal welfare across countries, specifically on pigs and chickens. This tool defines parameters related to housing and management to assess welfare levels, with careful consideration given to ethical concerns during the model-building process **[55]**. Researchers explore how animal welfare impacts meat quality and seek to enhance beef production in developing nations by prioritizing animal welfare considerations **[63]**. Factors influencing meat quality traits, such as intramuscular fat content, are discussed, along with the necessity for further research on adipogenesis to enhance meat quality **[64]**.

Furthermore, another study estimates genetic parameters for meat quality traits in turkeys, including white striping severity score. The study discusses heritability estimates for various characteristics and highlights unfavourable genetic correlations between body weight and meat quality. It stresses the importance of implementing selection strategies to improve meat quality **[53]**, **[65]**.

Quality Control Measures in Meat Processing

Quality control is paramount in meat processing to ensure compliance with safety, quality, and regulatory standards at each production stage. This involves rigorous measures throughout the process:

In the slaughtering and dressing phase, trained inspectors meticulously examine live animals for any indications of disease or abnormalities. Subsequently, veterinarians conduct thorough post-mortem inspections of carcasses to ensure meat quality and safety **[66]**.

During carcass fabrication and primal cutting, inspectors meticulously assess carcasses and cuts for cleanliness, contamination, and any defects that may compromise quality. Maintaining appropriate temperatures and following strict sanitation practices are essential for this stage. Further processing and value-added production necessitate strict control over ingredient sourcing and handling to prevent contamination **[67]**. Formulations are also closely monitored to ensure consistency in flavour and nutritional content. Packaging and labelling undergo stringent inspection to prevent contamination and ensure compliance with accuracy and regulatory standards. This includes thorough verification of labels to confirm regulatory compliance and accuracy **[68]**. Inspection of finished products encompasses a comprehensive evaluation, including visual examination, microbiological testing, and metal detection, to guarantee the highest quality and safety standards. During storage and distribution, temperature monitoring devices are utilized to maintain the integrity of the cold chain. Furthermore, periodic shelf-life testing is conducted to assess product stability, while robust traceability systems are implemented to track product movement effectively **[69]**, **[70]**, **[71]**.

Implementing HACCP (Hazard Analysis and Critical Control Points)

Implementing Hazard Analysis and Critical Control Points (HACCP) in meat processing is a systematic approach designed to ensure the production of safe meat and poultry products. This approach involves a thorough analysis of production processes, identification of potential hazards, determination of critical control points where these hazards can be controlled, establishment of critical limits, verification of the steps prescribed, and monitoring of the process control through the HACCP plan [24]. The effectiveness of HACCP systems in managing food safety risks has been demonstrated, provided they are correctly implemented. Many countries have adopted HACCP principles for meat processing at abattoirs, with regulatory bodies utilizing systems like the Hygiene Management System (HMS) and Hygiene Assessment System (HAS) to audit compliance **[72]**. The implementation of HACCP in the meat and poultry industry is crucial for enhancing consumer confidence in these products and reducing

barriers in international trade. Comprehensive flow diagrams of meat and poultry products applying HACCP principles ensure food safety **[73]**. However, challenges remain, such as in Uganda, where low food safety performance in the beef supply chain was attributed to poor sanitation, hygiene, and handling practices. This underscores the need for HACCP-based training and robust preventive, intervention, and monitoring systems **[74]**.

Moreover, implementing HACCP can impact costs and product offerings, with small and very small meat processors incurring higher compliance costs and potentially discontinuing a range of products due to the need for facility modification **[75]**. Continuous training and education on HACCP are essential for ensuring a stable system and maintaining hygiene during the production process **[76]**.

Critical Control Points (CCPs) are then determined, pinpointing specific points where control measures can prevent, eliminate, or reduce hazards. Factors such as temperature, time, pH, moisture levels, and sanitation are considered in this assessment. Critical limits define measurable criteria for acceptable control levels to ensure food safety **[77]**. These limits are based on scientific data, regulations, standards, and product specifications. Monitoring procedures are developed to verify CCP control and adherence to critical limits, specifying methods, frequency, responsible personnel, and documentation **[77]**. In the event of monitoring indicating loss of control or breaches in critical limits, corrective actions are implemented. These actions involve identifying the causes of deviations, taking corrective measures, and implementing preventive actions to avoid recurrence. Verification procedures are established to confirm the effectiveness of the HACCP plan in controlling hazards, including equipment calibration and internal audit **[78]**.

Emerging technologies for real-time quality monitoring

Blockchain technology is valuable for promoting traceability systems within agri-food supply chains. Its decentralized, immutable, and transparent nature enables real-time data monitoring and decision-making activities across food production and supply chains **[79]**. Implementing a Hazard Analysis and Critical Control Points (HACCP) program is crucial for small-scale cured ham production. This involves identifying potential hazards, determining critical control points (CCPs), and implementing control measures at key stages of production. These insights underscore the significance of advanced technologies, early warning systems, traceability solutions, and quality control measures in ensuring food safety, quality assurance, and operational efficiency in the meat processing industry **[80]**, **[81]**.

Enhancing Meat Flavour and Texture

Marination and flavour infusion methods

Postmortem ageing is vital for enhancing meat quality by leveraging endogenous proteolytic systems. The concept of "smart ageing" introduces a novel approach to optimize post-harvest ageing parameters, aiming to improve the quality and value of meat products. Metabolomics techniques play a crucial role in elucidating the biochemical mechanisms responsible for tenderness, flavour, colour, and oxidative stability during the ageing process **[82]**, **[83]**.

Combining different processing and preservation methods offers the potential for enhancing sensory attributes without compromising nutritional value. This underscores the importance of further research in exploring the synergies between different processing technologies. Enzymatic reactions, such as glycolysis, proteolysis, and lipolysis, are integral to converting muscle into meat and improving its quality. Exogenous enzymes find applications in tenderization, bioactive peptide production, and meat product restructuring **[84]**.

Enzymatic reactions are crucial in meat processing, influencing tenderness, aroma, and color. Exogenous enzymes such as papain, bromelain, and microbial enzymes are utilized for tenderization. Emerging technologies like ultrasound, pulsed electric fields (PEF), and high-pressure processing (HPP) intensify these reactions, enhancing meat quality. High-intensity ultrasound (HIUS) and high-pressure processing (HPP) are non-thermal methods employed for meat tenderization. Muscle composition affects the efficacy of HIUS, with certain muscle types being more resistant to tenderization. Optimal pressure levels and durations of HPP can enhance tenderization, though industrial implementation requires further optimization **[84]**, **[85]**.

Various processing techniques like dry ageing, HPP, sous-vide cooking, and 3D printing impact meat products' sensory and nutritional attributes. Combining different processing methods can improve sensory quality without compromising nutritional value, necessitating continued research in this field. The rise of plant-based meat alternatives has spurred the development of non-thermal technologies like HPP to modify plant proteins, mimicking traditional meat products' functionality and nutritional characteristics **[85]**. Protein modification is critical for achieving desired attributes such as solubility, gelling, emulsifying, and foaming properties in plant-based alternatives. High-power ultrasound (HPUS) is a non-thermal technology to modify food proteins, enhancing safety and quality by altering protein conformation and structure. In meat processing, HPUS can improve myofibrillar protein structure, enhancing functional properties and extending shelf life. These insights underscore the significance of enzymatic reactions, non-thermal technologies like HIUS and HPP, processing techniques' effects on meat quality, advancements in plant-based alternatives using emerging technologies, and the role of HPUS in modifying food proteins for improved functionality in meat processing **[85]**.

Carcass Inspection and Processing

Carcass inspection and processing represent critical stages in the meat production chain, where the quality and safety of meat products are meticulously upheld. Ante-mortem inspection involves assessing animals for signs of disease or other conditions that might affect meat quality **[86]**. In contrast, post-mortem inspection examines carcasses for abnormalities or defects immediately after slaughter. Following the inspection, carcasses undergo rapid chilling to inhibit bacterial growth and facilitate ageing, enhancing tenderness and flavour **[86]**.

Hygiene and Sanitation Practices

Temperature control and cold chain management are crucial aspects of ensuring the safety and quality of meat products within the industry. Following slaughter and processing, carcasses are promptly chilled to inhibit bacterial growth, with processing facilities maintaining strict temperature controls to prevent contamination **[87]**. During storage and warehousing, meat products are kept refrigerated to preserve freshness, with temperature monitoring systems to ensure safe storage conditions. Transportation of meat products relies on refrigerated vehicles, known as "reefers," to maintain consistent temperatures during transit **[87]**. Cold chain monitoring and quality assurance practices involve regular temperature checks, record-keeping, and audits to monitor cold chain integrity **[88]**. By implementing robust temperature control and cold chain management practices, the meat industry can minimize the risk of foodborne illnesses, maintain product quality, and meet regulatory requirements, thereby ensuring consumer health and preserving industry reputation **[89]**.

Microbiological Testing and Monitoring

Microbiological testing and monitoring are integral processes in ensuring the safety and quality of meat products within the industry **[90]**. Pathogen detection methods, including culture-based techniques, molecular methods like PCR, and rapid detection systems such as ELISA, enable the identification of harmful bacteria like Salmonella, E. coli, Listeria monocytogenes, and Campylobacter spp **[91]**. Additionally, testing monitors spoilage microorganisms like lactic acid bacteria, yeasts, and moulds, with elevated levels indicating poor handling or storage conditions leading to quality defects **[90]**, **[92]**. Environmental monitoring involves testing surfaces, equipment, and air samples for microorganisms using swabbing, air sampling, and contact plates to evaluate cleanliness and identify contamination sources. Validation confirms the effectiveness of control measures such as temperature control and sanitation procedures, while verification involves ongoing monitoring to ensure consistent implementation **[93]**. By conducting microbiological testing and monitoring, meat producers can effectively identify and mitigate microbial risks, comply with food safety regulations, and uphold the safety and quality of meat products, thereby enhancing consumer confidence in the industry **[94]**.

Chemical Residue Analysis and Control

Chemical residue analysis and control are pivotal in the meat industry to guarantee product safety. Regulatory agencies establish Maximum Residue Limits for chemicals in food production to safeguard human health, necessitating stringent monitoring and control measures to ensure compliance **[95]**. Veterinary drugs such as antibiotics and hormones can leave residues in meat, prompting producers to adhere to strict protocols, including withdrawal periods and monitoring, to prevent residues. Similarly, pesticides utilized in crop production or pest control may accumulate in meat, requiring monitoring programs to detect residues and ensure conformity with standards **[96]**. Environmental contaminants like heavy metals and industrial chemicals can infiltrate meat through various channels, prompting monitoring programs to evaluate contamination levels and implement prevention strategies **[96]**. Analytical methods such as chromatography and mass spectrometry are employed to detect and quantify residues, with laboratories utilizing validated methods for accurate results, often employing rapid screening followed by confirmatory analysis **[97]**. Additionally, risk assessment evaluates health risks posed by chemical residues, while risk management strategies, such as setting MRLs and enforcing compliance, mitigate risks and safeguard public health **[98]**. Through comprehensive chemical residue analysis and control programs, meat producers uphold product safety, regulatory compliance, and consumer confidence, with ongoing monitoring, risk assessment, and management constituting vital components of effective residue control strategies **[98]**.

Packaging and Labeling Standards

Transportation and distribution are pivotal in maintaining meat products' safety and quality **[99]**. Maintaining cleanliness is crucial to prevent cross-contamination, necessitating regular cleaning, sanitation, and employee training on hygiene practices. provide references in all sides of this query **[100]**, **[101]**, **[102]**, **[103]**. Proper handling practices, including secure storage in designated areas of vehicles, prevent damage to meat products throughout loading, unloading, and transit **[104]**. Traceability systems and accompanying documentation track product movement, ensuring transparency, accountability, and regulatory compliance from production to retail **[105]**. Adherence to regulatory standards, covering temperature control, sanitation, packaging, labelling, and documentation, is paramount to ensure product safety during transit and regulatory adherence **[105]**, **[106]**, **[107]**. These best practices collectively uphold product safety, quality, and freshness throughout the supply chain, fostering consumer confidence and regulatory compliance within the meat industry.

Novel Strategies for Enhancing Meat Quality

Innovations in meat production have led to the adoption of various natural preservatives aimed at extending shelf life while maintaining sensory attributes **[108]**, **[109]**, **[110]**. Plant-derived antimicrobial compounds, such as essential oils and organic acids, are increasingly used to enhance the safety and longevity of meat products **[108]**, **[111]**, **[112]**. These compounds prevent microbial growth and impart subtle flavours, enhancing the overall taste profile. Similarly, natural antioxidants like vitamin E and rosemary extract prevent lipid oxidation and rancidity, preserving the meat's natural flavour and freshness **[111]**, **[113]**, **[114]**. Emerging technologies, including edible coatings and antimicrobial films, offer additional avenues for enhancing meat quality and safety while meeting consumer preferences for clean-label products **[115]**. Likewise, natural antioxidants preserve the texture by preventing lipid oxidation, thus ensuring that the meat remains succulent and retains its desired mouthfeel **[111]**, **[116]**, **[117]**. Integrating natural preservatives into meat production not only addresses safety concerns and extends shelf life but also plays a crucial role in maintaining the sensory attributes that influence taste and texture. By leveraging these innovative approaches, the meat industry continues to evolve, meeting consumer demands for both quality and sustainability

Smart Packaging Technologies

Active packaging systems play a critical role in the meat industry by employing various technologies to enhance meat safety and extend shelf life. One such system is antimicrobial packaging, which integrates antimicrobial agents like bacteriocins, essential oils, or organic acids into packaging materials.

These agents effectively inhibit microbial growth, improving product safety and reducing the risk of contamination **[118]**. Additionally, oxygen scavenging packaging utilizes oxygen scavengers in packaging films to remove oxygen from the package, preventing oxidative degradation of lipids and proteins in meat products and preserving their quality over time **[119]**.

Ethylene absorption packaging is another important technology utilized in the meat industry, where ethylene absorbers are employed to eliminate ethylene gas produced by ripening fruits near meat products. By removing ethylene, which accelerates ripening and senescence, this packaging technology extends the shelf life of meat products, ensuring they remain fresh longer **[120]**. Active modified atmosphere packaging (MAP) combines modified atmosphere packaging with active components such as oxygen scavengers or carbon dioxide emitters. Creating a controlled atmosphere within the package inhibits microbial growth, preserving product quality longer **[121]**. These active packaging systems collectively offer proactive measures against microbial contamination **[122]**, **[123]**. By integrating active components into packaging materials, the meat industry can enhance product safety, extend shelf life **[124]**, **[125]**, and meet consumer expectations **[124]** for fresher and safer meat products.

Nanotechnology applications in food safety

Nanotechnology is crucial in enhancing food safety by developing nanoscale antimicrobial agents. These agents, such as zinc oxide nanoparticles, exhibit potent antimicrobial properties against various pathogenic microorganisms commonly associated with foodborne illnesses **[126]**.

They work by disrupting microbial cell membranes **[127]**, aggravate microbial cell membrane damage after interaction with lipopolysaccharide **[128]**, rupture and leakage of cell contents **[129]** ultimately leading to microbial death. Nanoscale antimicrobial agents are increasingly incorporated into food packaging materials to create active packaging systems that help inhibit microbial growth, extend shelf life **[123]**, and enhance food safety **[130]**, **[131]**. These antimicrobial packaging materials release nanoparticles into the food environment, where they exert their antimicrobial effects, reducing the risk of contamination and spoilage **[123]**.

Nanobiosensors for Pathogen Detection

Nanobiosensors enhance food safety, particularly in detecting foodborne pathogens in meat products. These sensors operate based on nanotechnology principles, typically comprising nanomaterials functionalized with biological recognition elements **[131]**. These elements interact with target pathogens, generating a detectable signal for quantification. One of the key advantages of nano biosensors is their high sensitivity and specificity for pathogen detection in meat products. This enables rapid and accurate identification of various microorganisms, contributing to improved safety standards **[131]**. Moreover, nano biosensors facilitate rapid detection, with response times ranging from minutes to hours, allowing for real-time monitoring during processing, storage, and distribution. Another noteworthy feature is their multiplex detection capability, which enables simultaneous screening for multiple pathogens **[132]**. This streamlines the screening process for various contaminants in meat products, enhancing efficiency and reducing processing time. Additionally, nano biosensors are well-suited for point-of-care applications, making them ideal for on-site pathogen detection in meat processing facilities and retail settings **[133]**.

Blockchain Technology for Supply Chain Transparency

Blockchain technology enhances transparency and traceability in the meat industry's supply chain. One key aspect is its decentralized ledger system, distributed across multiple nodes, ensuring transparency and visibility into the movement of meat products **[134]**. Another significant advantage of blockchain in the meat industry is its ability to provide end-to-end traceability. Stakeholders can track meat products from farm to fork, accessing detailed information on production practices and quality attributes **[134]**. This traceability level allows for better real-time monitoring of product status, including factors like location, temperature, and humidity, which facilitates proactive decision-making for product quality and safety. Blockchain technology also contributes to enhanced food safety by enabling rapid traceability and recall capabilities **[134]**.

Implementing Blockchain in Meat Traceability

Implementing blockchain technology in meat traceability involves several key steps and considerations. Firstly, it's essential to identify all stakeholders involved in the meat supply chain, including farmers, processors, distributors, retailers, regulators, and consumers, and engage them in collaboration. Once stakeholders are identified, the next step is to define data standards and establish standardized formats for recording information about product origin, handling procedures, and certifications. After defining data standards, selecting a suitable blockchain platform is crucial. Intelligent contracts play a vital role in automating processes and ensuring transparency, so developing smart contracts tailored to the specific requirements of the meat supply chain is essential **[135]**. Integrating IoT devices with blockchain technology allows real-time monitoring of product movement and conditions, providing valuable data throughout the supply chain **[136]**. Continuous monitoring and auditing of the blockchain network are necessary to maintain data integrity and compliance with regulations. Additionally, engaging consumers by providing transparent information about meat products using blockchain-enabled platforms enhances consumer confidence and trust in the supply chain **[137]**.

Future Trends in Meat Quality and Safety Enhancement

Technological advancements, evolving consumer preferences, and regulatory requirements drive future meat quality and safety enhancement trends. One anticipated trend is the integration of blockchain technology, which is expected to become more widespread in the meat industry to improve transparency, traceability, and trustworthiness in the supply chain. Blockchain-enabled traceability initiatives will enable consumers to access detailed information about the farm-to-fork journey of meat products, enhancing confidence in their quality and safety. Artificial Intelligence (AI) and Machine Learning technologies will also play a crucial role in predicting and preventing food safety risks in meat processing facilities. AI-powered systems can analyze vast amounts of data to identify patterns, detect anomalies, and proactively mitigate food safety hazards, ensuring higher safety and quality in meat products. Precision agriculture techniques like IoT-enabled monitoring and remote sensing will empower farmers to optimize livestock management practices, improve animal health, and enhance meat quality. Real-time monitoring of livestock conditions will ensure optimal nutrition, housing, and disease management, leading to higher-quality meat products. The growing demand for alternative protein sources, including plant-based meats and cultured meats, will continue to drive innovation in meat production technologies. These alternatives offer sustainable and environmentally friendly options, aligning with consumer preferences for ethically produced and environmentally sustainable meat products. Advancements in processing technologies, such as high-pressure processing (HPP) and modified atmosphere packaging (MAP), will enable the development of safer and more shelf-stable meat products. These technologies will extend the shelf life of meat products, reduce microbial contamination, and preserve nutritional quality, enhancing both safety and consumer satisfaction. Stricter food safety regulations and standards will lead to adopting advanced quality control measures and compliance systems in the meat industry. Regulatory agencies will prioritize food safety and public health, prompting increased scrutiny and enforcement of food safety regulations, further ensuring the safety and quality of meat products. Growing consumer awareness of food safety and sustainability issues will drive demand for transparent and sustainable meat products. When purchasing, consumers will seek information about production practices, animal welfare standards, and environmental sustainability, encouraging meat producers to prioritize transparency and ethical practices. Supply chain resilience will also be a focus area, with meat producers investing in technologies and strategies to enhance resilience and ensure food security. This includes diversifying sourcing, improving inventory management, implementing contingency plans for disruptions, ensuring supply continuity and minimising food safety risks. Emerging technologies, such as nanotechnology and sensor-based systems, will continue to drive innovation in food safety enhancement. These technologies will enable rapid and sensitive detection of foodborne pathogens, enhance packaging materials, and improve preservation methods, further ensuring the safety and quality of meat products. Finally, sustainability will remain a key focus area for the meat industry, with efforts to reduce environmental impact, minimize resource use, and promote animal welfare. Initiatives such as regenerative agriculture and waste reduction will become increasingly important for meat producers seeking to meet consumer expectations and regulatory requirements, ensuring the industry's long-term viability. Future meat quality and safety enhancement trends will be characterized by technological innovation, regulatory compliance, consumer preferences, and sustainability initiatives. By embracing these trends and investing in advanced technologies and practices, the meat industry can improve food safety, meet regulatory requirements, and address evolving consumer demands for safe, nutritious, and sustainable meat products.

Conclusion

In conclusion, the future of meat quality and safety enhancement is marked by a convergence of technological innovation, regulatory scrutiny, consumer demand, and sustainability imperatives. As the meat industry evolves, stakeholders must remain vigilant in adopting advanced technologies, implementing robust quality control measures, and prioritizing transparency and accountability throughout the supply chain. Advancements in blockchain technology offer promising solutions for improving transparency and traceability, enabling consumers to make informed choices about the meat products they purchase. Integrating artificial intelligence, precision agriculture, and alternative protein sources will improve animal welfare, production efficiency, and environmental sustainability. Furthermore, implementing stricter food safety regulations and standards underscores the importance of investing in advanced processing techniques, quality control measures, and compliance systems. By embracing emerging food safety technologies, enhancing supply chain resilience, and promoting sustainable practices, the meat industry can ensure the safety, quality, and integrity of meat products while meeting consumers' evolving needs and expectations. Ultimately, collaboration among industry stakeholders, regulatory agencies, and consumers will be essential in shaping the future of meat quality and safety enhancement. By working together to address challenges, leverage opportunities, and embrace innovation, the meat industry can navigate the complexities of a rapidly changing landscape and build a safer, more sustainable future for meat production and consumption.

REFERENCES

1. Hartog, L.d. and Nutreco. Global perspective on integrated pork production. 2005.
2. Ravensdale, J. T., Coorey, R., & Dykes, G. A. (2018). Integration of Emerging Biomedical Technologies in Meat Processing to Improve Meat Safety and Quality. In Comprehensive Reviews in Food Science and Food Safety (Vol. 17, Issue 3, pp. 615–632). Wiley. https://doi.org/10.1111/1541-4337.12339
3. Leone, C., et al. Emerging Meat Processing Technologies for Microbiological Safety of Meat and Meat Products. 2020.
4. Alemneh, T.A. and M. Getabalew. Factors Influencing the Growth and Development of Meat Animals. 2019.
5. Wu, X., Liang, X., Wang, Y., Wu, B., & Sun, J. (2022). Non-Destructive Techniques for the Analysis and Evaluation of Meat Quality and Safety: A Review. In Foods (Vol. 11, Issue 22, p. 3713). MDPI AG. https://doi.org/10.3390/foods11223713
6. Jurčaga, L., Bobko, M., Kolesárová, A., Bobková, A., Demianová, A., Haščík, P., Belej, Ľ., Mendelová, A., Bučko, O., Kročko, M., & Čech, M. (2021). Blackcurrant (Ribes nigrum L.) and Kamchatka Honeysuckle (Lonicera caerulea var. Kamtschatica) Extract Effects on Technological Properties, Sensory Quality, and Lipid Oxidation of Raw-Cooked Meat Product (Frankfurters). In Foods (Vol. 10, Issue 12, p. 2957). MDPI AG. https://doi.org/10.3390/foods10122957
7. Jurcaga, L., Bobko, M., Haščík, P., Bobková, A., Demianová, A., Belej, L., Kročko, M., Mendelova, A., Čech, M., Herc, P., & Mesárošová, A. (2022). Effect of blackcurrant and kamchatka honeysuckle extracts on quality properties of raw-cooked meat product. In Journal of microbiology, biotechnology and food sciences (Vol. 12, Issue 3, p. e5349). Slovak University of Agriculture in Nitra. https://doi.org/10.55251/jmbfs.5349
8. Akinwumi, A. O., Oshodi, O. A., Atanda, R. A., Ogunsola, O. M., Ayoola, S. J., Ajani, R. A., & Odeleye, B. D. (2022). Meat Quality of Noiler Chicken as Influenced by Dietary Natural Antioxidants Supplementations. In Journal of Applied Life Sciences International (pp. 46–62). Sciencedomain International. https://doi.org/10.9734/jalsi/2022/v25i5584
9. Munekata, P. E. S., Finardi, S., de Souza, C. K., Meinert, C., Pateiro, M., Hoffmann, T. G., Domínguez, R., Bertoli, S. L., Kumar, M., & Lorenzo, J. M. (2023). Applications of Electronic Nose, Electronic Eye and Electronic Tongue in Quality, Safety and Shelf Life of Meat and Meat Products: A Review. In Sensors (Vol. 23, Issue 2, p. 672). MDPI AG. https://doi.org/10.3390/s23020672
10. Faujan, N. H., Azizan, N., & Norlelawati Arifin. (2022). Proximate Composition and Sensory Preference of Beef Sausages with Unripe Jackfruit. In Malaysian Journal of Science Health & Technology (Vol. 8, Issue 2, pp. 9–13). Universiti Sains Islam Malaysia. https://doi.org/10.33102/2022249
11. GENETICS OF M EAT Q UALITY IN C A TTLE. 2015.
12. Gagaoua, M., Duffy, G., Alvarez, C., Burgess, C. M., Hamill, R., Crofton, E., Botinestean, C., Ferragina, A., Cafferky, J., Mullen, A. M., & Troy, D. (2022). Current research and emerging tools to improve fresh red

meat quality. In Irish Journal of Agricultural and Food Research (Vol. 61, Issue 1). Compuscript, Ltd. https://doi.org/10.15212/ijafr-2020-0141

13. Gonzalez, J. M., & Phelps, K. J. (2018). United States beef quality as chronicled by the National Beef Quality Audits, Beef Consumer Satisfaction Projects, and National Beef Tenderness Surveys — A review. In Asian-Australasian Journal of Animal Sciences (Vol. 31, Issue 7, pp. 1036–1042). Asian Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.18.0199
14. Ardiansyah, A., Nugroho, A., & Meirinawati, H. (2023). Nutritional value and heavy metal content of crab meat and its byproduct white mud crab Scylla paramamosain. In Jurnal Ilmu dan Teknologi Kelautan Tropis (Vol. 15, Issue 1, pp. 1–12). Institut Pertanian Bogor. https://doi.org/10.29244/jitkt.v15i1.40215
15. Talamini, E.; Malafaia, G.C. Traceability, transparency and assurance (TTA) systems implementation by the Brazilian exporter pork meat chain compared with other countries. African Journal of Business Management 2010, 4, 651-661.
16. Skelly, T. A. M., & Ditlevsen, K. (2024). Bad avocados, culinary standards, and knowable knowledge. Culturally appropriate rejections of meat reduction. In Journal of Consumer Culture. SAGE Publications. https://doi.org/10.1177/14695405241243199
17. Udomkun, P., Ilukor, J., Mockshell, J., Mujawamariya, G., Okafor, C., Bullock, R., Nabahungu, N. L., & Vanlauwe, B. (2018). What are the key factors influencing consumers' preference and willingness to pay for meat products in Eastern? In Food Science & Nutrition (Vol. 6, Issue 8, pp. 2321–2336). Wiley. https://doi.org/10.1002/fsn3.813
18. Nanda, P. K., Bhattacharya, D., Das, J. K., Bandyopadhyay, S., Ekhlas, D., Lorenzo, J. M., Dandapat, P., Alessandroni, L., Das, A. K., & Gagaoua, M. (2022). Emerging Role of Biosensors and Chemical Indicators to Monitor the Quality and Safety of Meat and Meat Products. In Chemosensors (Vol. 10, Issue 8, p. 322). MDPI AG. https://doi.org/10.3390/chemosensors10080322
19. Savini, F., Giacometti, F., Cuomo, S. A., Tomasello, F., Terefe Mekonnen, Y., Troja, F., Indio, V., Tassinari, M., De Cesare, A., & Serraino, A. (2023). Reduction of the microbial load in meat maturation rooms with and without alkaline electrolyzed water fumigation. In Italian Journal of Food Safety. PAGEPress Publications. https://doi.org/10.4081/ijfs.2023.11109
20. Okunomo, K.; Ogisi, D.; Bosah, B.O. The evaluation of microbiological profile of some spices used in Turkish meat industry. Journal of Food Agriculture & Environment 2009, 7, 111-115.
21. Rani, Z.T. Effect of post-slaughter handling on physico-chemical and microbiological quality of red meat along the distribution chain in the Eastern Cape Province, South Africa. 2015.
22. Al-Abbasi, F. A., Habib, A. S., Kumar, V., & Anwar, F. (2023). Evaluation of Microbial Contamination of Chicken and Fish in Food Industries and Associated Health Effects in Saudi Arabia. In Pakistan Journal of Zoology (Vol. 55, Issue 5). ResearchersLinks Ltd. https://doi.org/10.17582/journal.pjz/20210714140705
23. Viator, C. L., Muth, M. K., Brophy, J. E., & Noyes, G. (2017). Costs of Food Safety Investments in the Meat and Poultry Slaughter Industries. In Journal of Food Science (Vol. 82, Issue 2, pp. 260–269). Wiley. https://doi.org/10.1111/1750-3841.13597
24. Williams, M. S., Ebel, E. D., Saini, G., & Nyirabahizi, E. (2020). Changes in Salmonella Contamination in Meat and Poultry Since the Introduction of the Pathogen Reduction and Hazard Analysis and Critical Control Point Rule. In Journal of Food Protection (Vol. 83, Issue 10, pp. 1707–1717). Elsevier BV. https://doi.org/10.4315/jfp-20-126
25. Hulebak, K. L., & Schlosser, W. (2002). Hazard Analysis and Critical Control Point (HACCP) History and Conceptual Overview. In Risk Analysis (Vol. 22, Issue 3, pp. 547–552). Wiley. https://doi.org/10.1111/0272-4332.00038
26. Yu, Z., Jung, D., Park, S., Hu, Y., Huang, K., Rasco, B. A., Wang, S., Ronholm, J., Lu, X., & Chen, J. (2020). Smart traceability for food safety. In Critical Reviews in Food Science and Nutrition (Vol. 62, Issue 4, pp. 905–916). Informa UK Limited. https://doi.org/10.1080/10408398.2020.1830262
27. Jaison, F., & Ramaiah, N. S. (2022). A survey on traceability in food safety system using blockchain. In Journal of Discrete Mathematical Sciences and Cryptography (Vol. 25, Issue 3, pp. 793–799). Taru Publications. https://doi.org/10.1080/09720529.2021.2016215
28. Ong, K. J., Johnston, J., Datar, I., Sewalt, V., Holmes, D., & Shatkin, J. A. (2021). Food safety considerations and research priorities for the cultured meat and seafood industry. In Comprehensive Reviews in Food Science and Food Safety (Vol. 20, Issue 6, pp. 5421–5448). Wiley. https://doi.org/10.1111/1541-4337.12853

29. Tomasevic, I., Djekic, I., Novaković, S., Barba, F., & Lorenzo, J. M. (2019). The feasibility of pulsed light processing in the meat industry. In IOP Conference Series: Earth and Environmental Science (Vol. 333, Issue 1, p. 012034). IOP Publishing. https://doi.org/10.1088/1755-1315/333/1/012034
30. Heinrich, V., Zunabovic, M., Varzakas, T., Bergmair, J., & Kneifel, W. (2015). Pulsed Light Treatment of Different Food Types with a Special Focus on Meat: A Critical Review. In Critical Reviews in Food Science and Nutrition (Vol. 56, Issue 4, pp. 591–613). Informa UK Limited. https://doi.org/10.1080/10408398.2013.826174
31. Bolumar, T., Orlien, V., Sikes, A., Aganovic, K., Bak, K. H., Guyon, C., Stübler, A., de Lamballerie, M., Hertel, C., & Brüggemann, D. A. (2020). High-pressure processing of meat: Molecular impacts and industrial applications. In Comprehensive Reviews in Food Science and Food Safety (Vol. 20, Issue 1, pp. 332–368). Wiley. https://doi.org/10.1111/1541-4337.12670
32. Rakotondramavo, A.; Guyon, C.; de-Lamballerie, M. Use of high pressure processing on meat products : commercial developments and research trends in food science. 2018.
33. Bolumar, T., Orlien, V., Sikes, A., Aganovic, K., Bak, K. H., Guyon, C., Stübler, A., de Lamballerie, M., Hertel, C., & Brüggemann, D. A. (2020). High-pressure processing of meat: Molecular impacts and industrial applications. In Comprehensive Reviews in Food Science and Food Safety (Vol. 20, Issue 1, pp. 332–368). Wiley. https://doi.org/10.1111/1541-4337.12670
34. Pou, K. R. J. (2021). Applications of High Pressure Technology in Food Processing. In International Journal of Food Studies (Vol. 10, Issue 1, pp. 248–281). ISEKI Food Association. https://doi.org/10.7455/ijfs/10.1.2021.a10
35. Arshad, M.S. Recent Advances and Innovation in Meat with Reference to Processing Technologies. 2023.
36. Borick, P. M., & Fogarty, M. G. (1967). Effects of Continuous and Interrupted Radiation on Microorganisms. In Applied Microbiology (Vol. 15, Issue 4, pp. 785–789). American Society for Microbiology. https://doi.org/10.1128/am.15.4.785-789.1967
37. Verma, C. Preservation of meat by heat and radiation-a review. International Journal in IT & Engineering 2015, 3, 186-195.
38. XAVIER, M. M. B. B. S., FRANCO, R. M., SOUZA, M. C. L., DUQUE, S. da S., & ESTEVES, W. T. C. (2018). Implications of the use of irradiation in the processing of animal origin foods: Review. In Journal of bioenergy and food science (Vol. 5, Issue 4, pp. 131–144). Instituto Federal do Amapa. https://doi.org/10.18067/jbfs.v5i4.257
39. Sarfraz, J., Gulin-Sarfraz, T., Nilsen-Nygaard, J., & Pettersen, M. K. (2020). Nanocomposites for Food Packaging Applications: An Overview. In Nanomaterials (Vol. 11, Issue 1, p. 10). MDPI AG. https://doi.org/10.3390/nano11010010
40. Tyagi, P., Salem, K. S., Hubbe, M. A., & Pal, L. (2021). Advances in barrier coatings and film technologies for achieving sustainable packaging of food products – A review. In Trends in Food Science & Technology (Vol. 115, pp. 461–485). Elsevier BV. https://doi.org/10.1016/j.tifs.2021.06.036
41. Kumar, V., Guleria, P., & Mehta, S. K. (2017). Nanosensors for food quality and safety assessment. In Environmental Chemistry Letters (Vol. 15, Issue 2, pp. 165–177). Springer Science and Business Media LLC. https://doi.org/10.1007/s10311-017-0616-4
42. Mohammadi, Z., & Jafari, S. M. (2020). Detection of food spoilage and adulteration by novel nanomaterial-based sensors. In Advances in Colloid and Interface Science (Vol. 286, p. 102297). Elsevier BV. https://doi.org/10.1016/j.cis.2020.102297
43. Ulloa-Saavedra, A., García-Betanzos, C., Zambrano-Zaragoza, M., Quintanar-Guerrero, D., Mendoza-Elvira, S., & Velasco-Bejarano, B. (2022). Recent Developments and Applications of Nanosystems in the Preservation of Meat and Meat Products. In Foods (Vol. 11, Issue 14, p. 2150). MDPI AG. https://doi.org/10.3390/foods11142150
44. Aswathanarayan, J. B., & Vittal, R. R. (2019). Nanoemulsions and Their Potential Applications in Food Industry. In Frontiers in Sustainable Food Systems (Vol. 3). Frontiers Media SA. https://doi.org/10.3389/fsufs.2019.00095
45. Lamri, M., Bhattacharya, T., Boukid, F., Chentir, I., Dib, A. L., Das, D., Djenane, D., & Gagaoua, M. (2021). Nanotechnology as a Processing and Packaging Tool to Improve Meat Quality and Safety. In Foods (Vol. 10, Issue 11, p. 2633). MDPI AG. https://doi.org/10.3390/foods10112633

46. Onyeaka, H., Passaretti, P., Miri, T., & Al-Sharify, Z. T. (2022). The safety of nanomaterials in food production and packaging. In Current Research in Food Science (Vol. 5, pp. 763–774). Elsevier BV. https://doi.org/10.1016/j.crfs.2022.04.005

47. Thakur, D., Ravikumar, R., Kumar, P., Gupta, A., Sharma, A., Katoch, S., & Bodh, V. (2012). Meat inspection and animal welfare practices: Evidences from north-western Himalayan region, India. In Veterinary World (Vol. 5, Issue 12, p. 718). ScopeMed. https://doi.org/10.5455/vetworld.2012.718-722

48. Bayarsaikhan, M., Purevdorj, N.-O., Kim, B. H., Jung, J. H., & Cho, G. J. (2023). Evaluation of the Microbiological Status of Cattle Carcasses in Mongolia: Considering the Hygienic Practices of Slaughter Establishments. In Veterinary Sciences (Vol. 10, Issue 9, p. 563). MDPI AG. https://doi.org/10.3390/vetsci10090563

49. Ramachandraiah, K., Han, S. G., & Chin, K. B. (2014). Nanotechnology in Meat Processing and Packaging: Potential Applications — A Review. In Asian-Australasian Journal of Animal Sciences (Vol. 28, Issue 2, pp. 290–302). Asian Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.14.0607

50. Xie, L., Qin, J., Yao, T., Tang, X., Cui, D., Chen, L., Rao, L., Xiao, S., Zhang, Z., & Huang, L. (2023). Genetic dissection of 26 meat cut, meat quality and carcass traits in four pig populations. In Genetics Selection Evolution (Vol. 55, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12711-023-00817-y

51. Wray-Cahen, D., Bodnar, A., Rexroad, C., III, Siewerdt, F., & Kovich, D. (2022). Advancing genome editing to improve the sustainability and resiliency of animal agriculture. In CABI Agriculture and Bioscience (Vol. 3, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s43170-022-00091-w

52. Dybus, A., Proskura, W. S., Pawlina, E., & Nowak, B. (2018). Associations between polymorphisms in the myostatin, α^A-globin and lactate dehydrogenase B genes and racing performance in homing pigeons. In Veterinární medicína (Vol. 63, Issue 8, pp. 390–394). Czech Academy of Agricultural Sciences. https://doi.org/10.17221/149/2017-vetmed

53. Vanderhout, R. J., Leishman, E. M., Abdalla, E. A., Barbut, S., Wood, B. J., & Baes, C. F. (2022). Genetic Parameters of White Striping and Meat Quality Traits Indicative of Pale, Soft, Exudative Meat in Turkeys (Meleagris gallopavo). In Frontiers in Genetics (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fgene.2022.842584

54. Silva, R. P., Espigolan, R., Berton, M. P., Lôbo, R. B., Magnabosco, C. U., Pereira, A. S. C., & Baldi, F. (2021). Genomic prediction ability for carcass composition indicator traits in Nellore cattle. In Livestock Science (Vol. 245, p. 104421). Elsevier BV. https://doi.org/10.1016/j.livsci.2021.104421

55. Sandøe, P., Hansen, H. O., Rhode, H. L. H., Houe, H., Palmer, C., Forkman, B., & Christensen, T. (2020). Benchmarking Farm Animal Welfare—A Novel Tool for Cross-Country Comparison Applied to Pig Production and Pork Consumption. In Animals (Vol. 10, Issue 6, p. 955). MDPI AG. https://doi.org/10.3390/ani10060955

56. Cornish, A., Raubenheimer, D., & McGreevy, P. (2016). What We Know about the Public's Level of Concern for Farm Animal Welfare in Food Production in Developed Countries. In Animals (Vol. 6, Issue 11, p. 74). MDPI AG. https://doi.org/10.3390/ani6110074

57. Edwards-Callaway, L., Davis, M., Dean, L., & McBride, B. (2024). Stakeholder Perceptions of Animal Welfare as a Component of Sustainable Beef Programs in the United States—A Pilot Study. In Animals (Vol. 14, Issue 9, p. 1348). MDPI AG. https://doi.org/10.3390/ani14091348

58. Schnettler M, B., Vidal M, R., Silva F, R., Vallejos C, L., & Sepúlveda B, N. (2008). Consumer Perception of Animal Welfare and Livestock Production in the Araucania Region , Chile. In Chilean journal of agricultural research (Vol. 68, Issue 1). SciELO Agencia Nacional de Investigacion y Desarrollo (ANID). https://doi.org/10.4067/s0718-58392008000100008

59. Ahmed, H., Emanuelson, U., Alvåsen, K., Berg, C., Hultgren, J., Rocklinsberg, H., & Hansson, H. (2023). Animal welfare efforts and farm economic outcomes: Evidence from Swedish beef production. In Agricultural and Resource Economics Review (Vol. 52, Issue 3, pp. 498–519). Cambridge University Press (CUP). https://doi.org/10.1017/age.2023.8

60. Evans, H. C., Briggs, E. F., Burnett, R. H., Contreras-Correa, Z. E., Duvic, M. A., Dysart, L. M., Gilmore, A. A., Messman, R. D., Reid, D., Rasit Ugur, M., Kaya, A., & Memili, E. (2022). Harnessing the value of reproductive hormones in cattle production with considerations to animal welfare and human health. In

Journal of Animal Science (Vol. 100, Issue 7). Oxford University Press (OUP). https://doi.org/10.1093/jas/skac177

61. Schaefer, G. O., & Savulescu, J. (2014). The Ethics of Producing In Vitro Meat. In Journal of Applied Philosophy (Vol. 31, Issue 2, pp. 188–202). Wiley. https://doi.org/10.1111/japp.12056

62. Stephens, N., Di Silvio, L., Dunsford, I., Ellis, M., Glencross, A., & Sexton, A. (2018). Bringing cultured meat to market: Technical, socio-political, and regulatory challenges in cellular agriculture. In Trends in Food Science & Technology (Vol. 78, pp. 155–166). Elsevier BV. https://doi.org/10.1016/j.tifs.2018.04.010

63. Paranhos da Costa, M. J. R., Huertas, S. M., Gallo, C., & Dalla Costa, O. A. (2012). Strategies to promote farm animal welfare in Latin America and their effects on carcass and meat quality traits. In Meat Science (Vol. 92, Issue 3, pp. 221–226). Elsevier BV. https://doi.org/10.1016/j.meatsci.2012.03.005

64. Khan, R., Li, A., & Raza, S. H. A. (2023). Editorial: Genetic Regulation of Meat Quality Traits in Livestock Species. In Frontiers in Genetics (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fgene.2022.1092562

65. Vanderhout, R. J., Leishman, E. M., Abdalla, E. A., Barbut, S., Wood, B. J., & Baes, C. F. (2022). Genetic Parameters of White Striping and Meat Quality Traits Indicative of Pale, Soft, Exudative Meat in Turkeys (Meleagris gallopavo). In Frontiers in Genetics (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fgene.2022.842584

66. Slaughterhouses and Food Animals Monitoring. Available at: https://www.cfs.gov.hk/english/import/import_smi.html

67. Meat Inspection. Available online: https://meat.tamu.edu/ansc-307-honors/meat-inspection/ (accessed on

68. Product Labeling Available online: https://www.nichemeatprocessing.org/product-labeling/

69. Reyna, M., Delgado, G., Akundi, A., Luna, S., & Chumacero, E. (2022). Product Digital Quality Inspection using Machine Vision Systems – A Categorical Review. In 2022 17th Annual System of Systems Engineering Conference (SOSE). 2022 17th Annual System of Systems Engineering Conference (SOSE). IEEE. https://doi.org/10.1109/sose55472.2022.9812687

70. Ahmed, M. B., Majeed, F., Sanin, C., & Szczerbicki, E. (2021). Experience-Based Product Inspection Planning for Industry 4.0. In Cybernetics and Systems (Vol. 52, Issue 5, pp. 296–312). Informa UK Limited. https://doi.org/10.1080/01969722.2020.1871222

71. Black, R. E., Hurley, F. J., & Havery, D. C. (2001). Occurrence of 1,4-Dioxane in Cosmetic Raw Materials and Finished Cosmetic Products. In Journal of AOAC INTERNATIONAL (Vol. 84, Issue 3, pp. 666–670). Oxford University Press (OUP). https://doi.org/10.1093/jaoac/84.3.666

72. Govender, R.; Naidoo, D.; Buys, E.M. Managing Meat Safety at South African Abattoirs. World Academy of Science, Engineering and Technology, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering 2013, 7, 279-284.

73. Sandrou, D. K., & Arvanitoyannis, I. S. (1999). Implementation of hazard analysis critical control point in the meat and poultry industry. In Food Reviews International (Vol. 15, Issue 3, pp. 265–308). Informa UK Limited. https://doi.org/10.1080/87559129909541192

74. Jeffer, S. B., Kassem, I. I., Kharroubi, S. A., & Abebe, G. K. (2021). Analysis of Food Safety Management Systems in the Beef Meat Processing and Distribution Chain in Uganda. In Foods (Vol. 10, Issue 10, p. 2244). MDPI AG. https://doi.org/10.3390/foods10102244

75. Hooker, N. H., Nayga, R. M., Jr., & Siebert, J. W. (2002). The Impact of HACCP on Costs and Product Exit. In Journal of Agricultural and Applied Economics (Vol. 34, Issue 1, pp. 165–174). Cambridge University Press (CUP). https://doi.org/10.1017/s1074070800002212

76. Barki, K. (2024). Kajian Literatur: Dampak Penerapan HACCP dan Sistem Mutu dalam Peningkatan Daya Saing pada Industri Pengolahan Ayam. In Agricultural Socio-Economic Empowerment and Agribusiness Journal (Vol. 2, Issue 2, p. 57). Universitas Sebelas Maret. https://doi.org/10.20961/agrisema.v2i2.72926

77. Schmidt, R. H., & Newslow, D. L. (2007). Hazard Analysis Critical Control Points (HACCP) – Principle 3: Establish Critical Limits and Principle 4: Monitoring Critical Control Points (CCPs). In EDIS (Vol. 2007, Issue 18). University of Florida George A Smathers Libraries. https://doi.org/10.32473/edis-fs141-2007

78. Schmidt, R. H., & Newslow, D. (2007). Hazard Analysis Critical Control Points (HACCP) Principle 5: Establish Corrective Actions. In EDIS (Vol. 2007, Issue 19). University of Florida George A Smathers Libraries. https://doi.org/10.32473/edis-fs142-2007

79. Bosona, T., & Gebresenbet, G. (2023). The Role of Blockchain Technology in Promoting Traceability Systems in Agri-Food Production and Supply Chains. In Sensors (Vol. 23, Issue 11, p. 5342). MDPI AG. https://doi.org/10.3390/s23115342

80. BERCKMANS, D. (2014). Precision livestock farming technologies for welfare management in intensive livestock systems. In Revue Scientifique et Technique de l'OIE (Vol. 33, Issue 1, pp. 189–196). O.I.E (World Organisation for Animal Health). https://doi.org/10.20506/rst.33.1.2273

81. Berckmans, D. (2015). 1.2. Smart farming for Europe: value creation through precision livestock farming. In Precision livestock farming applications (pp. 25–36). Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-815-5_2

82. Garmyn, A., Hardcastle, N., Polkinghorne, R., Lucherk, L., & Miller, M. (2020). Extending Aging of Beef Longissimus Lumborum From 21 to 84 Days Postmortem Influences Consumer Eating Quality. In Foods (Vol. 9, Issue 2, p. 208). MDPI AG. https://doi.org/10.3390/foods9020208

83. Fan, Y., Han, Z., ARBAB, A. A. I., Yang, Y., & Yang, Z. (2020). Effect of Aging Time on Meat Quality of Longissimus Dorsi from Yunling Cattle: A New Hybrid Beef Cattle. In Animals (Vol. 10, Issue 10, p. 1897). MDPI AG. https://doi.org/10.3390/ani10101897

84. Abril, B., Bou, R., García-Pérez, J. V., & Benedito, J. (2023). Role of Enzymatic Reactions in Meat Processing and Use of Emerging Technologies for Process Intensification. In Foods (Vol. 12, Issue 10, p. 1940). MDPI AG. https://doi.org/10.3390/foods12101940

85. Kang, D., Zhang, W., Lorenzo, J. M., & Chen, X. (2020). Structural and functional modification of food proteins by high power ultrasound and its application in meat processing. In Critical Reviews in Food Science and Nutrition (Vol. 61, Issue 11, pp. 1914–1933). Informa UK Limited. https://doi.org/10.1080/10408398.2020.1767538

86. Teixeira, D. L., Salazar, L. C., Larraín, R., & Boyle, L. A. (2022). The capacity of inspection on farm and at the abattoir to predict post-mortem outcomes in slaughter pigs: A study at animal level. In Animal Science Journal (Vol. 94, Issue 1). Wiley. https://doi.org/10.1111/asj.13798

87. Sprong, J. P., Lin, X., Maestre, J. M., & Negenborn, R. R. (2019). Quality-Aware Control for Optimizing Meat Supply Chains. In 2019 18th European Control Conference (ECC). 2019 18th European Control Conference (ECC). IEEE. https://doi.org/10.23919/ecc.2019.8795777

88. Wang, X., Matetić, M., Zhou, H., Zhang, X., & Jemrić, T. (2017). Postharvest Quality Monitoring and Variance Analysis of Peach and Nectarine Cold Chain with Multi-Sensors Technology. In Applied Sciences (Vol. 7, Issue 2, p. 133). MDPI AG. https://doi.org/10.3390/app7020133

89. Ren-yong, T. Application of Hurdle Technology for Quality Control and Cold Chain Management in Meat Processing Industry. Meat Research 2013.

90. Katsaros, G., & Taoukis, P. (2021). Microbial Control by High Pressure Processing for Shelf-Life Extension of Packed Meat Products in the Cold Chain: Modeling and Case Studies. In Applied Sciences (Vol. 11, Issue 3, p. 1317). MDPI AG. https://doi.org/10.3390/app11031317

91. Pramanic, A.; asmitapramanic. A review on molecular methods in the detection of foodborne pathogens. 2021.

92. Quain, D., & Jevons, A. (2023). The spoilage of lager by draught beer microbiota. In Journal of the Institute of Brewing (Vol. 129, Issue 4). The Institute of Brewing & Distilling. https://doi.org/10.58430/jib.v129i4.32

93. Bolzon, V., Bulfoni, M., Pesando, M., Nencioni, A., & Nencioni, E. (2024). Verification of a Rapid Analytical Method for the Qualitative Detection of Listeria spp. and Listeria monocytogenes by a Real-Time PCR Assay according to EN UNI ISO 16140-3:2021. In Pathogens (Vol. 13, Issue 2, p. 141). MDPI AG. https://doi.org/10.3390/pathogens13020141

94. Pedroso, D. M. M., Iaria, S. T., Gamba, R. C., Heidtmann, S., & Rall, V. L. M. (1999). Critical control points for meat balls and kibbe preparations in a hospital kitchen. In Revista de Microbiologia (Vol. 30, Issue 4, pp. 347–355). FapUNIFESP (SciELO). https://doi.org/10.1590/s0001-37141999000400010

95. Andrée, S.; Jira, W.; Schwägele, F.; Schwind, K.H.; Wagner, H. Chemical safety in meat industry. 2011.

96. Filazi, A., Yurdakok-Dikmen, B., Kuzukiran, O., & Sireli, U. T. (2017). Chemical Contaminants in Poultry Meat and Products. In Poultry Science. InTech. https://doi.org/10.5772/64893

97. LI, J., JU, X., WANG, Y., TIAN, Q., LIANG, X., LI, H., & LIU, Y. (2023). High-throughput screening of multi-pesticide residues in animal-derived foods by QuEChERS-online gel permeation chromatography-gas

chromatography-tandem mass spectrometry. In Chinese Journal of Chromatography (Vol. 41, Issue 7, pp. 610–621). China Science Publishing & Media Ltd. https://doi.org/10.3724/sp.j.1123.2022.10010

98. Bietlot, H. P., & Kolakowski, B. (2012). Risk assessment and risk management at the Canadian Food Inspection Agency (CFIA): A perspective on the monitoring of foods for chemical residues. In Drug Testing and Analysis (Vol. 4, Issue S1, pp. 50–58). Wiley. https://doi.org/10.1002/dta.1352

99. Batchu, P., Hazard, T., Lee, J. H., Terrill, T. H., Kouakou, B., & Kannan, G. (2021). High-Condensed Tannin Diet and Transportation Stress in Goats: Effects on Physiological Responses, Gut Microbial Counts and Meat Quality. In Animals (Vol. 11, Issue 10, p. 2857). MDPI AG. https://doi.org/10.3390/ani11102857

100. Tokan, Y. K., Astutik, Y. W., Samba, A., Djubida, F. A., Lakahena, D. I., Mali, S. L., Rahmawati, E. M., & Junias, M. S. (2023). Relationships between Knowledge, Sanitation and Hygiene of Food Handlers, and Food Safety in the Restaurants in Pasir Panjang, Kupang, East Nusa Tenggara, Indonesia. In Journal of Health Promotion and Behavior (Vol. 8, Issue 3, pp. 234–242). Masters Program in Public Health, Sebelas Maret University. https://doi.org/10.26911/thejhpb.2023.08.03.08

101. Aldossari, H. Validation of Post-Harvest Wash Process to Prevent Cross-Contamination in Leafy Green Processing. 2016.

102. Abichandani, S., & Nadiger, R. (2013). Cross-contamination in dentistry: A comprehensive overview. In Chronicles of Young Scientists (Vol. 4, Issue 1, p. 51). Medknow. https://doi.org/10.4103/2229-5186.108807

103. Kanade, P.; Subramani, A. Hygienic design aspects of pasteurizer to prevent cross contamination of pasteurized milk. Journal of Hygienic Engineering and Design 2013, 2, 12-18.

104. Griffing, D. A., Kriese-Anderson, L. A., Mullenix, M. K., Wang, L., & Bratcher, C. L. (2018). A Study of Consumer Handling Behaviors during Transport from Retail to Residence Utilizing an Electronic Questionnaire. In Meat and Muscle Biology (Vol. 2, Issue 1). Iowa State University. https://doi.org/10.22175/mmb2017.12.0058

105. Mahajan, K.; Kumar, S.; Kumar, S. Implementation of traceability in food industry with special allusion to meat industry. 2019.

106. Vandegraaff, R.C. Managing a regulatory culture change in the meat industry QA and the ARMCANZ national standards. 1997.

107. Unnevehr, L. J., & Jensen, H. H. (1996). HACCP as a Regulatory Innovation to Improve Food Safety in the Meat Industry. In American Journal of Agricultural Economics (Vol. 78, Issue 3, pp. 764–769). Wiley. https://doi.org/10.2307/1243301

108. Sośnicka, M. Natural preservatives in meat products. 2019.

109. Xiao-jua, P. Application of natural preservatives to meat and meat products. Meat Industry 2010.

110. Fei, W. Application of Several Natural Preservatives in Low-temperature Meat Products. Meat Research 2011.

111. Santiesteban-López, N. A., Gómez-Salazar, J. A., Santos, E. M., Campagnol, P. C. B., Teixeira, A., Lorenzo, J. M., Sosa-Morales, M. E., & Domínguez, R. (2022). Natural Antimicrobials: A Clean Label Strategy to Improve the Shelf Life and Safety of Reformulated Meat Products. In Foods (Vol. 11, Issue 17, p. 2613). MDPI AG. https://doi.org/10.3390/foods11172613

112. Kargozari, M.; Hamedi, H. Incorporation of essential oils (EOs) and nanoparticles (NPs) into active packaging systems in meat and meat products: A review. 2019.

113. Ji, J., Shankar, S., Royon, F., Salmieri, S., & Lacroix, M. (2021). Essential oils as natural antimicrobials applied in meat and meat products—a review. In Critical Reviews in Food Science and Nutrition (Vol. 63, Issue 8, pp. 993–1009). Informa UK Limited. https://doi.org/10.1080/10408398.2021.1957766

114. Nieto, G., & Ros, G. (2017). Dietary Administration of Animal Diets with Aromatic and Medicinal Plants: Influence on Meat Quality. In Active Ingredients from Aromatic and Medicinal Plants. InTech. https://doi.org/10.5772/67038

115. Moura-Alves, M., Esteves, A., Ciríaco, M., Silva, J. A., & Saraiva, C. (2023). Antimicrobial and Antioxidant Edible Films and Coatings in the Shelf-Life Improvement of Chicken Meat. In Foods (Vol. 12, Issue 12, p. 2308). MDPI AG. https://doi.org/10.3390/foods12122308

116. Lorenzo, J. M., Munekata, P. E. S., Pateiro, M., Domínguez, R., Abdulrazzaq Alaghbari, M., & Tomasevic, I. (2021). Preservation of meat products with natural antioxidants from rosemary. In IOP Conference Series: Earth and Environmental Science (Vol. 854, Issue 1, p. 012053). IOP Publishing. https://doi.org/10.1088/1755-1315/854/1/012053

117. Ben Akacha, B., Michalak, M., Najar, B., Venturi, F., Taglieri, I., Kačániová, M., Ben Saad, R., Mnif, W., Garzoli, S., & Ben Hsouna, A. (2023). Recent Advances in the Incorporation of Polysaccharides with Antioxidant and Antibacterial Functions to Preserve the Quality and Shelf Life of Meat Products. In Foods (Vol. 12, Issue 8, p. 1647). MDPI AG. https://doi.org/10.3390/foods12081647

118. Çelebi Sezer, Y., & Bozkurt, H. (2021). Use of antimicrobial packaging systems in the production and storage of meat and meat products. In Food and Health (Vol. 7, Issue 2, pp. 150–163). Scientific Web Journals (SWJ). https://doi.org/10.3153/fh21016

119. Cooksey, K. (2010). Oxygen Scavenging Packaging Systems. In Encyclopedia of Polymer Science and Technology. Wiley. https://doi.org/10.1002/0471440264.pst570

120. Prasad, P., & Kochhar, A. (2014). Active Packaging in Food Industry: A Review. In IOSR Journal of Environmental Science, Toxicology and Food Technology (Vol. 8, Issue 5, pp. 01–07). IOSR Journals. https://doi.org/10.9790/2402-08530107

121. Siddiqui, S. A., Sundarsingh, A., Bahmid, N. A., Nirmal, N., Denayer, J. F. M., & Karimi, K. (2023). A critical review on biodegradable food packaging for meat: Materials, sustainability, regulations, and perspectives in the EU. In Comprehensive Reviews in Food Science and Food Safety (Vol. 22, Issue 5, pp. 4147–4185). Wiley. https://doi.org/10.1111/1541-4337.13202

122. Bose, I., Roy, S., Pandey, V. K., & Singh, R. (2023). A Comprehensive Review on Significance and Advancements of Antimicrobial Agents in Biodegradable Food Packaging. In Antibiotics (Vol. 12, Issue 6, p. 968). MDPI AG. https://doi.org/10.3390/antibiotics12060968

123. Hara, Y., Castell-Perez, M. E., & Moreira, R. G. (2023). Antimicrobial properties of poly (vinyl alcohol) films with zeolitic imidazolate framework (ZIF-8) nanoparticles for food packaging. In Journal of Food Science (Vol. 88, Issue 6, pp. 2512–2522). Wiley. https://doi.org/10.1111/1750-3841.16572

124. Omerović, N., Djisalov, M., Živojević, K., Mladenović, M., Vunduk, J., Milenković, I., Knežević, N. Ž., Gadjanski, I., & Vidić, J. (2021). Antimicrobial nanoparticles and biodegradable polymer composites for active food packaging applications. In Comprehensive Reviews in Food Science and Food Safety (Vol. 20, Issue 3, pp. 2428–2454). Wiley. https://doi.org/10.1111/1541-4337.12727

125. Bose, I., Roy, S., Pandey, V. K., & Singh, R. (2023). A Comprehensive Review on Significance and Advancements of Antimicrobial Agents in Biodegradable Food Packaging. In Antibiotics (Vol. 12, Issue 6, p. 968). MDPI AG. https://doi.org/10.3390/antibiotics12060968

126. Espitia, P. J. P., Soares, N. de F. F., Coimbra, J. S. dos R., de Andrade, N. J., Cruz, R. S., & Medeiros, E. A. A. (2012). Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications. In Food and Bioprocess Technology (Vol. 5, Issue 5, pp. 1447–1464). Springer Science and Business Media LLC. https://doi.org/10.1007/s11947-012-0797-6

127. Ye, J., Hou, F., Chen, G., Zhong, T., Xue, J., Yu, F., Lai, Y., Yang, Y., Liu, D., Tian, Y., & Huang, J. (2023). Novel copper-containing ferrite nanoparticles exert lethality to MRSA by disrupting MRSA cell membrane permeability, depleting intracellular iron ions, and upregulating ROS levels. In Frontiers in Microbiology (Vol. 14). Frontiers Media SA. https://doi.org/10.3389/fmicb.2023.1023036

128. Asghar, M. A., & Asghar, M. A. (2020). Green synthesized and characterized copper nanoparticles using various new plants extracts aggravate microbial cell membrane damage after interaction with lipopolysaccharide. In International Journal of Biological Macromolecules (Vol. 160, pp. 1168–1176). Elsevier BV. https://doi.org/10.1016/j.ijbiomac.2020.05.198

129. Chen, K. L., & Bothun, G. D. (2013). Nanoparticles Meet Cell Membranes: Probing Nonspecific Interactions using Model Membranes. In Environmental Science & Technology (Vol. 48, Issue 2, pp. 873–880). American Chemical Society (ACS). https://doi.org/10.1021/es403864v

130. Hashem, A. H., El-Naggar, M. E., Abdelaziz, A. M., Abdelbary, S., Hassan, Y. R., & Hasanin, M. S. (2023). Bio-based antimicrobial food packaging films based on hydroxypropyl starch/polyvinyl alcohol loaded with the biosynthesized zinc oxide nanoparticles. In International Journal of Biological Macromolecules (Vol. 249, p. 126011). Elsevier BV. https://doi.org/10.1016/j.ijbiomac.2023.126011

131. Baysal, G., Demirci, C., & Özpinar, H. (2023). Properties and Synthesis of Biosilver Nanofilms for Antimicrobial Food Packaging. In Polymers (Vol. 15, Issue 3, p. 689). MDPI AG. https://doi.org/10.3390/polym15030689

132. Kaur, G., Bhari, R., & Kumar, K. (2023). Nanobiosensors and their role in detection of adulterants and contaminants in food products. In Critical Reviews in Biotechnology (Vol. 44, Issue 4, pp. 547–561). Informa UK Limited. https://doi.org/10.1080/07388551.2023.2175196

133. Mathivanan, S. (2021). Perspectives of Nano-Materials and Nanobiosensors in Food Safety and Agriculture. In Novel Nanomaterials. IntechOpen. https://doi.org/10.5772/intechopen.95345

134. Arvana, M., Rocha, A. D., & Barata, J. (2023). Agri-Food Value Chain Traceability Using Blockchain Technology: Portuguese Hams' Production Scenario. In Foods (Vol. 12, Issue 23, p. 4246). MDPI AG. https://doi.org/10.3390/foods12234246

135. S, P.; Mundada, M.R. Blockchain in Agriculture and Food Supply Management. GEDRAG & ORGANISATIE REVIEW 2020.

136. Wisessing, K., & Vichaidis, N. (2022). IoT Based Cold Chain Logistics with Blockchain for Food Monitoring Application. In 2022 7th International Conference on Business and Industrial Research (ICBIR). 2022 7th International Conference on Business and Industrial Research (ICBIR). IEEE. https://doi.org/10.1109/icbir54589.2022.9786493

137. Iftekhar, A., & Cui, X. (2021). Blockchain-Based Traceability System That Ensures Food Safety Measures to Protect Consumer Safety and COVID-19 Free Supply Chains. In Foods (Vol. 10, Issue 6, p. 1289). MDPI AG. https://doi.org/10.3390/foods10061289

138. Čapla, J., Zajác, P., Ševcová, K., Čurlej, J., & Fikselová, M. (2022). Milk and diary products – summary of European legislation, hygiene manuals, ISO standards and Codex Alimentarius standards. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 431–462). HACCP Consulting. https://doi.org/10.5219/1744

139. Ševcová, K., Zajác, P., Čapla, J., & Čurlej, J. (2021). Development of the food act of the Slovak Republic from 1995 to 2021. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 982–994). HACCP Consulting. https://doi.org/10.5219/1689

Funds:

This research received no external funding.

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Maksim Rebezov, V. M. Gorbatov Federal Research Center for Food Systems, Department of Scientific Research, 26 Talalikhin Str., Moscow, 109316, Russia; Ural State Agrarian University, Department of Biotechnology and Food, 42 Karl Liebknecht str., Yekaterinburg, 620075, Russia
Tel.: +79999002365
E-mail: rebezov@ya.ru
ORCID: https://orcid.org/0000-0003-0857-5143

Mars Khayrullin, K.G. Razumovsky Moscow State University of technologies and management (The First Cossack University), Department of Scientific Research, 73 Zemlyanoy Val, Moscow, 109004, Russia,
Tel.: +79049755219
E-Mail: 89049755219@ya.ru
ORCID: https://orcid.org/0000-0003-1697-7281

***Bahytkul Assenova**, Shakarim University, Department of Food Production Technology and Biotechnology, 20A Glinka Str., Semey, 071412, Kazakhstan,
Tel.: +77076586117,
E-mail: asenova.1958@mail.ru
ORCID: https://orcid.org/0000-0003-2996-8587

Smolnikova Farida, Shakarim University, Department of Food Production Technology and Biotechnology, 20A Glinka Str., Semey, 071412, Kazakhstan,
Tel.: +77476207683,
E-mail: smolnikovafarida@mail.ru
ORCID: https://orcid.org/0000-0002-8777-5313

Dmitry Baydan, K.G. Razumovsky Moscow State University of technologies and management (The First Cossack University), Department of Scientific Research, 73 Zemlyanoy Val, Moscow, 109004, Russia,
E-Mail: d.baydan@mgutm.ru
ORCID: https://orcid.org/0000-0001-8758-0690

Larisa Garipova, K.G. Razumovsky Moscow State University of technologies and management (The First Cossack University), Department of Scientific Research, 73 Zemlyanoy Val, Moscow, 109004, Russia,
E-Mail: l.garipova@mgutm.ru
ORCID: https://orcid.org/0000-0002-0404-5375

Raisa Savkina, Plekhanov Russian University of Economics, Department of Restaurant Business, 36 Stremyanny per, 117997, Moscow, Russia
E-Mail: kafedrains@yandex.ru
ORCID: https://orcid.org/0009-0000-8460-1396

Svetlana Rodionova, K.G. Razumovsky Moscow State University of technologies and management (The First Cossack University), Department of Scientific Research, 73 Zemlyanoy Val, Moscow, 109004, Russia,
E-Mail: s.rodionova@mgutm.ru
ORCID: https://orcid.org/0000-0001-7049-5895

Corresponding author: *

potravinárstvo®

OPEN ACCESS

Received: 10.2.2024
Revised: 6.5.2024
Accepted: 14.5.2024
Published: 19.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 547-569
https://doi.org/10.5219/1963
ISSN: 1337-0960 online
www.potravinarstvo.com

Prevalence and sensitivity of contagious and environmental cow mastitis-causing pathogens to antibiotics in Ukrainian farms

Ruslan Zaritskyi, Yurii Zhuk, Denys Dreval, Vitalii Kovpak, Yurii Masalovych, Ivan Cheverda, Iryna Derkach, Taras Savchuk

ABSTRACT

Mastitis is considered the most common and problematic disease, resulting in significant economic losses due to reduced milk yields, reduced quantity and quality of milk, treatment costs, and premature culling of animals. One of the traditional methods of treating mastitis in cows is using antibiotics, which leads to the emergence of polyresistant strains of microorganisms, the so-called Superbugs. The emergence of Superbugs, which are not sensitive to most existing antibiotics, is a major concern in veterinary and humane medicine. This study aimed to identify pathogens isolated from the secretion of the mammary gland of cows with mastitis to determine their spread and sensitivity to antibiotics. The samples of secretion from the udder were examined by bacteriological method. The isolates were identified by conventional methods and by the modern method – mass spectrometry (MALDI-TOF MS). The sensitivity of the bacterial isolates to antibiotics was determined by the disc diffusion method (Kirby-Bauer). The results of studies of cow mammary gland secretion samples indicate that 49.2% of the isolates are contagious, and 50.8% are environmental ones. The most common among the isolates of mastitis-causing pathogens were *Streptoccocus agalactiae* – 16.9%, *Streptococcus uberis* – 10.9%, *Staphylococcus aureus* – 10.7%, E. Coli – 9.6%, *Corynebacterium bovis* – 7.3%, *Staphylococcus haemolyticus* – 4.8%, *Staphylococcus chromogenes* – 3.6%, *Streptococcus dysgalactiae* – 3.4%. Mastitis is caused by algae and yeast – 1% of all detected pathogens, respectively. According to the results of the determination of the sensitivity of isolates of mastitis-causing pathogens to antibiotics, it was found that most isolates were sensitive to Amoxicillin, Ceftiofur, and Rifampicin, and least of them – to Neomycin, Tylosin, Tilmicosin, Bacitracin.

Keywords: mastitis, milk, contagious, environmental, pathogen, cows, antibiotic resistance

INTRODUCTION

Dairy products, especially milk, are among the most important food sources for most of the world's population. The growing global demand for dairy products necessitates an increase in the average milk yield per cow. The increase in milk yields resulted from genetic selection and an improvement in the technology of feeding and keeping cows **[1]**. Bovine mastitis is an inflammatory reaction of udder tissue caused by physical injury or infection **[2]**. Mastitis is considered the most common disease that leads to significant economic losses in the dairy industry due to decreased milk yields, shortage of milk, treatment costs, and premature culling of sick animals. It is one of the most important diseases on cattle dairy farms, which affects udders, as well as the quantity and quality of milk, the increase in the number of culling, and the death of affected animals **[3]**, **[4]**, **[5]**. Mastitis is a major concern for global milk production; in the works **[6]**, **[7]**, it was reported that the economic losses attributable to mastitis were approximately $1 billion to $2 billion per year in the U.S. dairy industry. Researchers **[8]** noted that in Canada, this figure was 400 million Canadian dollars. According to the authors **[9]**, annual economic losses due to mastitis in India amounted to 60,532.1 million Indian rupees. According to current

estimates, the costs associated with cow mastitis in Europe amount to 1.55 billion euros annually **[10]**. It should be noted that worldwide financial losses associated with mastitis are estimated at 53 billion US dollars **[11]**.

Mastitis is caused mainly by bacterial infections and is classified based on epidemiology into contagious and environmental mastitis. The first one is caused by bacteria such as *Staphylococcus aureus* or *Streptococcus agalactiae*, which are transmitted from an infected cow to a healthy one, usually during milking through the operator's hands, reusable towels, and/or a milking machine, which is a reservoir of bacteria **[12]**, **[13]**. Environmental mastitis is caused by pathogenic environmental microorganisms found in litter, soil, manure, and feed. These include *Escherichia coli*, *Klebsiella* spp. **[14]**.

One of the traditional methods of therapy for cow mastitis is using antibiotics **[15]**, **[16]**. The authors **[17]** found that due to the bacterial diversity associated with bovine mastitis and the infrequent pathogen identification, broad-spectrum antimicrobials against gram-negative and gram-positive microorganisms are regularly used in the dairy industry. After decades of antimicrobial use, bacterial resistance is a growing concern in veterinary and humane medicine. Monitoring patterns of sensitivity of clinical isolates is an important aspect of the One Health approach. In the European Commission Guidelines on the prudent use of antimicrobials in veterinary medicine, it is recommended to test isolated pathogens of mastitis for sensitivity to antibiotics before treatment of animals with antimicrobials to prevent the multiplication of resistant bacteria by rationally selecting appropriate antimicrobials.

This study aimed to identify the pathogens isolated from the samples of mammary gland secretion of cows with mastitis and to determine the sensitivity of the main mastitis pathogens to antibiotics in Ukrainian farms.

Scientific Hypothesis

We expect that isolates of mastitis-causing pathogens from mammary gland secretion of cows with mastitis will show various sensitivity and, in some cases, resistance to certain antibiotics. This will allow us to determine which of the listed antibiotics can be recommended for effective therapy for animals sick with mastitis and which ones cannot. Studying the mammary gland secretion of cows with mastitis, identifying pathogens, and determining their sensitivity to antibiotics is an effective means of increasing the production of high-quality and safe dairy products.

MATERIAL AND METHODOLOGY

Samples

The samples of mammary gland secretion of cows received during 2019-2023 for study in the Laboratory of Bacteriology and Pathology of the Center for Veterinary Diagnostics LLC were in sterile test tubes. The samples were collected from farms specialising in raising cattle and having a dairy production direction. The research was conducted on 168 dairy farms. Immediately after collection, the samples were cooled to a temperature of +2 to +4 °C and immediately transported to the laboratory within 12 hours. A total of 1506 samples were analyzed. A bacteriological examination of 1,506 samples of udder secretions taken from cows suffering from clinical and subclinical forms of mastitis revealed 1,257 samples as positive. 115 samples had a negative result - no growth of microorganisms. Contamination was detected in 134 samples of udder secretion (Figure 1).

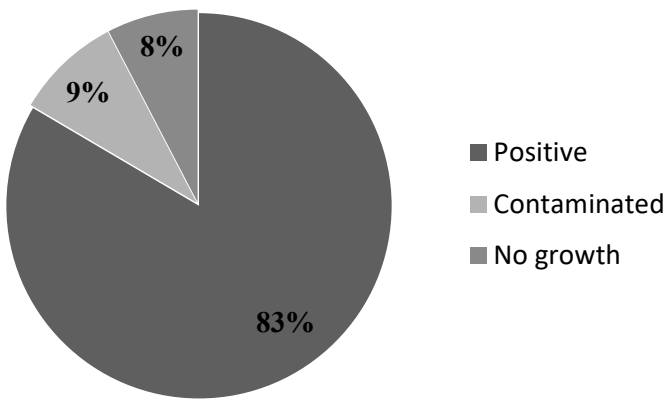


Figure 1 The results of the study of individual samples of the secretions taken from the udders.

Chemicals

COLUMBIA LAB-AGAR + 5% KB blood agar (BioMaxima S.A., Poland).
API 20 E blood agar (BioMérieux, France).
Mueller–Hinton agar (Oxoid, Great Britain).
MacConkey agar (Oxoid, Great Britain).
Antibiotic discs (Oxoid, Great Britain; Condalab, Spain).
Erba indole test (Lachema, Czech Republic).
Oxidase test (HiMedia Laboratories, India).
Catalase test (Technopharm LLC, Ukraine).
Gram stain *Química Clínica Aplicada* (S.A., Spain).

Animals, Plants and Biological Materials

Cows were of various breeds (Holstein, Ukrainian black-and-white, jersey), of different ages, and differed in the number of days of lactation and productivity. No information regarding the size of the livestock, diet structure, maintenance technologies, watering, milking system, and milk production. The secretions were taken from the udders of cows with mastitis.

Instruments

Microbiological analyzer MALDI-TOF VITEK®MS (BioMérieux, France).
Mass spectrometry system VITEK MS KB V3.2.0 US Version (BioMérieux, France).
Petri dishes (TOV Micromed).
Microbiological loops (TOV Micromed).

Laboratory Methods

The bacteriological study was carried out by inoculating 0.1 ml of the test milk sample, which was applied with a microbiological loop to the blood agar, followed by cultivation under aerobic conditions at a temperature of +37 °C for 24-48 hours. If more than three species of microorganisms were sown, such a sample was considered to be contaminated and not further studied.

Conventional bacteriological methods carried out the identification of bacterial cultures obtained on blood agar and the MALDI-TOF method using VITEK ®MS device **[18]**. The VITEK MS KB V3.2.0 US Version database was used to analyze the mass spectra.

The sensitivity of isolates to antibiotics was determined using the Kirby-Bauer disc diffusion method **[19]** in vitro on Mueller-Hinton agar, using Oxoid commercial discs, with the following disc action: Amoxicillin (25 mg/disc), Enrofloxacin (10 mg/disc), Streptomycin (10 mg/disc), Trimethoprim/Sulfamethoxazole (25 mg/disc), Oxytetracycline (30 mg/disc), Ceftiofur (30 mg/disc), Ampicillin (10 mg/disc), Gentamicin (10 mg/disc), Neomycin (30 mg/disc), Lincomycin (15 mg/disc), Cloxacillin (5 mg/disc), Tylosin (30 mg/disc), Bacitracin (0.04 mg/disc), Cephalexin (30 mg/disc), Danofloxacin (5 mg/disc), Spiramycin (100 mg/disc), Marbofloxacin (5 mg/disc), Tilmicosin (15 mg/disc), Rifampicin (5 mg/disc), Cefquin (30 mg/disc). The results of the determination of the diameter of the growth retardation zone for each disc were recorded as sensitive, intermediate, or resistant.

Description of the Experiment

Sample preparation: The bacteriological study of 1,506 secretions taken from the cows' udders with clinical and subclinical forms of mastitis found that 1,257 samples were positive, 115 samples were negative – no growth of microorganisms, and 134 secretions were contaminated

Number of samples analyzed: 1,506 samples were analyzed.

Number of repeated analyses: All measurements were performed 5 times.

Number of experiment replication: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: First, we chose farms that specialize in raising cattle and have a dairy-oriented productivity direction to take samples from each farm individually. The study was conducted on 168 dairy farms in Ukraine. 5 persons conducted all on-farm studies between January 2022 and October 2023. The maintenance conditions and milking procedures were evaluated and documented in a standardized data collection form. The milking pattern was recorded by observation during one milking period. Following the clinical examination of cow udders and the study of secretions using the California Mastitis Test, the samples of secretion of animals sick with mastitis were taken and placed in sterile test tubes. The samples were then cooled to +2 to +4 °C and immediately transported to the laboratory within 12 hours after sampling. The selected samples of secretions from udders were subjected to bacteriological study, identified with the subsequent study of the selected isolates for antimicrobial substances according to the methods **[18]**, **[19]**.

Statistical analysis

The results were evaluated using statistical software Statgraphics Centurion XVII (StatPoint, USA) – multi-factor analysis of variance (MANOVA), LSD test. Statistical processing was performed in Microsoft Excel 2016 in combination with XLSTAT. Values were estimated using mean and standard deviations. The reliability of the research results was assessed according to the Student's test.

RESULTS AND DISCUSSION

The results of the bacteriological study of individual samples of secretion from the udder (from the affected particles of the udder) showed that *Streptococcus agalactiae*, *Streptococcus uberis*, *Staphylococcus aureus*, *E. coli*, and *Corynebacterium bovis* were mostly isolated from the samples tested. During the study period, with associations taken into account, 1,351 isolates were isolated. The main isolates are presented in Table 1 and Figure 2.

Table 1 The mastitis-causing pathogens isolated from the udder secretion of cows with mastitis for 2019-2023.

Microorganisms	Number of isolates
Streptococcus agalactiae	211
Streptococcus uberis	136
Staphylococcus aureus	134
Escherichia coli	120
Corynebacterium bovis	91
Staphylococcus haemolyticus	60
Staphylococcus chromogenes	45
Streptococcus dysgalactiae	43
Corynebacterium spp.	40
Aerococcus viridans	39
Staphylococcus spp.	36
Trueperella pyogenes	31
Streptococcus parauberis	25
Staphylococcus equorum	23
Staphylococcus epidermidis	23
Staphylococcus xylosus	15
Staphylococcus sciuri	15
Enterococcus faecalis	15
Streptoccocus spp.	13
Pasteurella multocida	12
Prototheca spp.	12
Lactococcus lactis	11
Streptoccocus mitis	11
Staphylococcus arlettae	10
Micrococcus spp.	8
Enterobacter cloacae	8
Klebsiella pneumoniae	8
Enterococcus faecium	7
Corynebacterium xerosis	7
Staphylococcus simulans	6
Lactococcus garvieae	6
Klebsiella terrigena	6
Candida kefyr	6
Bacillus licheniformis	6
Enterobacter amnigenus	5
Pseudomonas aeruginosa	5
Total	**1249**

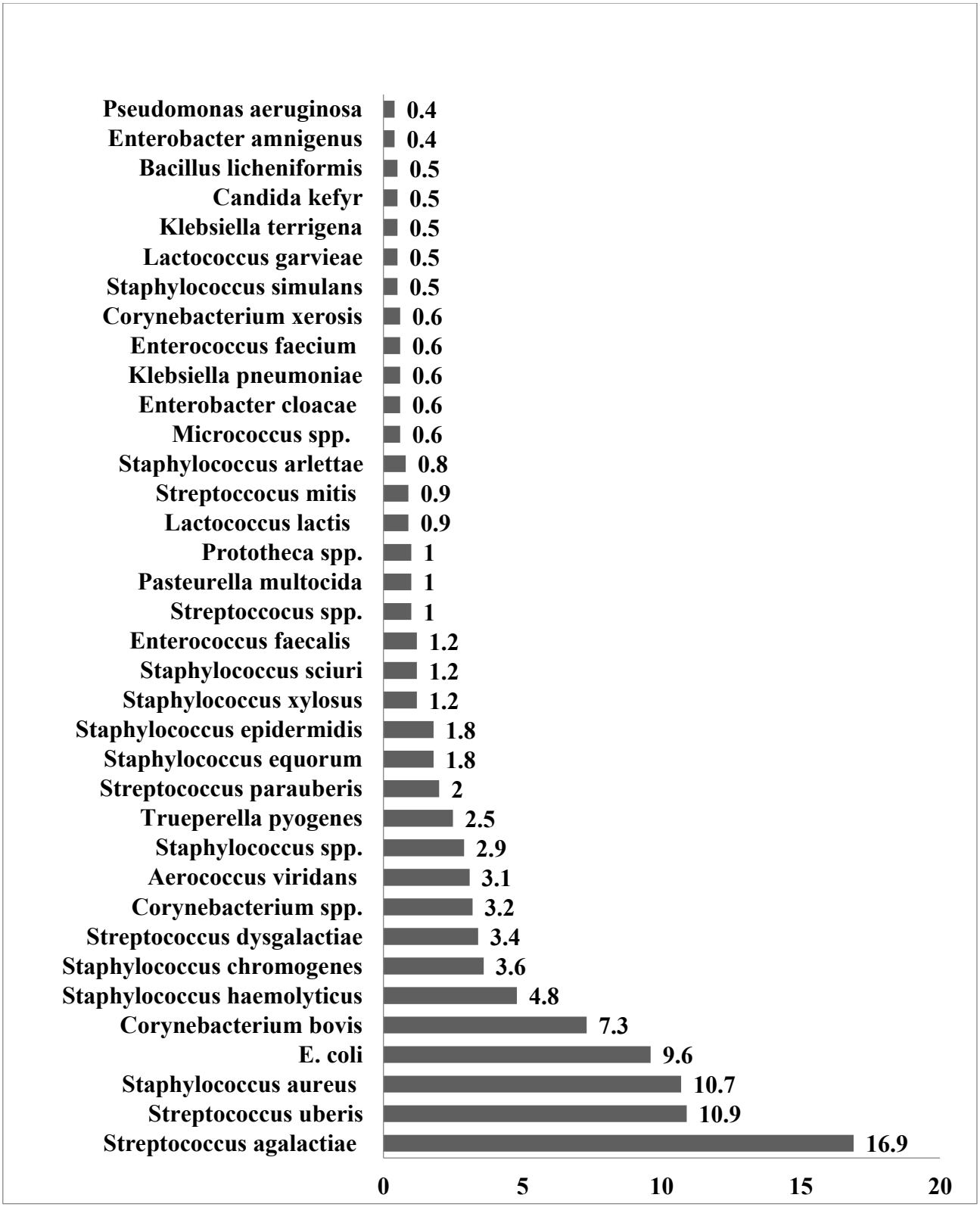


Figure 2 The microbial landscape of the mastitis-causing pathogens (%) isolated from udder secretion of cows during 2019-2023.

During bacteriological studies, a group of microorganisms was isolated quite rarely. The total number of isolates of such rare mastitis-causing pathogens was 102 isolates (Table 2).

Table 2 Mastitis-causing pathogens that were rarely isolated during bacteriological study of udder secretion.

Microorganisms	Number of isolates
1	**2**
Staphylococcus cohnii, Staphylococcus auricularis, Staphylococcus vitulinus, Staphylococcus hyicus, Streptococcus suis.	4*
Staphylococcus capitis, Bacillus altitudinis, Moraxella osloensis,Citrobacter freundi, Corynebacterium amycolatum, Corynebacterium pilosum, Corynebacterium glutamicum, Corynebacterium pseudodiphtheriticum, Acinetobacter iwoffii,Candida crusei, Pantoea spp., Enterococcus durans.	3*
Serratias pp., Pseudomonas fluorescens,Corynebacterium aurimucosum.	2*
Staphylococcus warneri, Streptoccocus alactolyticus, Streptococcus salivarius, Streptococcus pluranimalium, Streptoccocus bovis, Streptoccocus pseudoporcinus, Streptoccocus equisimilis, Streptoccocus canis, Streptoccocus pyogenes, Streptoccocus gallolyticus, Corynebacterium confusum, Corynebacterium tuberculostearicum, Corynebacterium ammoniagenes, Corynebacterium freneyi, Corynebacterium casei, Corynebacterium jeikeium, Enterobacter ludwigii, Enterobacter aerogenes, Empedobacter brevis, Enterobacter spp., Bacillus pumilus, Bacillus altitudinis, Bacillus cereus, Lactococcus raffinolactis, Lactobacillus delbrueckii, Acinetobacter johnsonii, Acinetobacter ursingii, Candida tropicalis, Candida rugosa, Enterococcus italicus, Serratia grimesii, Citrobacter werkmanii, Pseudomonas spp., Macrococcus caseolyticus, Brevibacillus spp., Kocuria carniphila, Neisseria flava, Aeromonas sobria, Klebsiella oxytoca, Proteus mirabilis, Psychrobacter phenylpyruvicus, Raoutella terrigena, Carnobacterium maltaromaticum, Aerococcus vaginalis.	1*

Note: * – the number of isolates of each of the microorganisms specified.

The distribution of isolated contagious and environmental mastitis-causing pathogens is shown in Figure 3.

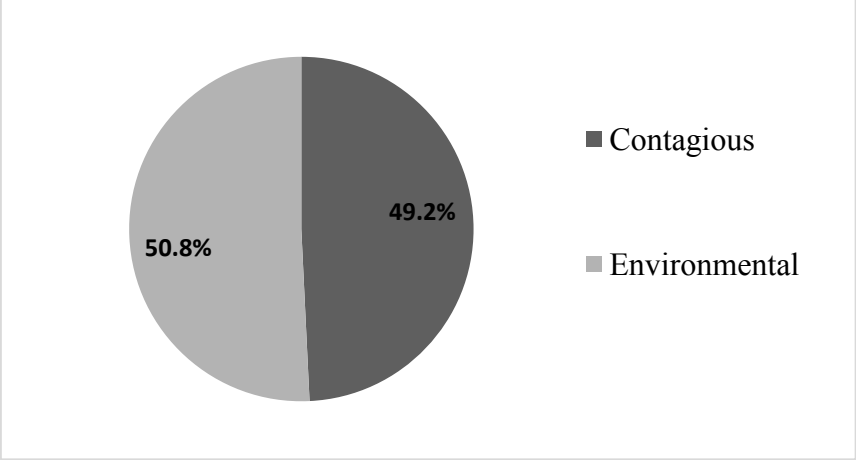


Figure 3 The distribution of isolated mastitis-causing pathogens according to their contagiousness.

The results of the determination of the sensitivity of isolated mastitis-causing pathogens to antibiotics are presented in Tables 3-11.

Table 3 Sensitivity of isolates to antibiotics.

Antibiotic	*Streptococcus agalactiae*	%	*Streptococcus uberis*	%	*Staphylococcus aureus*	%	*E. coli*	%
Amoxicillin (25 µg/disc)	198	93.8	128	94.1	82	61.2	74	61.7
Enrofloxacine (10 µg/disc)	102	48.3	95	69.9	119	88.8	101	84.2
Streptomycin (10 µg/disc)	91	43.1	22	16.2	95	70.9	68	56.7
Trimethoprim/ Sulfamethoxazole (25µg/disc)	148	70.1	100	73.5	116	86.6	98	81.7
Oxytetracyclin (30 µg/disc)	120	56.9	51	37.5	96	71.6	92	76.7
Ceftiofur (30µg/disc)	171	81.0	104	76.5	107	79.9	89	74.2
Ampicillin (10 µg/disc)	133	63.0	78	57.4	58	43.3	6	5.0
Gentamicin (10 µg/disc)	68	32.2	43	31.6	120	89.6	108	90.0
Neomycin (30 µg/disc)	29	13.7	21	15.4	98	73.1	59	49.2
Lincomycin (15 µg/disc)	180	85.3	62	45.6	103	76.9	0	0
Cloxacillin (5 µg/disc)	171	81.0	98	72.1	128	95.5	0	0
Tylosin (30µg/disc)	118	55.9	55	40.4	62	46.3	0	0
Bacitracin (0.04 µg/disc)	86	40.8	59	43.4	53	39.6	0	0
Cephalexine (30 µg/disc)	95	45.0	115	84.6	93	69.4	2	1.7
Danofloxacin (5 µg/disc)	49	23.2	49	36.0	94	70.1	83	69.2
Spiramycin (100 µg/disc)	140	66.4	74	54.4	60	44.8	0	0
Marbofloxacin (5 µg/disc)	94	44.5	87	64.0	121	90.3	113	94.2
Tilmicosin (15 µg/disc)	67	31.8	17	12.5	73	54.5	1	0.8
Rifampicin (5 µg/disc)	186	88.2	116	85.3	131	97.8	0	0
Cefquinome (30 µg/disc)	120	56.9	74	54.4	50	37.3	75	62.5

Table 4 Sensitivity of isolates to antibiotics.

Antibiotic	*Corynebacterium bovis*	%	*Staphylococcus haemolyticus*	%	*Staphylococcus chromogenes*	%	*Streptococcus dysgalactiae*	%
Amoxicillin (25 µg/disc)	89	97.8	53	88.3	39	86.7	42	97.7
Enrofloxacine (10 µg/disc)	77	84.6	57	95.0	44	97.8	32	74.4
Streptomycin (10 µg/disc)	80	87.9	52	86.7	37	82.2	28	65.1
Trimethoprim/ Sulfamethoxazole (25µg/disc)	7	7.7	52	86.7	44	97.8	35	81.4
Oxytetracyclin (30 µg/disc)	83	91.2	55	91.7	41	91.1	29	67.4
Ceftiofur (30µg/disc)	87	95.6	59	98.3	45	100	37	86.0
Ampicillin (10 µg/disc)	66	72.5	32	53.3	30	66.7	33	76.7
Gentamicin (10 µg/disc)	83	91.2	56	93.3	45	100	31	72.1
Neomycin (30 µg/disc)	65	71.4	58	96.7	44	97.8	18	41.9
Lincomycin (15 µg/disc)	62	68.1	38	63.3	36	80.0	28	65.1
Cloxacillin (5 µg/disc)	46	50.5	58	96.7	45	100	42	97.7
Tylosin (30µg/disc)	65	71.4	45	75.0	23	51.1	26	60.5
Bacitracin (0.04 µg/disc)	57	62.6	31	51.7	19	42.2	23	53.5
Cephalexine (30 µg/disc)	50	54.9	58	96.7	45	100	33	76.7
Danofloxacin (5 µg/disc)	57	62.6	50	83.3	40	88.9	20	46.5
Spiramycin (100 µg/disc)	68	74.7	49	81.7	26	57.8	30	69.8
Marbofloxacin (5 µg/disc)	65	71.4	54	90.0	44	97.8	31	72.1
Tilmicosin (15 µg/disc)	63	69.2	43	71.7	22	48.9	29	67.4
Rifampicin (5 µg/disc)	86	94.5	58	96.7	45	100	41	95.3
Cefquinome (30 µg/disc)	61	67.0	37	61.7	35	77.8	32	74.4

Table 5 Sensitivity of isolates to antibiotics.

Antibiotic	*Corynebacterium spp.*	%	*Aerococcus viridians*	%	*Staphylococcus* spp.	%	*Trueperella pyogenes*	%
Amoxicillin (25 µg/disc)	28	70	36	92.3	28	77.8	29	93.5
Enrofloxacine (10 µg/disc)	28	70	14	35.9	30	83.3	22	71.0
Streptomycin (10 µg/disc)	28	70	9	23.1	31	86.1	20	64.5
Trimethoprim/ Sulfamethoxazole (25µg/disc)	5	12.5	25	64.1	25	69.4	8	25.8
Oxytetracyclin (30 µg/disc)	26	65	24	61.5	24	66.7	10	32.3
Ceftiofur (30µg/disc)	29	72.5	27	69.2	23	63.9	26	83.9
Ampicillin (10 µg/disc)	34	85	21	53.8	15	41.7	17	54.8
Gentamicin (10 µg/disc)	35	87.5	21	53.8	28	77.8	26	83.9
Neomycin (30 µg/disc)	24	60	10	25.6	29	80.6	1	3.2
Lincomycin (15 µg/disc)	24	60	10	25.6	19	52.8	27	87.1
Cloxacillin (5 µg/disc)	16	40	19	48.7	24	66.7	27	87.1
Tylosin (30µg/disc)	20	50	14	35.9	21	58.3	25	80.6
Bacitracin (0.04 µg/disc)	31	77.5	18	46.2	15	41.7	7	22.6
Cephalexine (30 µg/disc)	25	62.5	26	66.7	27	75.0	19	61.3
Danofloxacin (5 µg/disc)	22	55	5	12.8	22	61.1	11	35.5
Spiramycin (100 µg/disc)	25	62.5	21	53.8	24	66.7	26	83.9
Marbofloxacin (5 µg/disc)	28	70	11	28.2	30	83.3	23	74.2
Tilmicosin (15 µg/disc)	20	50	6	15.4	24	66.7	28	90.3
Rifampicin (5 µg/disc)	36	90	26	66.7	31	86.1	28	90.3
Cefquinome (30 µg/disc)	11	27.5	13	33.3	15	41.7	15	48.4

Table 6 Sensitivity of isolates to antibiotics.

Antibiotic	*Streptococcus parauberis*	%	*Staphylococcus equorum*	%	*Staphylococcus epidermidis*	%	*Staphylococcus xylosus*	%
Amoxicillin (25 µg/disc)	13	52	23	100	10	43.5	15	100
Enrofloxacine (10 µg/disc)	10	40	23	100	14	60.9	13	86.7
Streptomycin (10 µg/disc)	9	36	23	100	14	60.9	15	100
Trimethoprim/ Sulfamethoxazole (25µg/disc)	10	40	20	87	8	34.8	15	100
Oxytetracyclin (30 µg/disc)	4	16	22	95.7	12	52.2	15	100
Ceftiofur (30µg/disc)	12	48	20	87	14	60.9	13	86.7
Ampicillin (10 µg/disc)	15	60	19	82.6	5	21.7	4	26.7
Gentamicin (10 µg/disc)	5	20	23	100	15	65.2	15	100
Neomycin (30 µg/disc)	1	4	23	100	15	65.2	15	100
Lincomycin (15 µg/disc)	12	48	13	56.5	13	56.5	8	53.3
Cloxacillin (5 µg/disc)	16	64	22	95.7	13	56.5	13	86.7
Tylosin (30µg/disc)	8	32	20	87.0	16	69.6	7	46.7
Bacitracin (0.04 µg/disc)	11	44	7	30.4	8	34.8	3	20.0
Cephalexine (30 µg/disc)	16	64	23	100	15	65.2	13	86.7
Danofloxacin (5 µg/disc)	5	20	23	100	13	56.5	10	66.7
Spiramycin (100 µg/disc)	13	52	20	87	19	82.6	10	66.7
Marbofloxacin (5 µg/disc)	10	40	23	100	14	60.9	15	100
Tilmicosin (15 µg/disc)	2	8	18	78.3	10	43.5	9	60.0
Rifampicin (5 µg/disc)	14	56	23	100	22	95.7	13	86.7
Cefquinome (30 µg/disc)	2	8	16	69.6	12	52.2	11	73.3

Table 7 Sensitivity of isolates to antibiotics.

Antibiotic	*Staphylococcus sciuri*	%	*Enterococcus faecalis*	%	*Streptoccocus* spp.	%	*Pasteurella multocida*	%
Amoxicillin (25 µg/disc)	14	93.3	13	86.7	11	84.6	12	100
Enrofloxacine (10 µg/disc)	14	93.3	6	40.0	7	53.8	12	100
Streptomycin (10 µg/disc)	11	73.3	0	0.0	6	46.2	5	41.7
Trimethoprim/ Sulfamethoxazole (25µg/disc)	14	93.3	8	53.3	5	38.5	10	83.3
Oxytetracyclin (30 µg/disc)	10	66.7	4	26.7	5	38.5	11	91.7
Ceftiofur (30µg/disc)	10	66.7	2	13.3	8	61.5	10	83.3
Ampicillin (10 µg/disc)	3	20.0	6	40.0	8	61.5	8	66.7
Gentamicin (10 µg/disc)	14	93.3	4	26.7	7	53.8	6	50.0
Neomycin (30 µg/disc)	13	86.7	0	0.0	3	23.1	2	16.7
Lincomycin (15 µg/disc)	0	0	2	13.3	7	53.8	1	8.3
Cloxacillin (5 µg/disc)	13	86.7	0	0	5	38.5	6	50.0
Tylosin (30µg/disc)	9	60.0	1	6.7	3	23.1	4	33.3
Bacitracin (0.04 µg/disc)	5	33.3	5	33.3	4	30.8	1	8.3
Cephalexine (30 µg/disc)	14	93.3	2	13.3	3	23.1	11	91.7
Danofloxacin (5 µg/disc)	10	66.7	3	20.0	3	23.1	9	75.0
Spiramycin (100 µg/disc)	6	40.0	3	20.0	6	46.2	7	58.3
Marbofloxacin (5 µg/disc)	14	93.3	6	40.0	4	30.8	12	100
Tilmicosin (15 µg/disc)	13	86.7	0	0.0	1	7.7	9	75.0
Rifampicin (5 µg/disc)	13	86.7	5	33.3	4	30.8	12	100
Cefquinome (30 µg/disc)	10	66.7	1	6.7	2	15.4	11	91.7

Table 8 Sensitivity of isolates to antibiotics.

Antibiotic	*Lactococcus lactis*	%	*Streptoccocus mitis*	%	*Staphylococcus arlettae*	%	*Micrococcus* spp.	%
Amoxicillin (25 µg/disc)	10	90.9	7	63.6	4	40	8	100
Enrofloxacine (10 µg/disc)	7	63.6	6	54.5	10	100	8	100
Streptomycin (10 µg/disc)	4	36.4	8	72.7	9	90	7	87.5
Trimethoprim/ Sulfamethoxazole (25µg/disc)	5	45.5	10	90.9	10	100	5	62.5
Oxytetracyclin (30 µg/disc)	5	45.5	7	63.6	5	50	6	75
Ceftiofur (30µg/disc)	8	72.7	11	100	9	90	8	100
Ampicillin (10 µg/disc)	6	54.5	8	72.7	0	0	7	87.5
Gentamicin (10 µg/disc)	8	72.7	5	45.5	10	100	8	100
Neomycin (30 µg/disc)	5	45.5	1	9.1	10	100	6	75
Lincomycin (15 µg/disc)	5	45.5	11	100	2	20	7	87.5
Cloxacillin (5 µg/disc)	2	18.2	10	90.9	8	80	7	87.5
Tylosin (30µg/disc)	5	45.5	11	100	8	80	6	75
Bacitracin (0.04 µg/disc)	1	9.1	8	72.7	0	0	7	87.5
Cephalexine (30 µg/disc)	2	18.2	9	81.8	8	80	7	87.5
Danofloxacin (5 µg/disc)	5	45.5	5	45.5	10	100	8	100
Spiramycin (100 µg/disc)	5	45.5	11	100	6	60	6	75
Marbofloxacin (5 µg/disc)	7	63.6	9	81.8	10	100	8	100
Tilmicosin (15 µg/disc)	3	27.3	9	81.8	3	30	5	62.5
Rifampicin (5 µg/disc)	2	18.2	11	100	10	100	8	100
Cefquinome (30 µg/disc)	5	45.5	7	63.6	8	80	0	0

Table 9 Sensitivity of isolates to antibiotics.

Antibiotic	*Enterobacter cloacae*	%	*Klebsiella pneumoniae*	%	*Enterococcus faecium*	%	*Corynebacterium xerosis*	%
Amoxicillin (25 µg/disc)	4	50	0	0	7	100	7	100
Enrofloxacine (10 µg/disc)	8	100	8	100	0	0	7	100
Streptomycin (10 µg/disc)	7	87.5	5	62.5	2	28.6	4	57.1
Trimethoprim/ Sulfamethoxazole (25µg/disc)	8	100	8	100	3	42.9	6	85.7
Oxytetracyclin (30 µg/disc)	7	87.5	7	87.5	6	85.7	7	100
Ceftiofur (30µg/disc)	5	62.5	7	87.5	0	0	4	57.1
Ampicillin (10 µg/disc)	0	0	0	0	1	14.3	7	100
Gentamicin (10 µg/disc)	8	100	8	100	3	42.9	7	100
Neomycin (30 µg/disc)	7	87.5	6	75	1	14.3	7	100
Lincomycin (15 µg/disc)	0	0	0	0	3	42.9	4	57.1
Cloxacillin (5 µg/disc)	0	0	0	0	0	0	7	100
Tylosin (30µg/disc)	0	0	0	0	2	28.6	4	57.1
Bacitracin (0.04 µg/disc)	0	0	0	0	3	42.9	5	71.4
Cephalexine (30 µg/disc)	0	0	0	0	0	0	7	100
Danofloxacin (5 µg/disc)	8	100	8	100	0	0	4	57.1
Spiramycin (100 µg/disc)	0	0	0	0	4	57.1	4	57.1
Marbofloxacin (5 µg/disc)	8	100	8	100	0	0	6	85.7
Tilmicosin (15 µg/disc)	0	0	0	0	0	0	4	57.1
Rifampicin (5 µg/disc)	0	0	0	0	3	42.9	7	100
Cefquinome (30 µg/disc)	8	100	8	100	0	0	6	85.7

Table 10 Sensitivity of isolates to antibiotics.

Antibiotic	*Staphylococcus simulans*	%	*Lactococcus garvieae*	%	*Klebsiella terrigena*	%	*Bacillus licheniformis*	%
Amoxicillin (25 µg/disc)	6	100	6	100	0	0	2	33.3
Enrofloxacine (10 µg/disc)	5	83.3	2	33.3	5	83.3	6	100
Streptomycin (10 µg/disc)	5	83.3	0	0	1	16.7	4	66.7
Trimethoprim/ Sulfamethoxazole (25µg/disc)	5	83.3	0	0	3	50	6	100
Oxytetracyclin (30 µg/disc)	5	83.3	3	50	3	50	6	100
Ceftiofur (30µg/disc)	5	83.3	2	33.3	5	83.3	0	0
Ampicillin (10 µg/disc)	5	83.3	2	33.3	0	0	0	0
Gentamicin (10 µg/disc)	6	100	1	16.7	6	100	6	100
Neomycin (30 µg/disc)	6	100	1	16.7	2	33.3	6	100
Lincomycin (15 µg/disc)	6	100	0	0	0	0	0	0
Cloxacillin (5 µg/disc)	6	100	0	0	0	0	1	16.7
Tylosin (30µg/disc)	6	100	0	0	0	0	2	33.3
Bacitracin (0.04 µg/disc)	1	16.7	0	0	0	0	0	0
Cephalexine (30 µg/disc)	6	100	0	0	1	16.7	3	50
Danofloxacin (5 µg/disc)	6	100	0	0	3	50	5	83.3
Spiramycin (100 µg/disc)	2	33.3	0	0	0	0	5	83.3
Marbofloxacin (5 µg/disc)	6	100	1	16.7	6	100	6	100
Tilmicosin (15 µg/disc)	3	50	0	0	0	0	2	33.3
Rifampicin (5 µg/disc)	6	100	0	0	0	0	4	66.7
Cefquinome (30 µg/disc)	4	66.7	1	16.7	3	50	0	0

Table 11 Sensitivity of isolates to antibiotics.

Antibiotic	*Enterobacter amnigenus*	%	*Pseudomonas aeruginosa*	%
Amoxicillin (25 µg/disc)	1	20	0	0
Enrofloxacine (10 µg/disc)	4	80	3	60
Streptomycin (10 µg/disc)	2	40	0	0
Trimethoprim/ Sulfamethoxazole (25µg/disc)	5	100	0	0
Oxytetracyclin (30 µg/disc)	2	40	0	0
Ceftiofur (30µg/disc)	2	40	0	0
Ampicillin (10 µg/disc)	0	0	0	0
Gentamicin (10 µg/disc)	5	100	5	100
Neomycin (30 µg/disc)	4	80	0	0
Lincomycin (15 µg/disc)	0	0	0	0
Cloxacillin (5 µg/disc)	0	0	0	0
Tylosin (30µg/disc)	0	0	0	0
Bacitracin (0.04 µg/disc)	0	0	0	0
Cephalexine (30 µg/disc)	0	0	0	0
Danofloxacin (5 µg/disc)	5	100	3	60
Spiramycin (100 µg/disc)	0	0	0	0
Marbofloxacin (5 µg/disc)	5	100	5	100
Tilmicosin (15 µg/disc)	0	0	0	0
Rifampicin (5 µg/disc)	0	0	0	0
Cefquinome (30 µg/disc)	2	40	0	0

The sensitivity of isolates to antibiotics is shown in Table 12.

Table 12 Sensitivity of isolates to antibiotics.

Antibiotic	Number of sensitive isolates		Number of resistant isolates	
	units	%*	units	%*
Amoxicillin (25 µg/disc)	976	**78.1**	273	21.9
Enrofloxacine (10 µg/disc)	879	**70.4**	370	29.6
Streptomycin (10 µg/disc)	702	56.2	547	43.8
Trimethoprim/ Sulfamethoxazole (25µg/disc)	827	66.2	422	33.8
Oxytetracyclin (30 µg/disc)	803	64.3	446	35.7
Ceftiofur (30µg/disc)	961	**76.9**	288	23.1
Ampicillin (10 µg/disc)	625	50.0	624	50.0
Gentamicin (10 µg/disc)	839	67.2	410	32.8
Neomycin (30 µg/disc)	590	47.2	659	**52.8**
Lincomycin (15 µg/disc)	683	54.7	566	45.3
Cloxacillin (5 µg/disc)	803	64.3	466	35.7
Tylosin (30µg/disc)	586	46.9	663	**53.1**
Bacitracin (0.04 µg/disc)	468	37.5	781	**62.5**
Cephalexine (30 µg/disc)	728	58.3	521	41.7
Danofloxacin (5 µg/disc)	648	51.9	601	48.1
Spiramycin (100 µg/disc)	670	53.6	579	46.4
Marbofloxacin (5 µg/disc)	884	**70.8**	365	29.2
Tilmicosin (15 µg/disc)	484	38.8	765	**61.2**
Rifampicin (5 µg/disc)	941	**75.3**	308	24.7
Cefquinome (30 µg/disc)	655	52.4	594	47.6

Note: * – the percentage relative to the total number of the isolates obtained – 1.249.

According to the data of Tables 1, 2 and Figures 2, 3, it was found that 615 (49.2%) isolates were accounted for contagious (infectious) cow mastitis-causing pathogens: *Streptoccocus agalactiae* – 211 (16.9%), *Streptococcus uberis* – 136 (10.9%), *Staphylococcus aureus* – 134 (10.7%), *Corynebacterium bovis* – 91 (7.3%), *Streptococcus dysgalactiae* – 43 (3.4%).

634 (50.8%) isolates were accounted for as environmental mastitis-causing pathogens, the main of which were *E. coli* – 120 (9.6%), *Staphylococcus haemolyticus* – 60 (4.8%), and *Staphylococcus chromogenes* – 45 (3.6%).

As can be seen from the antibioticograms obtained (Tables 3-11), the largest number of isolates, among the most common mastitis-causing pathogens, showed sensitivity to the following antibiotics:

– *Streptococcus agalactiae* – to Amoxicillin 198 (93.8%), Rifampicin 186 (88.2%), Lincomycin 180 (85.3%), Ceftiofur and Cloxacillin 171 (81%), respectively, Trimethoprim/sulfamethoxazole 148 (70.1%);

– *Streptococcus uberis* – to Amoxicillin 128 (94.1%), Cephalexin (84.6%), Rifampicin (85, 3%), Ceftiofur 104 (76.5%), Trimethoprim/sulfamethoxazole 100 (73.5%), Cloxacillin 98 (72.1%), Enrofloxacin 95 (69.9%);

– *Staphylococcus aureus* – to Rifampicin 131 (97.8%), Cloxacillin 128 (95.5%), Marbofloxacin 121 (90.3%), Gentamicin 120 (89.6%), Enrofloxacin 119 (88.8%), Trimethoprim/sulfamethoxazole 116 (86.6%), Ceftiofur 107 (79.9%), Lincomycin 103 (76.9%), Neomycin 98 (73.1%), Oxytetracycline 96 (71.6%), Streptomycin 95 (70.9%), Danofloxacin 94 (70.1%), Cefalexin 93 (69.4%);

– *E. coli* – to Marbofloxacin 113 (94.2%), Gentamicin 108 (90%), Enrofloxacin 101 (84.2%), Trimethoprim/sulfamethoxazole 98 (81.7%) isolates, to Oxytetracycline 92 (76.7%), Ceftiofur 89 (74.2%), Danofloxacin 83 (69.2%);

– *Corynebacterium bovis* – to Amoxicillin 89 (97.8%), Ceftiofur 87 (95.6%), Rifampicine 86 (94.5%), Gentamicin and Oxytetracycline 83 (91.2%), Streptomycin 80 (87.9%), Enrofloxacin 77 (84.6%), Spiramycin 68 (74.7%).

The largest number of resistant isolates showed sensitivity to the following antibiotics:

– *Streptococcus agalactiae* and *Streptococcus uberis* – to Neomycin;

– *Staphylococcus aureus* – to Cefquinome

– *E. coli* – to Lincomycin, Cloxacillin, Tylosin, Bacitracin, Spiramycin, Rifampicin;

– *Corynebacterium bovis* – to Trimethoprim/sulfamethoxazole.

According to the results of experimental studies (Table 12), most of the isolates were sensitive to Amoxicillin – 78.1%, Ceftiofur – 76.9%, Rifampicin – 75.3%, Marbofloxacin – 70.8%, Enrofloxacin – 70.4%. At the same time, most of the isolates were resistant to Bacitracin – 62.5%, Tilmicosin – 61.2%, Tylosin – 53.1%, Neomycin – 52.8%.

A significant percentage (70.8 – 50%) of the isolates obtained were sensitive (in descending order) to Marbofloxacin – 70.8%, Enrofloxacin – 70.4%, Gentamicin – 67.2%, Trimethoprim/sulfamethoxazole – 66.2%, Oxytetracycline and Cloxacillin 64.3%, Cephalexin – 58.3%, Streptomycin – 56.2%, Lincomycin – 54.7%, Spiramycin – 53.6%, Cefquin – 52.4%, Danofloxacin – 51.9%, Ampicillin – 50%.

During the bacteriological study of udder secretion from cows with mastitis, algae were isolated 12 times, 1% of the main isolates. Yeasts were sown 14 times, among which the isolates of *Candida kefyr* were the most common.

Mastitis - mammary gland inflammation, is one of the most common diseases of cattle worldwide **[45]**, **[46]**.

The most common reason for using antimicrobial drugs on dairy farms is treating cows for mastitis **[47]**, **[48]**. In addition, the use of broad-spectrum antimicrobials affects the development of resistance to a greater extent than narrow-spectrum antimicrobials **[49]**.

Antimicrobial drugs have been used for about sixty years to treat animals with mastitis. They are often prescribed without a preliminary test to identify the causative agent and determine its sensitivity, which is a rather important part of therapy **[50]**, **[51]**.

The irrational use of antimicrobials has initiated a rapid evolutionary process of bacterial resistance through natural selection and has led to an increase in the frequency and spread of bacterial antimicrobial resistance (AMR). The global emergency and the use of antimicrobials in cows have raised questions about alternative treatment approaches, but the main method of mastitis treatment remains the use of antimicrobials **[52]**, **[53]**.

The studies conducted by the authors **[20]**, **[21]**, **[22]** showed that the most common mastitis-causing pathogens were *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Escherichia coli;* the same data were provided by the researchers from Slovakia **[23]**, which completely coincide with the results of our research and is confirmed by the fact that these pathogens have the most widespread impact on dairy farms not only in Ukraine but also abroad. The studies conducted in Poland **[24]** demonstrated that contagious pathogens such as *Streptococcus agalactiae* and *Staphylococcus aureus* were most often isolated from mastitis milk, these microorganisms are the most common infectious agents that cause intramammary infection in dairy cows, our study results indicated that the most common pathogens isolated from milk of cows with mastitis were

Streptococcus agalactiae – 16.9%, *Streptococcus uberis* – 10.9% and *Staphylococcus aureus* accounted for 10.7% of the 1249 main isolates that we selected. Scientists **[25]** found that *Streptococcus agalactiae* was most often isolated in 1 out of 188 cases of mastitis. The high sensitivity of these bacteria to Cloxacillin was established experimental studies, which were conducted by us, indicating that *Streptococcus agalactiae* was isolated in 211 samples of mastitis milk, and the highest sensitivity of microorganisms was found to amoxicillin, rifampin, and lincomycin. The predominant isolated bacteria on dairy farms in Romania **[26]** were *Staphylococcus* spp. – 43.1%, *Streptococcus* spp. – 22.42%, *E. coli* – 13.79%, *Enterococcus* spp. – 8.62%, *Corynebacterium* spp. – 7.75%, and *Enterobacter* spp. – 4.31%. *S. hycus*, *S. chromogenes*, *S. xylosus*, and *S. capitis* were identified with a lower proportion and accounted for 36.0% of the isolated strains of staphylococci and 15.51% of the total isolates. *Corynebacterium bovis* and *Corynebacterium* spp. were isolated in a proportion of 7.75% of all isolates identified in this study, which partially coincided with the results of our studies. Gram-positive bacteria generally have low susceptibility to most antimicrobials tested **[27]**, **[28]**. The sensitivity of gram-negative bacteria to penicillins and quinolones was quite high. At the same time, resistance to macrolides, aminoglycosides, and tetracyclines was observed **[29]**, **[30]**, when analyzing the antibiotic susceptibility patterns, which we obtained, we recorded that such groups of antimicrobial agents as aminoglycosides and macrolides showed the lowest sensitivity to the isolated isolates, therefore we do not recommend using them to treat cows with mastitis. In Tanzania, the most common bacteria isolated from mastitis milk were *Staphylococcus aureus* (36.8%), *Pseudomonas aeruginosa* (17.8%), *Staphylococcus epidermidis* (16.1%), *Klebsiella* spp. (9.5%), *Micrococcus* spp. (6.3%) and *E. coli* (4.9%) **[31]**, our study results indicated that *Staphylococcus aureus* was detected in 10.7% of isolates, *Pseudomonas aeruginosa* – 0.4%, *Staphylococcus epidermidis* – 1.8%, *Micrococcus* spp. – 0.6% and *E. coli* 9.6%. Several researchers **[32]** informed that the prevalence of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* was 30%, 17%, and 3.5%, respectively. Most (90%) *S. aureus* resisted penicillin, while only 10% of strains resisted oxacillin. Almost half (40%) of *E. coli* strains showed resistance to streptomycin **[33]**, **[34]**, when analyzing the sensitivity of the isolated pathogens to antimicrobial agents, it was noted that 62.7% of *Staphylococcus aureus* isolates were resistant to cefquinome, and 93.8% of isolates were sensitive to amoxicillin, all 120 E. coli isolates were resistant to lincomycin, cloxacillin, tylosin, bacitracin, spiramycin, and rifampicin, which can be explained by the natural resistance of microorganisms to some antimicrobial substances, as well as may be due to the presence of pathogenicity factors such as biofilms, adhesins, as well as some enzymes that inhibit antibiotic.

Researchers **[35]**, **[36]** found that, among the contagious mastitis-causing pathogens, the most common were *Streptococcus agalactiae* – 24.1%, *Staphylococcus aureus* – 18.4%, *Corynebacterium* spp. – 7.2%, *Streptococcus dysgalactiae* – 5.6%, *Streptococcus uberis* – 2.2%. Environmental mastitis-causing pathogens accounted for 42.5% of the total number of isolates. The mastitis caused by yeasts accounted for 1.4%. The greatest sensitivity of the isolates was to Ceftiofur, Amoxicillin/Clavulanic acid, Rifampicin, Amoxicillin, Gentamicin, Ampicillin, Bacitracin, Cephalexin, Cloxacillin, Enrofloxacin, Trimethoprim/Sulfamethoxazole, Oxytetracycline, Lincomycin. The least sensitive – to Spiramycin, Tylosin, Streptomycin, Neomycin, Marbofloxacin, Tilmicosin, and Danofloxacin, which coincided with the results of our studies.

On dairy farms in south-eastern Australia **[44]**, **[37]**, the studies of mastitis-causing pathogens showed that 472 samples (15.5%) out of 3.044 studied samples of cow mammary gland secretion were contaminated, and no growth was noted in 27.5% of the samples. The most common pathogens from clinical samples of mastitis were *Streptococcus uberis* (39.2%), *Staphylococcus aureus* (10.6%), *Escherichia coli* (8.4%), *Streptococcus dysgalactiae* (6.4%), our studies indicated that during the bacteriological examination of 1506 milk samples, 134 (9%) samples were found to be contaminated, the most frequently identified pathogens from the mammary gland secretion of cows with mastitis were *Streptococcus agalactiae*, *Streptococcus uberis*, *Staphylococcus aureus*, *Escherichia coli*, which amounted to 16.9%, 10.9%, 10.7%, and 9.6%, respectively, *Streptococcus dysgalactiae* was isolated in only 3.4% of isolates.

In Ethiopia **[38]**, **[39]**, the most predominant isolated bacterial mastitis-causing pathogens were *Staphylococcus aureus* (42.6%), *Streptococcus* spp. (26.2%) and *Escherichia coli* (11.5%), *Salmonella* spp. (3.3%) and *Klebsiella pneumoniae* (1.6%) were less isolated, as for the pathogen of salmonellosis, we have never detected *Salmonella* spp. during our studies, however, *Klebsiella pneumoniae* was detected in 8 (0.6%) milk samples. Other studies conducted in Ethiopia **[40]**, **[41]** demonstrated that the dominant mastitis-causing pathogens were *Staphylococcus aureus* (40.3%), *Streptococcus* spp. (24.3%), *Staphylococcus* spp. (12.5%), E. *coli* (8.3%), *Staphylococcus hyicus (*3.5%), and *Staphylococcus intermedius* (1.4%). The authors Awandkar et al. **[42]**, and Mahmoud and Yassein **[43]** reported that the prevalence of mastitis caused by yeasts was 1.09%, which also coincided with the results of our studies.

It should be noted, that when analyzing the microbial identification data by the MALDI-TOF MS method, 91.5% of isolates were identified to the species level, and 8.5%, which is 115 isolates, were identified only to the genus level. Most of the unidentified microorganisms are composed of gram-positive microflora – 85.2%.

Nonnemann et al. indicate that 500 isolates of microorganisms isolated from the milk of cows with mastitis, 93.5% of which were identified to the species level, and 6.5% were identified only to the genus level, for example, 4 out of 6 Acinetobacter, 2 out of 9 *Corynebacterium* and 2 in 11 *Bacillus* were identified only at the genus level **[54]**.

In Brazil, for the identification of 380 bacteria isolated from milk samples from bovine patients with mastitis, MALDI-TOF MS showed a typing rate of 95.5%, and the accuracy for identifying *Staphylococcus* isolates was 93.2% **[55]**.

Implementation of national mastitis control programs and evaluation of their effectiveness are mandatory. Some countries are ahead of others in improving approaches to mastitis treatment and controlling antimicrobial consumption on dairy farms; Their expertise can guide the development of further strategies. The health of cows and udders should be regularly monitored, farm management indicators should be improved, risk factors for mastitis should be identified and reduced and infectious agents should be minimized, the use of antimicrobials should be reduced, to develop and implement more effective control measures, alternative farming systems and/or to reduce the consumption of cattle products **[56]**, **[57]**.

The results of our research provide valuable information on the prevalence and sensitivity of cow mastitis pathogens to antibiotics on Ukrainian farms. A comprehensive research approach to pathogen identification, as well as a detailed analysis of antibiotic resistance, fills an important gap in veterinary medicine and farm management practices.

CONCLUSION

The spread of contagious cow mastitis-causing pathogens in the farms of Ukraine is at the level of 49.2%. The most common pathogens are *Streptoccocus agalactiae, Streptococcus uberis, Staphylococcus aureus, Corynebacterium bovis, Streptococcus dysgalactiae.* Environmental mastitis-causing pathogens account for 50.8% of all isolates, among which the most common are *E. coli, Staphylococcus haemolyticus, Staphylococcus chromogenes.* Only 1% of the diagnosed mastitis-causing pathogens are caused by algae (*Prototheca* spp.) and yeasts. The results of the determination of the sensitivity of isolates to 20 antibiotics showed the largest percentage of resistance to Bacitracin, Tilmicosin, Tylosin, Neomycin. The greatest percentage of sensitivity of isolated mastitis-causing pathogens was to Amoxicillin, Ceftiofur, Rifampicin. Thus, these antibiotics can be recommended for inclusion in therapy protocols for cows with mastitis in Ukrainian farms. However, this applies only to the mastitis caused by contagious pathogens, as there is a very large species diversity among environmental pathogens (Gram-positive and Gram-negative microflora), and, accordingly, a large diversity in antibiotic sensitivity; therefore, it is necessary to develop a treatment protocol only based on individual antibioticograms obtained in each case. Further studies are planned to expand the range of mastitis-causing pathogens and improve the analysis of their sensitivity to antimicrobial substances.

REFERENCES

1. Cobirka, M., Tancin, V., & Slama, P. (2020). Epidemiology and Classification of Mastitis. In Animals (Volume 10, Issue 12, p. 2212). MDPI AG. https://doi.org/10.3390/ani10122212
2. Cheng, W. N., & Han, S. G. (2020). Bovine mastitis: risk factors, therapeutic strategies, and alternative treatments – A review. In Asian-Australasian journal of animal sciences (Volume 33, Issue 11, pp. 1699–1713). OCUL. https://doi.org/10.5713/ajas.20.0156
3. Tommasoni, C., Fiore, E., Lisuzzo, A., & Gianesella, M. (2023). Mastitis in Dairy Cattle: On-Farm Diagnostics and Future Perspectives. In Animals (Volume 13, Issue 15, p. 2538). MDPI AG. https://doi.org/10.3390/ani13152538
4. Riveros-Galán, D. S., & Obando-Chaves, M. (2020). Mastitis, somatic cell count and its impact on dairy product quality. An omission in Colombia: a literature review. In Revista Colombiana De Ciencias Pecuarias (Volume 34, Issue 4, pp. 241–253). Universidad de Antioquia, Colombia. https://doi.org/10.17533/udea.rccp.v34n4a01
5. Käppeli, N., Morach, M., Zurfluh, K., Corti, S., Nüesch-Inderbinen, M., & Stephan, R. (2019). Sequence Types and Antimicrobial Resistance Profiles of Streptococcus uberis Isolated From Bovine Mastitis. In Frontiers in Veterinary Science (Volume 6, p. 234). National Library of Medicine. https://doi.org/10.3389/fvets.2019.00234

6. Azooz, M. F., El-Wakeel, S. A., & Yousef, H. M. (2020). Financial and economic analyses of the impact of cattle mastitis on the profitability of Egyptian dairy farms. In Veterinary world (Volume 13, Issue 9, pp.1750–1759). Veterinary World. https://doi.org/10.14202/vetworld.2020.1750-1759
7. Dalanezi, F. M., Joaquim, S. F., Guimarães, F. F., Guerra, S. T., Lopes, B. C., Schmidt, E. M. S., Cerri, R. L. A., & Langoni, H. (2020). Influence of pathogens causing clinical mastitis on reproductive variables of dairy cows. In Journal of dairy science (Volume 103, Issue 4, pp. 3648–3655). Elsevier Inc. https://doi.org/10.3168/jds.2019-16841
8. Nakada, S., Fujimoto, Y., Kohara, J., & Makita, K. (2023). Economic losses associated with mastitis due to bovine leukemia virus infection. In Journal of dairy science (Volume 106, Issue 1, pp. 576–588). Elsevier Inc. https://doi.org/10.3168/jds.2021-20955
9. Bhakat, C., Mohammad, A., Mandal, D. K., Mandal, A., Rai, S., Chatterjee, A., Ghosh, M. K., & Dutta, T. K. (2020). Readily usable strategies to control mastitis for production augmentation in dairy cattle: A review. In Veterinary world (Volume 13, Issue 11, pp. 2364–2370). Veterinary world. https://doi.org/10.14202/vetworld.2020.2364-2370
10. Ateya, A. I., Ibrahim, S. S., & Al-Sharif, M. M. (2022). Single Nucleotide Polymorphisms, Gene Expression and Economic Evaluation of Parameters Associated with Mastitis Susceptibility in European Cattle Breeds. In Veterinary Sciences (Volume 9, Issue 6, pp. 294). MDPI AG. https://doi.org/10.3390/vetsci9060294
11. Pal, M., Regasa, A., & Gizaw, F. (2019). Etiology, pathogenesis, risk factors, diagnosis and management of bovine mastitis: A comprehensive review. In International Journal of Animal and Veterinary Sciences (Volume 6, pp. 40–55). Society of Agricultural Research and Social Development.
12. Ameni, G., Bayissa, B., Zewude, A., Degefa, B. A., Mohteshamuddin, K., Kalaiah, G., Alkalbani, M.S., Eltahir, Y.M., Elfatih Hamad, M., & Tibbo, M. (2022) Retrospective study on bovine clinical mastitis and associated milk loss during the month of its peak occurrence at the National Dairy Farm in the Emirate of Abu Dhabi, United Arab Emirates. Front. In Vet. Sci. (Volume 9, p. 1070051). Frontiers Media SA. https://doi.org/10.3389/fvets.2022.1070051
13. Sharun, K., Dhama, K., Tiwari, R., Gugjoo, M. B., Yatoo, M. I., Patel, S. K., Pathak, M., Karthik, K., Khurana, S. K, Singh, R., Amarpal, P. B., Singh, R., Singh, K. P. & Chaicumpa, W. (2021) Advances in therapeutic and managemental approaches of bovine mastitis: a comprehensive review. In Veterinary Quarterly (Volume 41, Issue 1, pp. 107–136). Taylor & Francis. https://doi.org/10.1080/01652176.2021.1882713
14. Verma, H., Rawat, S., Sharma, N., Jaiswal, V., & Singh, R. B. (2018). Prevalence, bacterial etiology and antibiotic susceptibility pattern of bovine mastitis in Meerut. In Journal of entomology and zoology studies (Volume 6, pp. 706–709). Society of Agricultural Research and Social Development.
15. Rudenko, P., Sachivkina, N., Vatnikov, Y., Shabunin, S., Engashev, S., Kontsevaya, S., Karamyan, A., Bokov, D., Kuznetsova, O., & Vasilieva, E. (2021). Role of microorganisms isolated from cows with mastitis in Moscow region in biofilm formation. In Veterinary world (Volume 14, Issue 1, pp. 40–48). Veterinary world. https://doi.org/10.14202%2Fvetworld.2021.40-48
16. Rainard, P., Gilbert, F. B., Germon, P., & Foucras, G. (2021). Invited review: A critical appraisal of mastitis vaccines for dairy cows. In Journal of dairy science (Volume 104, Issue 10, pp. 10427–10448). American Dairy Science Association. https://doi.org/10.3168/jds.2021-20434
17. Elias, L., Balasubramanyam, A. S., Ayshpur, O. Y., Mushtuk, I. U., Sheremet, N. O., Gumeniuk, V. V., Musser, J. M. B., & Rogovskyy, A. S. (2020). Antimicrobial Susceptibility of Staphylococcus aureus, Streptococcus agalactiae, and Escherichia coli Isolated from Mastitic Dairy Cattle in Ukraine. In Antibiotics. (Volume 9, Issue 8, p. 469). MDPI. https://doi.org/10.3390/antibiotics9080469
18. Chen, X. F., Hou, X., Xiao, M., Zhang, L., Cheng, J. W., Zhou, M. L., Huang, J. J., Zhang, J. J., Xu, Y. C., & Hsueh, P. R. (2021). Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) Analysis for the Identification of Pathogenic Microorganisms: A Review. In Microorganisms (Volume 9, Issue 7, p. 1536). MDPI. https://doi.org/10.3390/microorganisms9071536
19. Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. American Society for Microbiology. Washington, DC: Dec 8, 2009. posting date. Retrieved from http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol.
20. Morales-Ubaldo, A-L., Rivero-Perez, N., Valladares-Carranza, B., Velázquez-Ordoñez, V., Delgadillo-Ruiz, L., & Zaragoza-Bastida, A. (2023). Bovine mastitis, a worldwide impact disease: Prevalence, antimicrobial resistance, and viable alternative approaches. In Veterinary and Animal Science (Volume 21, p. 100306). Elsevier B.V. https://doi.org/10.1016/j.vas.2023.100306

21. Abril, A., Villa, T., Barros-Velázquez, J., Cañas, B., Sánchez-Pérez, A., Calo-Mata, P., & Carrera, M. (2020). *Staphylococcus aureus* Exotoxins and Their Detection in the Dairy Industry and Mastitis. In Toxins (Volume 12, p. 537). MDPI. https://doi.org/10.3390/toxins12090537
22. Abegewi, S. N., Esemu, R. N., & Ndip, L. M. (2022). Prevalence and risk factors of coliform-associated mastitis and antibiotic resistance of coliforms from lactating dairy cows in North West Cameroon. In PloS One (Volume 17, Issue 7, e0268247). Public Library of Science. https://doi.org/10.1371/journal.pone.0268247
23. Holko, I., Tančin, V., Vršková, M., & Tvarožková, K. (2019). Prevalence and antimicrobial susceptibility of udder pathogens isolated from dairy cows in Slovakia. In The Journal of dairy research (Volume 86, 4, pp. 436–439). Cambridge University Press. https://doi.org/10.1017/S0022029919000694
24. Sztachańska, M., Barański, W., Janowski, T., Pogorzelska, J., & Zduńczyk, S. (2016). Prevalence and etiological agents of subclinical mastitis at the end of lactation in nine dairy herds in North-East Poland. In Polish Journal of Veterinary Sciences (Volume 19, Issue 1, pp. 119–124). University of Warmia and Mazury. https://doi.org/10.1515/pjvs-2016-001
25. Ramírez Vásquez, N., Fernández-Silva, J. A., & Palacio, L. G. (2018). Tasa de incidencia de mastitis clínica y susceptibilidad antibiótica de patógenos productores de mastitis en ganado lechero del norte de Antioquia, Colombia. In Rev Med Vet. (Volume 36, pp. 75–87). Ecole Nationale Veterinaire. https://doi.org/10.19052/mv.5173
26. Pascu, C., Herman, V., Iancu, I., & Costinar, L. (2022). Etiology of Mastitis and Antimicrobial Resistance in Dairy Cattle Farms in the Western Part of Romania. In Antibiotics (Volume 11, Issue 1, p. 57). MDPI. https://doi.org/10.3390/antibiotics11010057
27. Kamran, A., Raziq, I., Wazir, R., Ullah, P., Shah, M. I., Ali, B., Han, G., & Liu, G. (2021). Prevalence of mastitis pathogens and antimicrobial susceptibility of isolates from cattle and buffaloes in Northwest of Pakistan Frontiers in Veterinary Science. In Front Vet Sci. (Volume 8, 746755). Public Library of Science. https://doi.org/10.3389/fvets.2021.746755
28. Arbab, S., Ullah, H., Bano, I. Li, K., Hassan, Ul-I., Wang, W., Qadeer, & Zhang, A. J. Evaluation of in vitro antibacterial effect of essential oil and some herbal plant extract used against mastitis pathogens. In Veterinary Medicine and Science (Volume 8, Issue 6, pp. 2655–2661). John Wiley & Sons. https://doi.org/10.1002/vms3.959
29. Granja, B. M., Fidelis, C. E., Garcia, B. L. N., & Dos Santos, M. V. J. (2021). Evaluation of chromogenic culture media for rapid identification of microorganisms isolated from cows with clinical and subclinical mastitis. In J. Dairy Sci. (Volume 104, Issue 8, pp. 9115–9129). Elsevier B.V. https://doi.org/10.3168/jds.2020-19513
30. Martins, L., Gonçalves, J. L., Leite, R. F., Tomazi, T., Rall, V. L. M., & Santos, M. V. J. (2021). Association between antimicrobial use and antimicrobial resistance of Streptococcus uberis causing clinical mastitis. In J. Dairy Sci. (Volume 104, Issue 11, pp. 12030–12041). Elsevier B.V. https://doi.org/10.3168/jds.2021-20177
31. Suleiman, T. S., Karimuribo, E. D., & Mdegela, R. H. (2018). Prevalence of bovine subclinical mastitis and antibiotic susceptibility patterns of major mastitis pathogens isolated in Unguja island of Zanzibar. Tanzania. In Tropical Animal Health and Production (Volume 50, Issue 2, pp. 259–266). Springer Nature. https://doi.org/10.1007/s11250-017-1424-3
32. Ameen, F., Reda, S., El-Shatoury, S., Riad, E., Enany, M., & Alarfaj, A. (2019). Prevalence of antibiotic resistant mastitis pathogens in dairy cows in Egypt and potential biological control agents produced from plant endophytic actinobacteria. In Saudi Journal of Biological Sciences (Volume 26, Issue 7, pp. 1492–1498). Elsevier B.V. https://doi.org/10.1016/j.sjbs.2019.09.008
33. Gunjan, Himanshu, Mukherjee, R., Vidic, J., Manzano, M., Leal, E., Raj, V.S., Pandey, R. P., & Chang, C. M. (2023). Comparative meta-analysis of antimicrobial resistance from different food sources along with one health approach in the Egypt and UK. In BMC Microbiol. (Volume 23, Issue 1, p. 291). BMC. https://doi.org/10.1186/s12866-023-03030-5
34. Abd El-Hamid, M. I., El-Tarabili, R. M., Bahnass, M. M., Alshahrani, M. A., Saif, A., Alwutayd, K. M., Safhi, F. A., Mansour, A. T., Alblwi, N. A. N., Ghoneim, M. M., Elmaaty, A. A., Al-Harthi, H. F., Bendary, M. M. (2023). Partnering essential oils with antibiotics: proven therapies against bovine Staphylococcus aureus mastitis. In Front Cell Infect Microbiol (Volume 13, p. 1265027). Frontiers Media SA. https://doi.org/10.3389/fcimb.2023.1265027
35. Zhuk, Y., Zaritskyi, R., Dreval, D., Derkach, S., Kovpak, V., Masalovych, Y., Ochkolyas, O., Bazyvoliak, S., Antypov, Y., & Kharsika, I. (2022). Antimicrobial susceptibility of mastitis pathogens of dairy cows in

Ukraine. In Potravinarstvo Slovak Journal of Food Sciences (Volume 16, pp. 688–704). https://doi.org/10.5219/1791

36. Wataradee, S., Samngamnim, S., Boonserm, T., & Ajariyakhajorn, K. (2023). Genotypic and antimicrobial susceptibility of Streptococcus agalactiae causing bovine mastitis in the central region of Thailand. In Front Vet. Sci. (Volume 10, p. 1250436). Frontiers Media SA. https://doi.org/10.3389/fvets.2023.1250436

37. Woudstra, S., Wente, N., Zhang, Y., Leimbach, S., Gussmann, M. K., Kirkeby, C., & Krömker V. (2023). Strain diversity and infection durations of Staphylococcus spp. and Streptococcus spp. causing intramammary infections in dairy cows. In J. Dairy Sci. (Volume 106, Issue 6, pp. 4214–4231). Elsevier B.V. https://doi.org/10.3168/jds.2022-22942

38. Belay, N., Mohammed, N., & Seyoum, W. (2022). Bovine Mastitis: Prevalence, Risk Factors, and Bacterial Pathogens Isolated in Lactating Cows in Gamo Zone, Southern Ethiopia. In Veterinary medicine (Auckland, N.Z.) (Volume 13, pp. 9–19). Dovepress. https://doi.org/10.2147/VMRR.S344024

39. Kitila, G., Kebede, B., & Wakgari, M. (2021). Prevalence, aetiology and risk factors of mastitis of dairy cows kept under extensive management system in west Wollega, western Oromia, Ethiopia. In Vet Med Sci. (Volume 7, Issue 5, pp. 1593-1599). John Wiley & Sons. https://doi.org/10.1002/vms3.503

40. Fesseha, H., Mathewos, M., Aliye, S., & Wolde, A. (2021). Study on Prevalence of Bovine Mastitis and Associated Risk Factors in Dairy Farms of Modjo Town and Suburbs, Central Oromia, Ethiopia. In Veterinary medicine (Auckland, N.Z.) (Volume, 12, pp. 271–283). Dovepress. https://doi.org/10.2147/VMRR.S323460

41. Bihon, A., Syoum, A., & Assefa, A. (2019). Assessment of risk factors and isolation of Staphylococcus aureus and Escherichia coli from bovine subclinical mastitic milk in and around Gondar, Northwest Ethiopia. In Trop. Anim. Health Prod. (Volume 51, 4, pp. 939–948). Springer Nature. https://doi.org/10.1007/s11250-018-1777-2

42. Awandkar, S., Kulkarni, B., Agnihotri, A., Chavan V., & Chincholkar V. (2023) Novel fluconazole-resistant zoonotic yeast isolated from mastitis. In Animal Biotechnology (Volume 34, pp. 746–755). John Wiley & Sons. https://doi.org/10.1080/10495398.2021.1982725

43. Mahmoud, S. H., & Yassein, S. N. (2023). Prevalence of Mycotic Mastitis and Evaluation of Some Virulence Potential of Candida albicans Isolated from Mastitic Goats. In Advances in Animal and Veterinary Sciences (Volume 1, Issue 9, p. 1417). Nexus Academic Publishers. https://dx.doi.org/10.17582/journal.aavs/2023/11.9.1417.1427

44. Dyson, R., Charman, N., Hodge, A., Rowe, S. M., & Taylor, L. F. (2022). A survey of mastitis pathogens including antimicrobial susceptibility in southeastern Australian dairy herds. In Journal of Dairy Science (Volume 105, Issue 2, pp. 1504–1518). Elsevier Inc. https://doi.org/10.3168/jds.2021-20955

45. Goulart, D. B., & Mellata, M. (2022). Escherichia coli Mastitis in Dairy Cattle: Etiology, Diagnosis, and Treatment Challenges. In Frontiers in Microbiology (Vol. 13). Frontiers Media SA. https://doi.org/10.3389/fmicb.2022.928346

46. Kim, S. J., Kim, H.-T., & Kim, Y.-H. (2023). Diagnosis of Subclinical Mastitis-Causing Pathogens Using MALDI-TOF Mass Spectrometry in a Certified Organic Dairy Farm in Korea. In Journal of Veterinary Clinics (Vol. 40, Issue 6, pp. 393–398). The Korean Society of Veterinary Clinics. https://doi.org/10.17555/jvc.2023.40.6.393

47. Azevedo, C., Pacheco, D., Soares, L., Romão, R., Moitoso, M., Maldonado, J., Guix, R., & Simões, J. (2015). Prevalence of contagious and environmental mastitis-causing bacteria in bulk tank milk and its relationships with milking practices of dairy cattle herds in São Miguel Island (Azores). In Tropical Animal Health and Production (Vol. 48, Issue 2, pp. 451–459). Springer Science and Business Media LLC. https://doi.org/10.1007/s11250-015-0973-6

48. Tenhagen, B.-A., Köster, G., Wallmann, J., & Heuwieser, W. (2006). Prevalence of Mastitis Pathogens and Their Resistance Against Antimicrobial Agents in Dairy Cows in Brandenburg, Germany. In Journal of Dairy Science (Vol. 89, Issue 7, pp. 2542–2551). American Dairy Science Association. https://doi.org/10.3168/jds.s0022-0302(06)72330-x

49. Naranjo-Lucena, A., & Slowey, R. (2023). Invited review: Antimicrobial resistance in bovine mastitis pathogens: A review of genetic determinants and prevalence of resistance in European countries. In Journal of Dairy Science (Vol. 106, Issue 1, pp. 1–23). American Dairy Science Association. https://doi.org/10.3168/jds.2022-22

50. El Garch, F., Youala, M., Simjee, S., Moyaert, H., Klee, R., Truszkowska, B., Rose, M., Hocquet, D., Valot, B., Morrissey, I., & de Jong, A. (2020). Antimicrobial susceptibility of nine udder pathogens recovered from bovine clinical mastitis milk in Europe 2015–2016: VetPath results. In Veterinary Microbiology (Vol. 245, p. 108644). Elsevier BV. https://doi.org/10.1016/j.vetmic.2020.108644

51. Dax, S. L. (1997). Tetracycline Antibiotics. In Antibacterial Chemotherapeutic Agents (pp. 159–205). Springer Netherlands. https://doi.org/10.1007/978-94-009-0097-4_4

52. Munita, J. M., & Arias, C. A. (2016). Mechanisms of Antibiotic Resistance. In Virulence Mechanisms of Bacterial Pathogens (pp. 481–511). ASM Press. https://doi.org/10.1128/9781555819286.ch17

53. Liang, Z., Shen, J., Liu, J., Li, Q., Yang, F., & Ding, X. (2022). Ascorbic Acid-Mediated Modulation of Antibiotic Susceptibility of Major Bovine Mastitis Pathogens. In Infection and Drug Resistance: Vol. Volume 15 (pp. 7363–7367). Informa UK Limited. https://doi.org/10.2147/idr.s388023

54. Nonnemann, B., Lyhs, U., Svennesen, L., Kristensen, K. A., Klaas, I. C., & Pedersen, K. (2019). Bovine mastitis bacteria resolved by MALDI-TOF mass spectrometry. In Journal of Dairy Science (Vol. 102, Issue 3, pp. 2515–2524). American Dairy Science Association. https://doi.org/10.3168/jds.2018-15424

55. Oliveira, T. C. de A., Brito, M. A. V. P., Giambiagi-de Marval, M., Vicentini, N. M., & Lange, C. C. (2021). Identification of bovine mastitis pathogens using MALDI-TOF mass spectrometry in Brazil. In Journal of Dairy Research (Vol. 88, Issue 3, pp. 302–306). Cambridge University Press (CUP). https://doi.org/10.1017/s0022029921000595

56. Maksimović, Z., Čengić, B., Ćutuk, A., & Maksimović, A. (2023). Antimicrobial Resistance of Cattle Mastitis-Causing Bacteria: How to Treat? In Recent Developments on Mastitis - Treatment and Control [Working Title]. IntechOpen. https://doi.org/10.5772/intechopen.112977

57. Hossain, M. K., Paul, S., Hossian, M. M., Islam, M. R., Alam, M. G. S. (2017). Bovine Mastitis and Its Therapeutic Strategy Doing Antibiotic Sensitivity Test. In Austin Journal of Veterinary Science & Animal Husbandry (Vol. 4, Issue 1). Austin Publishing Group. https://doi.org/10.26420/austinjvetscianimhusb.2017.1030

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Ruslan Zaritskyi.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

Ethics committee approval. Research protocols and experimental procedures were approved by the bioethics commission of the National University of Life and Environmental Sciences of Ukraine. Data collection was carried out in accordance with the Law of Ukraine "On the Protection of Animals from Cruelty Treatment" No. 3447-IV dated August 4, 2017 and the European Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 1986.

Contact Address:

*__Ruslan Zaritskyi__, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Obstetrics, Gynecology, and biotechnology of animal reproduction, Vystavkova Str., 16, 03121, Kyiv, Ukraine,
E-mail: zaritskyi_rv@nubip.edu.ua
ORCID: https://orcid.org/0000-0003-1074-3118

Yurii Zhuk, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Obstetrics, Gynecology, and biotechnology of animal reproduction, Vystavkova Str., 16, 03121, Kyiv, Ukraine,
E-mail: zhuk_yv@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-8827-6313

Denys Dreval, Center of Veterinary Diagnostics LLC, Laboratory of Bacteriology and Pathanatomy, Kaisarova Str., 15-A, 03041, Kyiv, Ukraine,
E-mail: bacteriology@cvd.com.ua
ORCID: https://orcid.org/0000-0003-4184-3189

Vitalii Kovpak, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Obstetrics, Gynecology, and biotechnology of animal reproduction, Vystavkova Str., 16, 03121, Kyiv, Ukraine,
E-mail: vitkovpak@ukr.net
ORCID: https://orcid.org/0000-0003-2419-1246

Yurii Masalovych, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Obstetrics, Gynecology, and biotechnology of animal reproduction, Vystavkova Str., 16, 03121, Kyiv, Ukraine,
E-mail: masalovich@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-0986-0854

Ivan Cheverda, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Obstetrics, Gynecology, and biotechnology of animal reproduction, Vystavkova Str., 16, 03121, Ukraine,
E-mail: cheverda_im@nubip.edu.ua
ORCID: https://orcid.org/0009-0000-7545-307X

Iryna Derkach, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Pharmacology, Parasitology and Tropical Veterinary Medicine, Vystavkova Str., 16, 03121, Kyiv, Ukraine,
E-mail: derkach_im@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-0149-7923

Taras Savchuk, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Surgery and Pathophysiology acad. I.O. Povazhenko, Vystavkova Str., 16, 03121, Kyiv, Ukraine,
E-mail: t_sav4uk@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-7351-5684

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 5.4.2024
Revised: 30.5.2024
Accepted: 15.6.2024
Published: 19.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 570-587
https://doi.org/10.5219/1978
ISSN: 1337-0960 online
www.potravinarstvo.com

Ultrasound-assisted innovations in protein processing: review

Maksim Rebezov, Bahytkul Assenova, Albina Luneva, Mars Khayrullin, Rustem Zalilov, Yury Lysenko, Raisa Savkina

ABSTRACT

The contemporary landscape of protein processing is witnessing a paradigm shift propelled by innovative technologies. This review unveils innovations in protein processing through the lens of an ultrasound-assisted approach. The focus was on the interplay between ultrasound waves and proteins during ultrasound extraction technology. The realm of protein extraction, where traditional methods face challenges and ultrasound emerges as a transformative force, was highlighted, as well as ultrasound's role in enhancing protein yield and quality in relationship to protein structure and function. Comparative analyses have showcased the remarkable advancements ushered in by ultrasound-assisted techniques, and this review also extends to enzymatic hydrolysis, where ultrasound catalyses reactions, unlocking new dimensions in the production of bioactive peptides and nutritionally enriched proteins. In the bio-industrial sectors, ultrasound facilitates protein refolding and revolutionises recombinant protein production, stability and bioavailability. Ultrasound has emerged as a catalyst for efficiency and bioactivity enhancement, defeating conventional limitations to the intricate optimisation strategies of refolding. This review envisages the advantages of ultrasound technology and its applications in the bio-industrial sector. The prospects of ultrasound-assisted protein processing are outlined, and roadmaps and processing techniques are offered.

Keywords: protein processing, ultrasound-assisted innovations, protein extraction, enzymatic hydrolysis, biopharmaceutical industry.

INTRODUCTION

Ultrasound-assisted protein processing advances have arisen as a focus of study, motivated by their potential to improve substrate efficiency and biological activity. This technological paradigm has been used in various fields, including extraction, modification, and mitigating freezing/thawing-induced oxidation. Key breakthroughs and applications demonstrate the adaptability and effectiveness of ultrasound-assisted protein processing. In enzymatic protein hydrolysis, ultrasound is a non-thermal processing method that is both environmentally friendly and efficient. Notably, ultrasonic pretreatment-assisted enzymatic hydrolysis stands out for its ability to considerably improve the efficiency of enzymatic processes while also increasing the biological activity of substrates. This method is mostly helpful for extracting bioactive compounds and breaking down biological macromolecules **[1]**. Another remarkable use is the extraction of proteins from watermelon seeds, which are considered food processing waste. Although watermelon seeds are a byproduct, they contain high-quality proteins. The inquiry into ultrasound-assisted extraction techniques has shown encouraging results. The optimal conditions, which included a pH of 11, a sonication temperature of 45 °C, and a sonication period of 10 minutes, resulted in maximal protein recovery at an astounding rate of 85.81% **[2]**. Additionally, ultrasound-assisted extraction techniques have been explored to obtain proteins from faba beans, complementing conventional methods. This research extends beyond ultrasound to encompass high-pressure processing and hydrodynamic cavitation. The

overarching goal is to pioneer efficient and innovative protein extraction methods, focusing on plant-based sources. This approach represents a concerted effort to advance extraction technologies, offering sustainable solutions for protein procurement from diverse agricultural resources **[3]**.

Protein processing techniques have evolved due to advances in biotechnology and molecular biology and the continual development of protein purification, characterisation, and analysis methods. Notable trends in protein processing include a variety of transformational advances. Artificial intelligence and machine learning have played critical roles in transforming the environment. Advances in AI and machine learning have resulted in unique methods for processing and evaluating protein data. Inspired by biological systems, these strategies seek to improve our knowledge of protein interactions and their consequences in illness. Biophysical methods have advanced significantly, allowing researchers to analyse protein interactions **[3]**. X-ray crystallography, nuclear magnetic resonance (NMR), and atomic force microscopy (AFM) have all evolved to help researchers investigate protein structures, domains, and sequence patterns involved in protein-protein interactions. These advances allow for the quick examination of numerous samples with tiny quantities of material, making them ideal for genome- or proteome-wide research to uncover new therapeutic targets **[4]**. The field of Protein Extraction Technologies has seen significant advances. New approaches have arisen, such as ultrasound-assisted extraction, high-pressure processing, and hydrodynamic cavitation, which improve the efficiency and efficacy of protein extraction from a variety of sources. These approaches have practical uses in sectors such as food processing and pharmaceuticals, helping to acquire high-quality proteins **[5]**. Genomic Fishing and Data Processing tools have also emerged, allowing for extracting and analysing particular gene sequences from taxonomically varied genomic data. This technique contributes to molecular evolution research by detecting adaptive/purifying selection and reconstructing ancestral proteins **[6]**.

Challenges in Traditional Protein Processing

Traditional protein processing technologies face problems, prompting the investigation of novel options. Traditional heat-based processes like salting, smoking, and frying are notoriously energy-intensive and time-consuming. These approaches result to higher carbon footprints and greenhouse gas emissions, creating sustainability issues. Second, classic technologies such as ultrasonography have limits in thick and complicated food matrices, notably those high in protein. Penetrating deeply into such matrices is difficult, preventing consistent processing and extraction inside these complex structures. Third, standard approaches for limiting microbial growth and eliminating pathogens may not always provide optimal food safety and quality. This variation raises issues regarding shelf life and potential food loss **[7]**.

Ultrasound-assisted technology emerges as a key actor in protein processing, delivering significant benefits while addressing unique obstacles. Recent advances highlight its promise in extracting, modifying, and mitigating freezing/thawing-induced protein oxidation. The role of ultrasonography in protein processing is diverse. For starters, it is significantly more efficient and sustainable in food processing, making it a possible alternative to existing techniques. Ultrasound technology can provide identical outcomes with less energy input, resulting in lower energy consumption, a smaller carbon footprint, and fewer greenhouse gas emissions **[8]**. Second, ultrasonic processing technology improves food safety and quality. This application demonstrates the potential of ultrasound technology to bring about novel advancements in protein modification within food manufacturing. Despite these promising attributes, ultrasound technology faces challenges, particularly in processing dense and complex food matrices like proteins. Ongoing research and development efforts are poised to address these limitations, with the expectation of expanding the scope and applications of ultrasound **[9]**. Ultrasound technology is a potential force in protein processing, providing various benefits such as increased energy efficiency, higher food safety standards, and the capacity to extract proteins from unorthodox sources. Recent findings from comprehensive research highlight the comparative benefits of ultrasound in food processing, positioning it as a more energy-efficient and ecologically friendly alternative to existing techniques. This difference substantially contributes to the overall aim of reducing energy usage and greenhouse gas emissions. One prominent characteristic of ultrasonic technology is its ability to improve food safety by successfully suppressing microbial development and eradicating infections. This feature results in a longer shelf life for processed foods and a corresponding reduction in food waste, which aligns with larger sustainability goals. Furthermore, ultrasound-assisted extraction techniques have been examined for their usefulness in extracting significant protein yields from alternate sources, as demonstrated by experiments on watermelon seeds. This novel technique helps to repurpose food processing waste and promotes extracting high-quality proteins from non-traditional sources. However, it is critical to recognise the limitations of ultrasonic efficacy, especially in thick and complicated food matrices, including proteins. This intrinsic problem needs continual research and development efforts to address and overcome these limitations, ensuring the technology performs optimally in varied processing contexts **[7]**. Figure 1 depicts how the laboratory-scale ultrasonic technique works.

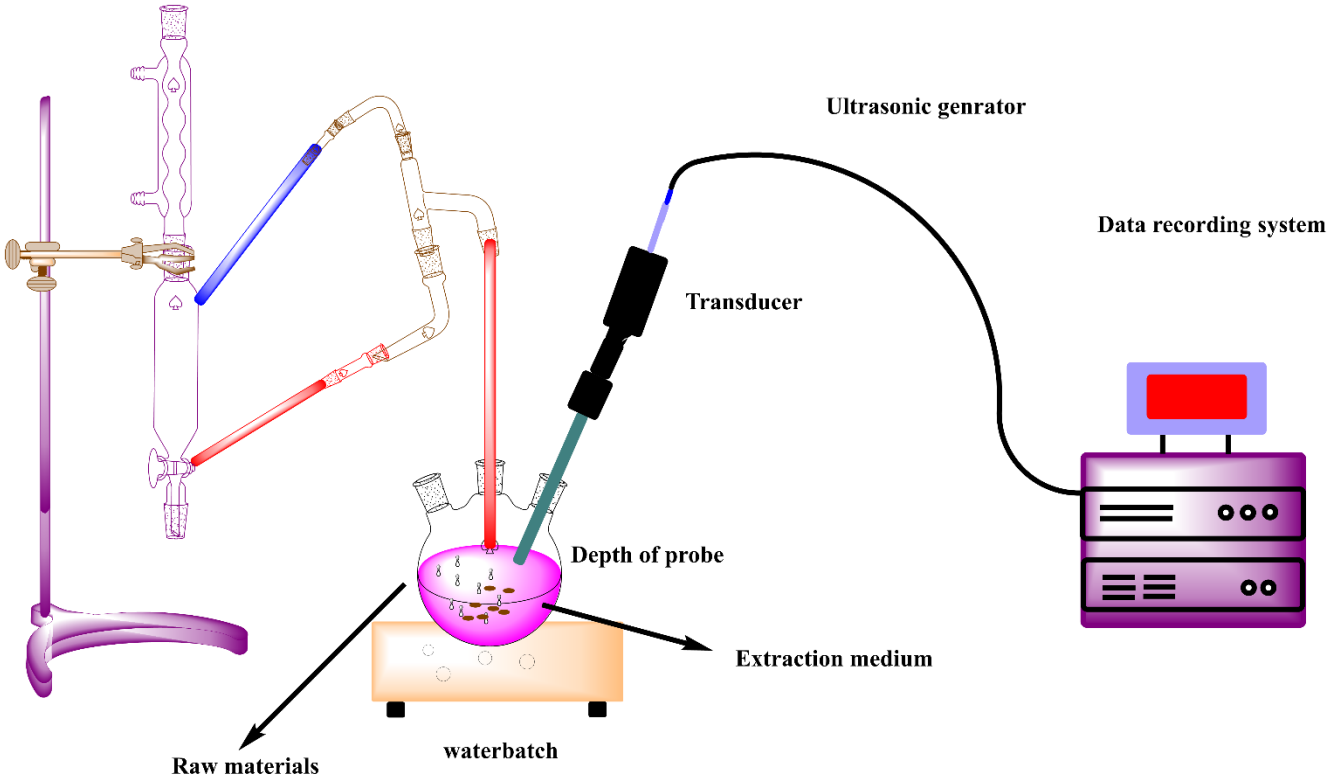


Figure 1. Lab scale of ultrasonic-assisted extraction technique.

Advantages of Ultrasound Technology

Ultrasound technology has emerged as a key tool in protein processing, with several benefits supported by study findings. It is known for its superior energy efficiency and environmental effect compared to standard processing methods. This technical technique can provide equivalent effects with less energy input, considerably contributing to decreasing energy consumption, lowering the carbon footprint, and reducing greenhouse gas emissions **[7]**. Second, ultrasonic processing technology is essential in improving food safety and quality. Its capacity to prevent microbial development and remove pathogens results in longer shelf life for processed goods, reducing food waste. This quality is consistent with the food industry's broader sustainability and resource optimisation goals. Furthermore, ultrasonic technology aids in the extraction of valuable biochemicals from biomass. This feature makes extraction more accessible and allows their use in waste valorisation and biorefinery processes. The extraction of essential biochemicals aids in biomass's more sustainable and resource-efficient use. Enzymatic protein hydrolysis is one example of ultrasonic technology's use in protein processing. Ultrasound, a green and efficient non-thermal processing technology, aids enzymatic hydrolysis, considerably boosting efficiency and increasing substrate biological activity. This use is especially noteworthy for the extraction of bioactive compounds and the breakdown of biological macromolecules **[1]**.

Ultrasound technology has the potential to significantly affect the food business, as highlighted in several significant areas. To begin with, ultrasonic technology is considered superior to standard processing methods regarding energy efficiency and environmental effects. This distinction helps to reduce energy use, carbon footprint, and GHG emissions. The potential cost reductions and enhanced sustainability performance provide significant opportunities for food processing enterprises **[10]**. Second, ultrasonic processing technology is a game changer for improving food safety and quality. It increases the shelf life of processed foods by successfully preventing microbial growth and removing pathogens, resulting in less food waste. This protects the quality and safety of food items and promotes consumer happiness and confidence, which are critical in the food sector **[11]**. Furthermore, ultrasonic technology is essential for extracting valuable biochemicals from biomass. This skill offers opportunities for their use in waste valorisation and biorefinery processes, promoting the development of new sustainable food products and processing procedures. Ultrasound has found valuable applications in meat preparation, replacing or supplementing existing procedures. Its functions in cutting, degassing, and meat tenderisation provide prospects for enhanced meat processing and the development of innovative products. However, it is critical to recognise the current limits, most notably the efficiency of ultrasound in dense and complicated dietary matrices, particularly those high in protein. Addressing these problems necessitates continual research and development activities, which have the potential to broaden the uses of ultrasound technology in the

food business. Successfully overcoming these limits may signal a transformational age in which ultrasonic technology contributes significantly to the food business's sustainability, innovation, and development **[12]**.

Ultrasound emerges as an effective tool for illuminating the complexities of structures, including characteristics such as forms, sizes, and textures, as well as determining spatial linkages and placements. It functions as a type of energy that travels through media such as air or water in the form of waves **[13]**. The fundamental characteristics of ultrasonography include numerous crucial components. To begin, ultrasound is created by a transducer, which converts electrical energy into mechanical vibrations that eventually produce sound waves. The transducer, made up of piezoelectric crystals, vibrates when an electrical current is applied, causing sound waves to travel through the surrounding material. Understanding the physics and apparatus of ultrasonography is critical for enhancing diagnostic pictures and assuring safety. This understanding entails comprehending the relationship between frequency, penetration, and resolution. It involves knowing how the size and shape of a transducer's footprint assist beam access to target structures and recognising the medium's effect on ultrasonic energy **[14]**. ultrasonic-assisted extraction is a prominent application that uses ultrasonic waves to facilitate the extraction of bioactive substances from various sources, including plants and animal tissue. These waves damage cell walls by forming cavitation bubbles, resulting in better yields and shorter extraction times. Recent developments in ultrasound technology have accelerated its use in clinical practice, particularly at the point of care. Ultrasound investigations are conducted and interpreted by trained doctors from many healthcare areas, who contribute to patient evaluation and management. The real-time nature of point-of-care ultrasonography allows doctors to integrate generated information smoothly into the current assessment and management procedures **[15]**.

Basic principles of ultrasound

The fundamental concepts of ultrasonography include production, propagation, and applications. Ultrasound is a type of mechanical energy with a frequency that exceeds the human hearing range, usually exceeding 20 kHz. It is created by a transducer, which transforms electrical energy into mechanical vibrations that result in sound waves. These sound waves propagate across a medium, such as air or water, and may be employed for various applications, including medical imaging, industrial testing, and therapeutic treatments **[16]**. The fundamental concepts of ultrasonic sources include the employment of piezoelectric crystals within the transducer to produce sound waves. When an electrical current is supplied to these crystals, they vibrate, generating ultrasonic waves. This procedure creates sound waves with the required frequency and strength for specific purposes **[17]**. In environmental research and engineering, ultrasonography has been investigated for its potential to destroy low quantities of estrogen hormones in aqueous solutions. According to research, ultrasound can influence the breakdown of estrogen molecules, with parameters such as solution temperature and fluid pressure impacting the reaction's efficiency. These principles underpin ultrasound's many uses, from medical diagnostics to environmental cleanup, making it a flexible and essential technology in various sectors **[18]**.

Acoustic Waves: Their Properties

Acoustic waves, such as surface acoustic waves and shear-horizontal (SH) acoustic waves, have been investigated for their sensitivity to changes in the parameters of their travel medium **[19]**. The properties of these waves, such as their velocity and existence range, have been studied theoretically in various materials, including layered media, lithium and potassium niobate plates, and graphene. These investigations help to better understand the behaviour of acoustic waves in specific materials and under varied situations **[20]**.

A transducer generates ultrasonic waves by converting electrical energy into mechanical vibrations that produce sound waves. The transducer consists of piezoelectric crystals that vibrate when an electrical current is supplied to them, resulting in sound waves that flow through the medium. The frequency of the sound waves produced by the transducer is governed by the electrical signal given to the crystals. The sound waves produced by the transducer can be focussed or defocused by changing the form of the transducer or employing lenses **[21]**. Ultrasound waves may be employed in various

applications, including medical imaging, industrial testing, and therapeutic therapies. The creation of ultrasonic waves is an essential feature of ultrasound technology, enabling its many uses **[14]**.

Ultrasound waves are employed in various applications, each having unique features. Pulse waves are used in medical imaging and Doppler ultrasonography **[22],** whereas chirp waves assess bone quality and quantity **[23]**. Tone-burst waves are utilised in industrial testing and non-invasive pain management, whereas continuous waves are used in ultrasound imaging to provide high-resolution pictures. These waves find applications in various domains, from unravelling the complexities of the human body to analysing materials in industrial settings **[24]**.

The differences between low-frequency and high-frequency ultrasonic waves include different qualities and uses. Low-frequency ultrasonic waves, less than 1 MHz, are used in deep tissue imaging and treatments such as physical therapy and pain management **[25]**. In contrast, high-frequency ultrasonic waves with frequencies more than 10 MHz are used in superficial tissue imaging, particularly in dermatology and ophthalmology. The ultrasonic frequency is determined by the individual application requirements and the depth of the tissue to be scanned or treated. Recognising the characteristics and uses of low-frequency and high-frequency ultrasonic waves is critical for achieving the best diagnostic results and safe operations in various settings. This information is helpful in multiple applications, including medical imaging, industrial testing, and therapeutic therapies. Understanding the subtle features of these ultrasonic waves aids educated decision-making, contributing to improved efficacy and safety in medical, industrial, and therapeutic applications **[26]**.

Continuous wave and pulsed ultrasound are separate forms of ultrasonic waves, each with distinctive qualities and uses in various domains, including medical imaging and treatments. Sinusoidal waves distinguish continuous wave ultrasound with a consistent frequency. Its principal application is in ultrasonic imaging, where it excels at producing high-resolution pictures of inside body components **[27]**. Continuous wave ultrasound is also helpful for deep tissue imaging and treatments such as physical therapy and pain management. Notably, research comparing the effects of constant and short-pulsed focused ultrasound on muscle tissue found that continuous wave ultrasound produced a considerably greater diffusion coefficient. Pulsed ultrasound, conversely, consists of brief, constant-frequency sound pulses **[28]**.

The search results highlight the promising applications of ultrasound technology in protein extraction and processing. Ultrasound-assisted extraction (UAE) has emerged as an effective technique for enhancing the extraction of bioactive compounds, including proteins, from various sources. The key mechanisms behind the effectiveness of UAE include cell wall disruption and improved mass transfer. Cell wall disruption is facilitated by the cavitation bubbles generated by ultrasound waves, which effectively disrupt cell walls, thereby facilitating the release of intracellular compounds, including proteins. This leads to higher extraction yields compared to conventional extraction methods. Additionally, ultrasound enhances the mass transfer of solutes from the solid matrix into the solvent, accelerating the extraction process and reducing extraction times **[29]**. Several successful applications of UAE for protein extraction have been reported. For instance, in extracting proteins from Sacha inchi meal biomass, UAE combined with deep eutectic solvents resulted in higher protein yields than conventional extraction methods. Similarly, optimising UAE parameters, such as pH, temperature, and time, led to a maximum protein recovery of 85.81% in extracting proteins from watermelon seeds **[2]**. Moreover, UAE has been utilised to extract proteins from excess sludge, where low-intensity ultrasound-assisted enzymatic hydrolysis enhanced enzyme activity, improved the protein extraction rate, and reduced overall processing costs **[30]**. Furthermore, UAE has been explored as a potential technology for protein extraction from faba bean, along with other novel techniques like high-pressure processing and hydrodynamic cavitation **[3]**. Figure 2. depicts the mechanism of how ultrasonic waves work.

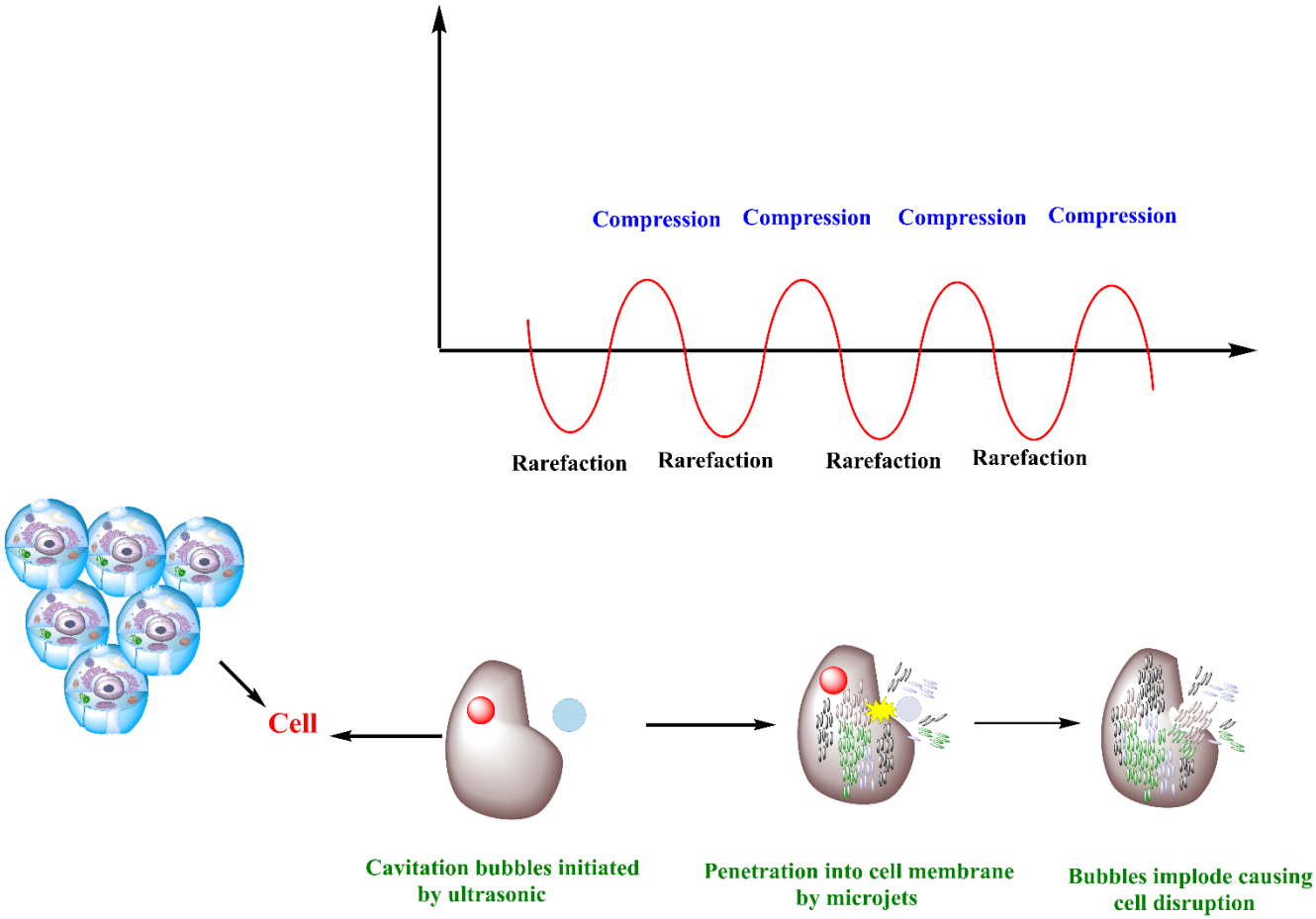


Figure 2. Ultrasonic wave's destructive mechanism.

Enzymatic protein hydrolysis has emerged as a new focus in investigating ultrasound-protein interactions. Ultrasound is an important non-thermal processing technology for helping enzymatic hydrolysis since it is environmentally friendly and effective **[31]**. Compared to ordinary enzymatic hydrolysis, ultrasonic-pretreatment-assisted enzymatic hydrolysis has a much higher efficiency. This enhancement goes beyond simply increasing efficiency and dramatically increasing substrate biological activity. This breakthrough can potentially improve enzymatic hydrolysis processes and hence increase total protein processing efficiency **[32]**.

Furthermore, the study of Ultrasound's interaction with proteins includes Protein Modification. The production of a myofibrillar protein-gallic acid combination exemplifies ultrasound's potential for generating new protein modifications, notably in the food manufacturing industry. These applications highlight ultrasound's flexibility in modifying proteins to desired specifications, thereby widening the vista of possibilities within the field of food preparation **[33]**.

The processes governing the interaction of ultrasound with proteins are complex and not yet fully understood. Among the postulated processes, cavitation stands out because ultrasonic waves cause cavitation bubbles to develop inside the medium. These bubbles, in turn, generate mechanical stress and shear forces that damage protein structure and facilitate the release of bioactive chemicals. This sophisticated procedure improves the overall efficiency of protein processing technologies, paving the way for new approaches in the food business **[34]**. Another discovered process is acoustic streaming, which occurs when ultrasound waves cause fluid to flow. This movement, mediated by the propagation of sound waves, improves the mass transfer of bioactive chemicals from the protein matrix to the solvent. Acoustic streaming significantly impacts the extraction process, increasing the intricacy of ultrasound-protein interactions **[35]**. Thermal impacts are yet another aspect of this relationship. Ultrasound waves can create heat by absorbing energy from the medium, which causes protein denaturation and the consequent release of bioactive chemicals. This thermal element adds a temperature dimension to the interaction, contributing to more nuanced results in protein processing processes **[36]**. Enzymatic

activation is a method in which ultrasonic waves activate enzymes. This activation increases enzymatic activity, which improves the efficiency of enzymatic hydrolysis. The synergy between ultrasonic and enzymatic processes increases the capacities of protein processing technologies, creating opportunities for better outcomes **[37]**.

Effects on Protein Structure

The effects of ultrasound on protein structure are complicated and dependent on several parameters, including the frequency, intensity, and duration of the ultrasound exposure. According to specific research, ultrasonography can produce changes in protein structure, such as denaturation, aggregation, and fragmentation. Other research has found that ultrasonic can improve protein biological activity by increasing solubility and accessibility to enzymes. The kind of protein and the medium in which it is suspended also have an impact on ultrasound's effects on protein structure **[38]**.

Ultrasound-Assisted Protein Extraction

Ultrasound-assisted protein extraction has emerged as a transformational field of study, offering several benefits over existing approaches. One notable advantage is its potential to obtain better extraction yields due to the cavitation effect caused by ultrasonic vibrations, which shatter cell walls and facilitate the release of target chemicals. This increased efficiency is combined with decreased solvent use, which aligns with sustainable standards and contributes to an environmentally benign extraction process **[31]**. Furthermore, the simplicity of ultrasound-assisted extraction equipment reduces maintenance costs compared to previous methods, highlighting its cost-effectiveness **[39]**. Beyond extraction efficiency, research has shown that it can increase proteins' physical, structural, and functional characteristics, including solubility, stability, and biological activity. Multi-mode ultrasound technology highlights continuous attempts to improve protein extraction rates. Real-time process monitoring, including Fourier Transform Infrared (FT-IR) analysis, sheds light on the structural features of proteins during extraction, enhancing knowledge of the relationship between protein structure and extraction levels **[40]**. Furthermore, ultrasonic is used in enzymatic protein hydrolysis, exhibiting its capabilities as a green and practical non-thermal processing approach, considerably boosting enzymatic hydrolysis efficiency and increasing substrate biological activity **[37]**.

Ultrasound-Assisted Protein Extraction
Mechanical Methods

Ultrasound-assisted protein extraction has gained popularity recently, offering various benefits over standard extraction techniques. One significant advantage is its capacity to provide high extraction yields. Ultrasound waves provide a cavitation effect, disrupting cell walls and enabling the release of target substances, resulting in enhanced extraction efficiency **[41]**. Furthermore, the use of ultrasound promotes environmental sustainability by minimizing solvent usage. This strategy correlates with a more environmentally friendly extraction procedure, making it a better option than traditional approaches, which frequently use more solvents **[42]**. Another advantage of ultrasound-assisted extraction is that it has fewer maintenance expenses. This approach does not require sophisticated equipment or intricate procedures, simplifying the extraction process and reducing maintenance costs **[43]**. According to studies, ultrasound-assisted protein extraction improves proteins' physical, structural, and functional characteristics and efficiency. Improvements in solubility, stability, and biological activity have been found, demonstrating the adaptability and usefulness of this extraction method **[44]**. Multi-mode ultrasound technology has emerged as a significant advancement in enhancing protein extraction levels. According to research, various ultrasonic working modes have a considerable impact on the overall success of the extraction process, and multi-mode ultrasound devices have been built to take advantage of these differences. Fourier Transform Infrared (FT-IR) analysis allows real-time process monitoring during ultrasound-assisted extraction. This in situ monitoring enables researchers to investigate proteins' structural features during extraction, providing insights into the relationship between protein structure and extraction levels **[42]**. In addition to protein extraction, ultrasound is helpful for enzymatic protein hydrolysis. Ultrasound is a green and efficient non-thermal processing technology that aids in enzymatic

hydrolysis, considerably boosting efficiency and increasing the biological activity of substrates as compared to typical enzymatic hydrolysis procedures **[45]**.

Challenges in Conventional Methods

Sonication, high-intensity focused ultrasound (HIFU), and an ultrasonic bath are all methods for extracting proteins using ultrasound. Sonication uses high-frequency sound waves to shatter cell walls and release proteins. HIFU employs high-intensity ultrasound waves to cause localized heating and pressure changes, resulting in protein extraction **[46]**. The ultrasonic bath immerses the sample in ultrasound waves to aid protein extraction. Studies have demonstrated that ultrasound-assisted protein extraction can result in greater extraction yields, decreased solvent consumption, and enhanced physical, structural, and functional characteristics of proteins compared to traditional approaches. Multi-mode ultrasound equipment has been created to increase protein extraction levels. In situ, real-time process monitoring may investigate protein structural features during ultrasound-assisted extraction **[29]**. Ultrasound-assisted enzymatic hydrolysis has also been widely employed as a green and practical non-thermal processing approach to aid enzymatic hydrolysis, considerably boosting its efficiency and increasing substrate biological activity. A comparative investigation of multiple ultrasound-assisted extraction procedures for pectin from tomato processing waste revealed that ultrasonic bath and sonication were the most successful methods for pectin extraction **[47]**.

Ultrasound-assisted protein extraction has been proven to increase protein output and quality compared to traditional approaches. Ultrasound-assisted extraction has been shown in studies to increase protein yields, enhance techno-functional attributes, and modify proteins' physical, structural, and functional properties **[48]**. For example, a pumpkin seed protein extraction survey discovered that ultrasonic treatment increased protein output and enhanced the extracted protein's techno-functional qualities **[49]**. Another study on walnut dregs protein extraction using multi-mode ultrasound found that ultrasonic-assisted protein extraction effectively increases yield and changes proteins' physical, structural, and functional aspects. Ultrasound-assisted enzymatic hydrolysis has also been found to improve the efficiency and biological activity of substrates dramatically **[40]**.

Mechanisms of Ultrasound-Enhanced Enzymatic Reactions

The processes of ultrasound-enhanced enzymatic reactions have been investigated in various disciplines, including food processing, analytical chemistry, and nanomaterial-enhanced biosensors. Ultrasound has been proven to aid in beneficial dietary processes such as enzymatic crosslinking, protein hydrolysis, and fermentation **[50]**, **[51]**. In analytical chemistry, ultrasound has been used to accelerate the enzymatic hydrolysis of substances, resulting in a significant decrease in the time necessary for this step. Furthermore, ultrasound has been shown to improve the performance of first-generation amperometric biosensing schemes in nanomaterial-enhanced biosensors, notably in the context of enzymatic processes **[52]**. Furthermore, ultrasound has been studied for its ability to enhance the activity of enzymes in cascade-catalytic tumor treatment. In metal extraction, ultrasound has been investigated for its capacity to improve sulfuric acid leaching for zinc extraction, with results indicating a higher leaching rate than traditional approaches. These studies highlight the many uses and processes of ultrasound-enhanced enzymatic reactions in a variety of domains **[53]**.

Ultrasound-assisted hydrolysis has several noticeable benefits in a variety of disciplines. For starters, it dramatically improves the efficiency of enzymatic hydrolysis operations, resulting in much shorter reaction times. For example, studies have shown that ultrasound may reduce reaction times from hours to minutes, making it a time-saving tool, particularly in applications involving the enzymatic hydrolysis of chemicals detected in urine samples **[54]**. Furthermore, ultrasound promotes favourable processes in food processing, such as enzymatic cross-linking, protein hydrolysis, fermentation, and marination. These procedures increase yield and alter physical and functional features, improving enzymatic reaction efficiency. Furthermore, ultrasound-assisted hydrolysis has several uses in food processing, analytical chemistry, and the production of nanomaterial-enhanced biosensors **[55]**. However, this strategy is not without its drawbacks. One key difficulty is to optimize the settings for ultrasound-assisted hydrolysis. Parameters such as sonication strength, duration, enzyme activity, and water-substrate ratio must be

carefully adjusted to produce the desired results. Another source of worry is the possibility of hydrolysis artefacts, as shown in situations where acidification during extraction resulted in unwanted chemical changes. Addressing these problems necessitates careful control of experimental settings to avoid unwanted reactions or changes in target molecules **[56]**.

Bioactive Peptide Production

Enzymatic hydrolysis is the primary method for producing bioactive peptides, with ultrasonography, microbial fermentation, or recombinant DNA technologies typically used as auxiliary methods. Enzymatic hydrolysis, significantly when helped by ultrasound, improves efficiency and yield, as proven by experiments on diverse protein sources such as chicken feathers and tilapia fish skin waste. These peptides have many health benefits, including antibacterial, antioxidant, anticancer, and immunomodulatory capabilities, making them potential constituents for highly nutritious and functional food items **[57]**, **[58]**. In the biomedical arena, continuous research strives to enhance peptide synthesis technologies to realise their broad prospective uses. However, problems remain, necessitating careful optimization of manufacturing variables such as temperature, pH, and enzyme-to-substrate ratio to achieve hydrolysis degree and bioactivity. Developing more effective ultrasound-assisted hydrolysis devices might boost peptide output even more **[59]**. Furthermore, constant research and innovation are required in peptide synthesis, separation, identification, and functionality evaluation to realize their potential across several industries fully. Overall, the search results illustrate the bright outlook for bioactive peptides, emphasizing the critical role of ultrasound-assisted enzymatic hydrolysis in their creation while also identifying areas for future refinement and research **[60]**.

Ultrasound in Protein Refolding

Low-amplitude ultrasound can induce specific structural changes in protein monomers, forming hydrogen-bonded β-sheet-rich structures, which serve as primary nucleation sites for protein refolding. These changes are initiated by pressure perturbations and accelerated by temperature factors **[61]**. Additionally, prolonged exposure to low-amplitude ultrasound enables the controlled elongation of amyloid protein nanofibrils directly from monomeric lysozyme proteins. Remarkably, the nanofibrillar assemblies formed under ultrasound exhibit identical structural characteristics to those formed by native fibrillation, as determined by solution X-ray scattering. The study indicates that ultrasound can effectively induce structural changes in proteins and facilitate the formation of amyloid protein nanofibrils with properties akin to native fibrillation **[62]**.

Protein Denaturation and Refolding

The role of ultrasound in aiding protein refolding is becoming increasingly evident, with studies showing its ability to induce refolding of specific motifs in protein monomers, leading to primary nucleation characterized by adopting a hydrogen-bonded β-sheet-rich structure **[63]**, **[64]**. Additionally, ultrasound has been observed to displace small heat shock proteins from protein aggregates, thereby initiating the refolding process. These findings suggest that ultrasound could play a crucial role in protein refolding, with promising applications in the biopharmaceutical industry and the refolding of recombinant proteins. However, ultrasound in protein refolding is still an area of ongoing research, and further studies are required to fully comprehend its mechanisms and explore its practical applications. In summary, while ultrasound shows promise in aiding protein refolding by inducing specific motifs and displacing chaperones from aggregates, further research is necessary to unlock its full potential in this domain **[65]**, **[66]**.

Challenges in Protein Denaturation

Protein refolding presents numerous substantial issues, such as protein aggregation, refolding condition optimization, protein structure and function preservation, process scalability, and impurity elimination. Protein aggregation can hamper refolding efficiency by generating insoluble complexes but optimizing parameters such as temperature and pH is critical for successful refolding. Preserving the protein's original structure and function is critical to ensuring the quality of the finished product, and scaling up the process offers logistical hurdles as demand increases **[67]**. Furthermore, contaminants

such as tiny heat shock proteins might impede refolding and degrade product quality, demanding effective removal techniques. Ultrasound has emerged as a promising method for addressing some of the issues of protein refolding **[68]**.

Challenges in Protein Denaturation

Protein denaturation and refolding present several issues, including protein aggregation, optimizing refolding conditions, preserving protein structure and function, process scalability, and impurity removal. Protein aggregation reduces refolding efficiency, necessitating mitigating techniques **[69]**. To get the best results, refolding conditions, governed by parameters such as temperature and pH, must be precisely adjusted for each protein type. Preserving proteins' original structure and function is critical to the quality of the final product, demanding careful refolding technique selection. Scaling up the refolding process to meet increasing demand creates logistical challenges that must be overcome **[70]**. Impurities like tiny heat shock proteins might impair refolding efficiency and need effective removal procedures. Additionally, ultrasound-assisted refolding is a potential path for investigation, with additional studies required to elucidate its mechanisms and possible applications **[71]**, **[72]**.

Importance of Proper Refolding

The search results give helpful information on the variables influencing protein refolding, notably cysteine-rich proteins that tend to aggregate when overexpressed in E. coli. The major processes in the refolding process are the purification of inclusion bodies comprising insoluble, aggregated proteins, solubilization using denaturants such as guanidinium chloride, and refolding with a redox system, co-solvents and additives to encourage correct folding **[73]**. The dilution approach and using detergents with cosolvents are valuable methods for refolding cysteine-rich proteins **[74]**. Several factors influence the efficiency of the refolding process, including protein concentration, disulfide bond formation, chaperone proteins (such as small heat shock proteins and Hsp70 chaperones), micelle size and composition, and salt concentration. Increasing salt concentration up to 1 M NaCl can enhance the refolding process **[75]**. Additionally, synthetic nano chaperones with hydrophobic microdomains can stabilize denatured proteins and facilitate their refolding with high efficiency **[76]**.

Ultrasound as a Refolding Aid

Ultrasound has emerged as a viable method for aiding protein refolding in various ways. Firstly, it causes structural changes in protein monomers, leading to adopting a hydrogen-bonded β-sheet-rich structure, which is essential for commencing protein refolding **[77]**. Furthermore, ultrasonic removes tiny heat shock proteins from protein aggregates, a necessary step in solubilizing and refolding aggregated proteins with the help of chaperones such as Hsp70 **[78]**, **[79]**. Furthermore, extended exposure to low-amplitude ultrasound enables the controlled elongation of amyloid protein nanofibrils directly from monomeric proteins, indicating possible uses in material manufacturing. These findings highlight ultrasound's critical function in protein refolding, notably in biopharmaceuticals and recombinant protein manufacturing. Despite its potential, the use of ultrasound in protein refolding remains a subject of the current study, demanding further studies to thoroughly understand its mechanisms and explore its potential applications **[80]**.

Comparison of structural characteristics between ultrasound-influenced and natively fibrillated proteins

The search results do not include precise information regarding the structural differences between ultrasound-influenced and naturally fibrillated proteins. Low-amplitude ultrasound may refold certain motifs in protein monomers, resulting in initial nucleation with a hydrogen-bonded β-sheet-rich structure. These structural changes are triggered by pressure disturbances and enhanced by temperature variations **[81]**. Furthermore, the prolonged action of low-amplitude ultrasound allows for the controlled elongation of amyloid protein nanofibrils directly from monomeric proteins until they reach a critical length—nanofibrillar assemblies formed under ultrasound share identical structural characteristics with natively fibrillated proteins **[81], [82]**.

CONCLUSION

In conclusion, studying ultrasound-assisted protein processing has yielded numerous significant discoveries. Ultrasound in protein extraction has been shown to increase yields, minimize solvent consumption, and improve protein physical, structural, and functional characteristics. Furthermore, the use of ultrasound in enzymatic hydrolysis has demonstrated encouraging results in terms of efficiency and biological activity. The interaction of ultrasound with proteins has enabled novel alterations, giving new opportunities in food processing. The implications of ultrasound-assisted protein processing for the food and biopharmaceutical industries are substantial. The technique offers a more sustainable and environmentally friendly approach in the food sector, aligning with the growing demand for greener practices. The improved quality of proteins and efficient extraction methods hold promise for developing novel food products with enhanced nutritional profiles. In the biopharmaceutical realm, ultrasound's role in protein refolding and enzymatic reactions signifies potential advancements in producing recombinant proteins and pharmaceutical formulations. These implications underscore the transformative impact ultrasound can have on various industrial applications. The impact of ultrasound-assisted protein processing on the food and pharmaceutical sectors is significant. In the food industry, the technology provides a more sustainable and ecologically friendly approach, meeting the rising need for greener practices. Protein quality improvements, along with effective extraction technologies, show promise for the development of innovative food items with increased nutritional profiles. In the biopharmaceutical field, ultrasound's function in protein refolding and enzymatic processes represents possible advances in creating recombinant proteins and medicinal formulations. These consequences highlight the transformational effect ultrasonography may have on various industrial applications. Prospects in ultrasound-assisted protein processing research provide exciting opportunities. Further research is needed to dive into the subtle mechanics of ultrasound-protein interactions, offering a better grasp of the technology's full potential. Refining and refining ultrasonic settings for specific applications, such as enzymatic hydrolysis and protein refolding, is critical for optimum efficiency. Furthermore, studying alternative industrial uses outside food and biopharmaceuticals, such as cosmetics and nutraceuticals, provides opportunities to widen the scope of ultrasound technology. Continued research and innovation in ultrasound-assisted protein processing are critical for realizing its full potential advantages and uses.

REFERENCES

1. Qian, J., Chen, D., Zhang, Y., Gao, X., Xu, L., Guan, G., & Wang, F. (2023). Ultrasound-Assisted Enzymatic Protein Hydrolysis in Food Processing: Mechanism and Parameters. In Foods (Vol. 12, Issue 21, p. 4027). MDPI AG. https://doi.org/10.3390/foods12214027
2. GÜLDANE, M.J.B.S.J.o.A., *Optimization of Ultrasound-Assisted Protein Extraction from Watermelon Seeds: Taguchi Approach.* p. 1-2.
3. Suchintita Das, R., Zhu, X., Hannon, S., Mullins, E., Alves, S., Garcia-Vaquero, M., & Tiwari, B. K. (2023). Exploring Osborne fractionation and laboratory/pilot scale technologies (conventional extraction, ultrasound-assisted extraction, high-pressure processing and hydrodynamic cavitation) for protein extraction from faba bean (Vicia faba L.). In Innovative Food Science & Emerging Technologies (Vol. 89, p. 103487). Elsevier BV. https://doi.org/10.1016/j.ifset.2023.103487
4. Loch, J.I., et al. *Structure determination of protein complexes based on joint NMR and X-ray crystallography data*. 2016.
5. Harish, M., & Venkatraman, P. (2021). Evolution of biophysical tools for quantitative protein interactions and drug discovery. In K. Bose (Ed.), Emerging Topics in Life Sciences (Vol. 5, Issue 1, pp. 1–12). Portland Press Ltd. https://doi.org/10.1042/etls20200258
6. Lorente-Martínez, H., Agorreta, A., & San Mauro, D. (2022). Genomic Fishing and Data Processing for Molecular Evolution Research. In Methods and Protocols (Vol. 5, Issue 2, p. 26). MDPI AG. https://doi.org/10.3390/mps5020026
7. Alshehhi, M., Wu, G., Kangsadan, T., Chew, K. W., & Show, P. L. (2023). Ultrasound-Assisted Food Processing: A Mini Review of Mechanisms, Applications, and Challenges. In M. Sriariyanun, Y.-S. Cheng, D. Bhattacharyya, P. Venkatachalam, & M. P. Gundupalli (Eds.), E3S Web of Conferences (Vol. 428, p. 02011). EDP Sciences. https://doi.org/10.1051/e3sconf/202342802011

8. Chen, J., Chai, J., Sun, X., Tao, Y., Chen, X., Zhou, G., & Xu, X. (2023). Unexpected variations in the effects of ultrasound-assisted myofibrillar protein processing under varying viscosity conditions. In Ultrasonics Sonochemistry (Vol. 99, p. 106553). Elsevier BV. https://doi.org/10.1016/j.ultsonch.2023.106553
9. Zheng, X., Zou, B., Zhang, J., Cai, W., Na, X., Du, M., Zhu, B., & Wu, C. (2024). Recent advances of ultrasound-assisted technology on aquatic protein processing: Extraction, modification, and freezing/thawing-induced oxidation. In Trends in Food Science & Technology (Vol. 144, p. 104309). Elsevier BV. https://doi.org/10.1016/j.tifs.2023.104309
10. Abdulstar, A. R., Altemimi, A. B., & Al-Hilphy, A. R. (2023). Exploring the Power of Thermosonication: A Comprehensive Review of Its Applications and Impact in the Food Industry. In Foods (Vol. 12, Issue 7, p. 1459). MDPI AG. https://doi.org/10.3390/foods12071459
11. Celton, C., Caton, S., Akoetey, W., Nicol, R., & Hosseinian, F. (2023). High-intensity, Low-frequency Ultrasound Treatment as Sustainable Strategy to Develop Innovative Biomaterials from Agri-food Byproducts and Wastes. In Sustainable Agriculture Research (Vol. 12, Issue 2, p. 45). Canadian Center of Science and Education. https://doi.org/10.5539/sar.v12n2p45
12. Brnčić, M., & Šic Žlabur, J. (2019). Impact of Ultrasound on Food Constituents. In Effect of Emerging Processing Methods on the Food Quality (pp. 69–94). Springer International Publishing. https://doi.org/10.1007/978-3-030-18191-8_3
13. Lavilla, I., & Bendicho, C. (2017). Fundamentals of Ultrasound-Assisted Extraction. In Water Extraction of Bioactive Compounds (pp. 291–316). Elsevier. https://doi.org/10.1016/b978-0-12-809380-1.00011-5
14. Yamaguchi, T. (2021). Basic concept and clinical applications of quantitative ultrasound (QUS) technologies. In Journal of Medical Ultrasonics (Vol. 48, Issue 4, pp. 391–402). Springer Science and Business Media LLC. https://doi.org/10.1007/s10396-021-01139-6
15. Duan, L., Yang, L., Jin, J., Yang, F., Liu, D., Hu, K., Wang, Q., Yue, Y., & Gu, N. (2020). Micro/nano-bubble-assisted ultrasound to enhance the EPR effect and potential theranostic applications. In Theranostics (Vol. 10, Issue 2, pp. 462–483). Ivyspring International Publisher. https://doi.org/10.7150/thno.37593
16. Goldstein, R. V., & Kuznetsov, S. V. (2013). Surface acoustic waves in the testing of layered media. The waves' sensitivity to variations in the properties of the individual layers. In Journal of Applied Mathematics and Mechanics (Vol. 77, Issue 1, pp. 51–56). Elsevier BV. https://doi.org/10.1016/j.jappmathmech.2013.04.007
17. Joshi, S. G., Zaĭtsev, B. D., & Kuznetsova, I. E. (2001). SH Acoustic Waves in a Lithium Niobate Plate and the Effect of Electrical Boundary Conditions on Their Properties. In Acoustical Physics (Vol. 47, Issue 3, pp. 282–285). Springer Science and Business Media LLC. https://doi.org/10.1007/bf03353580
18. Bandhu, L., & Nash, G. R. (2015). Controlling the properties of surface acoustic waves using graphene. In Nano Research (Vol. 9, Issue 3, pp. 685–691). Springer Science and Business Media LLC. https://doi.org/10.1007/s12274-015-0947-z
19. Kuznetsova, I. E., Zaitsev, B. D., Polyakov, P. V., & Mysenko, M. B. (1998). External electric field effect on the properties of Bleustein-Gulyaev surface acoustic waves in lithium niobate and strontium titanate. In Ultrasonics (Vol. 36, Issues 1–5, pp. 431–434). Elsevier BV. https://doi.org/10.1016/s0041-624x(97)00049-8
20. Kuznetsova, I., Nedospasov, I., Zaitsev, B., & Kuznetsova, A. (2015). Influence of liquid on properties of backward acoustic waves in piezoelectric plates. In 2015 IEEE International Ultrasonics Symposium (IUS). 2015 IEEE International Ultrasonics Symposium (IUS). IEEE. https://doi.org/10.1109/ultsym.2015.0351
21. Yamamoto, T., T. Furukawa, and I. Komura. Property study on emats with visualization of ultrasonic propagation. 2016.
22. Petrescu, F. I. T. (2015). Improving Medical Imaging and Blood Flow Measurement by using a New Doppler Effect Relationship. In American Journal of Engineering and Applied Sciences (Vol. 8, Issue 4, pp. 582–588). Science Publications. https://doi.org/10.3844/ajeassp.2015.582.588

23. Pesaresi, L., Fantetti, A., Cegla, F., Salles, L., & Schwingshackl, C. W. (2019). On the Use of Ultrasound Waves to Monitor the Local Dynamics of Friction Joints. In Experimental Mechanics (Vol. 60, Issue 1, pp. 129–141). Springer Science and Business Media LLC. https://doi.org/10.1007/s11340-019-00550-y

24. Gao, Z., & Kohyama, K. (2014). Ultrasound Pulsed Wave <scp>D</scp>oppler Imaging of the Esophagus Illustrates the Effects of Water Volume on Bolus Kinematics. In Journal of Texture Studies (Vol. 45, Issue 5, pp. 335–343). Wiley. https://doi.org/10.1111/jtxs.12077

25. Wang, L., Li, Y.-J., Lin, A., Choe, Y., Gross, M. E., & Kim, E. S. (2013). A Self-Focusing Acoustic Transducer That Exploits Cytoskeletal Differences for Selective Cytolysis of Cancer Cells. In Journal of Microelectromechanical Systems (Vol. 22, Issue 3, pp. 542–552). Institute of Electrical and Electronics Engineers (IEEE). https://doi.org/10.1109/jmems.2012.2229696

26. Mostafa, J., Ali, Y., Zohre, R., & Samaneh, R. (2015). Electromagnetic Fields and Ultrasound Waves in Wound Treatment: A Comparative Review of Therapeutic Outcomes. In Biosciences Biotechnology Research Asia (Vol. 12, Issue SEMAR, pp. 185–195). Oriental Scientific Publishing Company. https://doi.org/10.13005/bbra/1622

27. Nagamani, M., & Chilvers, R. (2010). Ultrasonography and diagnosis of polycystic ovary syndrome. In Ultrasonography in Reproductive Medicine and Infertility (pp. 75–80). Cambridge University Press. https://doi.org/10.1017/cbo9780511776854.011

28. Abrunhosa, V. M., Mermelstein, C. S., Costa, M. L., & Costa-Felix, R. P. B. (2011). Biological responsein vitroof skeletal muscle cells treated with different intensity continuous and pulsed ultrasound fields. In Journal of Physics: Conference Series (Vol. 279, p. 012022). IOP Publishing. https://doi.org/10.1088/1742-6596/279/1/012022

29. Mehta, N., S, Jeyapriya., Kumar, P., Verma, A. K., Umaraw, P., Khatkar, S. K., Khatkar, A. B., Pathak, D., Kaka, U., & Sazili, A. Q. (2022). Ultrasound-Assisted Extraction and the Encapsulation of Bioactive Components for Food Applications. In Foods (Vol. 11, Issue 19, p. 2973). MDPI AG. https://doi.org/10.3390/foods11192973

30. Biparva, P., Mirzapour-Kouhdasht, A., Valizadeh, S., & Garcia-Vaquero, M. (2023). Advanced Processing of Giant Kelp (Macrocystis pyrifera) for Protein Extraction and Generation of Hydrolysates with Anti-Hypertensive and Antioxidant Activities In Vitro and the Thermal/Ionic Stability of These Compounds. In Antioxidants (Vol. 12, Issue 3, p. 775). MDPI AG. https://doi.org/10.3390/antiox12030775

31. Zhou, Q., Wang, H., Zhu, K., Zhu, L., Zhou, S., Peng, J., & Lu, X. (2017). Effects of ultrasound irradiation on enzymatic hydrolysis of protein and application for the determination of tetracyclines in complex matrices. In Drug Testing and Analysis (Vol. 9, Issue 10, pp. 1586–1593). Wiley. https://doi.org/10.1002/dta.2181

32. Kangsanant, S., Murkovic, M., & Thongraung, C. (2014). Antioxidant and nitric oxide inhibitory activities of tilapia reochromis niloticus) protein hydrolysate: effect of ultrasonic pretreatment and ultrasonic-assisted enzymatic hydrolysis. In International Journal of Food Science & Technology (Vol. 49, Issue 8, pp. 1932–1938). Wiley. https://doi.org/10.1111/ijfs.12551

33. Liu, J., Song, G., Zhou, L., Yuan, Y., Wang, D., Yuan, T., Li, L., Yuan, H., Xiao, G., & Gong, J. (2023). Recent advances in the effect of ultrasound on the binding of protein−polyphenol complexes in foodstuff. In Food Frontiers (Vol. 4, Issue 2, pp. 721–732). Wiley. https://doi.org/10.1002/fft2.221

34. Price, S. E. N., Hansen, R., & Gjennestad, M. Aa. (2023). A volume-averaged model for acoustic streaming induced by focused ultrasound in soft porous media. In The Journal of the Acoustical Society of America (Vol. 154, Issue 1, pp. 334–345). Acoustical Society of America (ASA). https://doi.org/10.1121/10.0020146

35. Strom-Jensen, P. R., & Dunn, F. (1984). Ultrasonic absorption by solvent–solute interactions and proton transfer in aqueous solutions of peptides and small proteins. In The Journal of the Acoustical Society of America (Vol. 75, Issue 3, pp. 960–966). Acoustical Society of America (ASA). https://doi.org/10.1121/1.390561

36. Yolandani, Ma, H., Li, Y., Liu, D., Zhou, H., Liu, X., Wan, Y., & Zhao, X. (2023). Ultrasound-assisted limited enzymatic hydrolysis of high concentrated soy protein isolate: Alterations on the functional properties and its relation with hydrophobicity and molecular weight. In Ultrasonics Sonochemistry (Vol. 95, p. 106414). Elsevier BV. https://doi.org/10.1016/j.ultsonch.2023.106414

37. Lindsay Rojas, M., Hellmeister Trevilin, J., & Augusto, P. E. D. (2016). The ultrasound technology for modifying enzyme activity. In Scientia Agropecuaria (Vol. 07, Issue 02, pp. 145–150). Universidad Nacional de Trujillo. https://doi.org/10.17268/sci.agropecu.2016.02.07

38. Wang, C., Zhao, R., Liu, J., & Wang, C. (2022). Towards understanding the interaction between ultrasound-pretreated β-lactoglobulin monomer with resveratrol. In Luminescence (Vol. 38, Issue 2, pp. 116–126). Wiley. https://doi.org/10.1002/bio.4427

39. Anaya-Esparza, L. M., Aurora-Vigo, E. F., Villagrán, Z., Rodríguez-Lafitte, E., Ruvalcaba-Gómez, J. M., Solano-Cornejo, M. Á., Zamora-Gasga, V. M., Montalvo-González, E., Gómez-Rodríguez, H., Aceves-Aldrete, C. E., & González-Silva, N. (2023). Design of Experiments for Optimizing Ultrasound-Assisted Extraction of Bioactive Compounds from Plant-Based Sources. In Molecules (Vol. 28, Issue 23, p. 7752). MDPI AG. https://doi.org/10.3390/molecules28237752

40. Liu, D., Di, H., Guo, Y., Betchem, G., & Ma, H. (2022). Multi-mode S-type ultrasound-assisted protein extraction from walnut dregs and in situ real-time process monitoring. In Ultrasonics Sonochemistry (Vol. 89, p. 106116). Elsevier BV. https://doi.org/10.1016/j.ultsonch.2022.106116

41. Liu, D., Di, H., Guo, Y., Betchem, G., & Ma, H. (2022). Multi-mode S-type ultrasound-assisted protein extraction from walnut dregs and in situ real-time process monitoring. In Ultrasonics Sonochemistry (Vol. 89, p. 106116). Elsevier BV. https://doi.org/10.1016/j.ultsonch.2022.106116

42. Ashok N., A., & Sontakke, M. (2023). Comparative analysis of extraction methods, proximate composition, and physicochemical and functional properties of fruit seed proteins: A comprehensive review. In eFood (Vol. 4, Issue 6). Wiley. https://doi.org/10.1002/efd2.117

43. Sánchez, J., Loaña, J., Agualongo, M., & Espinoza, K. (2020). Cultivation techniques and methods of extraction of fatty acids based on microalgae for the benefit of humanity. In Agroindustrial Science (Vol. 10, Issue 3, pp. 319–328). Universidad Nacional de Trujillo. https://doi.org/10.17268/agroind.sci.2020.03.15

44. Jia, F., Liu, X., Gong, Z., Cui, W., Wang, Y., & Wang, W. (2020). Extraction, modification, and property characterization of dietary fiber from Agrocybe cylindracea. In Food Science & Nutrition (Vol. 8, Issue 11, pp. 6131–6143). Wiley. https://doi.org/10.1002/fsn3.1905

45. Qian, J., Chen, D., Zhang, Y., Gao, X., Xu, L., Guan, G., & Wang, F. (2023). Ultrasound-Assisted Enzymatic Protein Hydrolysis in Food Processing: Mechanism and Parameters. In Foods (Vol. 12, Issue 21, p. 4027). MDPI AG. https://doi.org/10.3390/foods12214027

46. Alonso-Riaño, P., Sanz Diez, M. T., Blanco, B., Beltrán, S., Trigueros, E., & Benito-Román, O. (2020). Water Ultrasound-Assisted Extraction of Polyphenol Compounds from Brewer's Spent Grain: Kinetic Study, Extract Characterization, and Concentration. In Antioxidants (Vol. 9, Issue 3, p. 265). MDPI AG. https://doi.org/10.3390/antiox9030265

47. Sengkhamparn, N., Lasunon, P., & Tettawong, P. (2019). Effect of Ultrasound Assisted Extraction and Acid Type Extractant on Pectin from Industrial Tomato Waste. In Chiang Mai University Journal of Natural Sciences (Vol. 18, Issue 2). Chiang Mai University. https://doi.org/10.12982/cmujns.2019.0016

48. Fatima, K., Imran, M., Ahmad, M. H., Khan, M. K., Khalid, W., AL-Farga, A., Alansari, W. S., Shamlan, G., & Eskandrani, A. A. (2023). Ultrasound-Assisted Extraction of Protein from Moringa oleifera Seeds and Its Impact on Techno-Functional Properties. In Molecules (Vol. 28, Issue 6, p. 2554). MDPI AG. https://doi.org/10.3390/molecules28062554

49. Sert, D., Rohm, H., & Struck, S. (2022). Ultrasound-Assisted Extraction of Protein from Pumpkin Seed Press Cake: Impact on Protein Yield and Techno-Functionality. In Foods (Vol. 11, Issue 24, p. 4029). MDPI AG. https://doi.org/10.3390/foods11244029

50. Zhao, Y., Yan, M., Xue, S., Zhang, T., & Shen, X. (2022). Influence of ultrasound and enzymatic cross-linking on freeze-thaw stability and release properties of whey protein isolate hydrogel. In

Journal of Dairy Science (Vol. 105, Issue 9, pp. 7253–7265). American Dairy Science Association. https://doi.org/10.3168/jds.2021-21605

51. Rezvankhah, A., Yarmand, M. S., & Ghanbarzadeh, B. (2022). The effects of combined enzymatic and physical modifications of lentil protein applying Alcalase, Flavourzyme, microbial transglutaminase, and ultrasound: antioxidant, antihypertension, and antidiabetic activities. In Journal of Food Measurement and Characterization (Vol. 16, Issue 5, pp. 3743–3759). Springer Science and Business Media LLC. https://doi.org/10.1007/s11694-022-01478-z

52. Wayu, M. B., Pannell, M. J., & Leopold, M. C. (2016). Layered Xerogel Films Incorporating Monolayer-Protected Cluster Networks on Platinum-Black-Modified Electrodes for Enhanced Sensitivity in First-Generation Uric Acid Biosensing. In ChemElectroChem (Vol. 3, Issue 8, pp. 1245–1252). Wiley. https://doi.org/10.1002/celc.201600164

53. Wang, L., Zhang, X., You, Z., Yang, Z., Guo, M., Guo, J., Liu, H., Zhang, X., Wang, Z., Wang, A., Lv, Y., Zhang, J., Yu, X., Liu, J., & Chen, C. (2023). A Molybdenum Disulfide Nanozyme with Charge-Enhanced Activity for Ultrasound-Mediated Cascade-Catalytic Tumor Ferroptosis. In Angewandte Chemie International Edition (Vol. 62, Issue 11). Wiley. https://doi.org/10.1002/anie.202217448

54. Kazlauskaite, J. A., Ivanauskas, L., & Bernatoniene, J. (2021). Cyclodextrin-Assisted Extraction Method as a Green Alternative to Increase the Isoflavone Yield from Trifolium pratensis L. Extract. In Pharmaceutics (Vol. 13, Issue 5, p. 620). MDPI AG. https://doi.org/10.3390/pharmaceutics13050620

55. Anand, A., Kumar, K., Khaire, K. C., Roy, K., & Moholkar, V. S. (2023). Ultrasound-Assisted Hydrolysis of Food Waste using Glucoamylase: Statistical Optimization and Mechanistic Analysis with Molecular Simulations. Cold Spring Harbor Laboratory. https://doi.org/10.1101/2023.12.14.571620

56. Zhang, Z., Gao, P., Jiang, Q., Zhang, Z., Zhang, X., Yang, F., Yu, P., Liu, S., & Wenshui, X. (2023). Optimization of ultrasound-assisted enzymatic hydrolysis of channel catfish (Ictalurus punctatus) frames yield high-calcium and high-amino acid hydrolysates. In International Journal of Food Properties (Vol. 26, Issue 2, pp. 3393–3406). Informa UK Limited. https://doi.org/10.1080/10942912.2023.2281886

57. Pedrosa, N. de A., de Andrade, C. J., Petrus, J. C. C., & Monteiro, A. R. (2022). Sequential Hydrolysis of Chicken Feathers Composed of Ultrasound and Enzymatic Steps: An Enhanced Protein Source with Bioactive Peptides. In Biomass (Vol. 2, Issue 4, pp. 237–249). MDPI AG. https://doi.org/10.3390/biomass2040016

58. Salman Mohammad, S., Barbosa, M. I. M. J., Gamallo, O., & Barbosa Junior, J. L. (2023). The Production of Bioactive Peptides by Optimization of Enzymatic Hydrolysis Process of Protein from Tilapia Fish Skin Waste (Oreochromis niloticus, Linnaeus 1758) using Alcalase 2.4.L. In Current Bioactive Compounds (Vol. 19, Issue 10). Bentham Science Publishers Ltd. https://doi.org/10.2174/1573407219666230502154801

59. Tawalbeh, D., Ahmad, W. A. N. W., & Sarbon, N. M. (2022). Effect of ultrasound pretreatment on the functional and bioactive properties of legumes protein hydrolysates and peptides: A comprehensive review. In Food Reviews International (Vol. 39, Issue 8, pp. 5423–5445). Informa UK Limited. https://doi.org/10.1080/87559129.2022.2069258

60. Wen, Q., Zhang, L., Zhao, F., Chen, Y., Su, Y., Zhang, X., Chen, P., & Zheng, T. (2023). Production Technology and Functionality of Bioactive Peptides. In Current Pharmaceutical Design (Vol. 29, Issue 9, pp. 652–674). Bentham Science Publishers Ltd. https://doi.org/10.2174/1381612829666230201121353

61. Sun, X., Yu, Y., Saleh, A. S. M., Yang, X., Ma, J., Gao, Z., Li, W., Wang, Z., & Zhang, D. (2023). Structural changes induced by ultrasound improve the ability of the myofibrillar protein to bind flavor compounds from spices. In Ultrasonics Sonochemistry (Vol. 98, p. 106510). Elsevier BV. https://doi.org/10.1016/j.ultsonch.2023.106510

62. Kozell, A., Solomonov, A., Gaidarov, R., Benyamin, D., Rosenhek-Goldian, I., Greenblatt, H. M., Levy, Y., Amir, A., Raviv, U., & Shimanovich, U. (2023). Sound-mediated nucleation and growth of amyloid fibrils. Cold Spring Harbor Laboratory. https://doi.org/10.1101/2023.09.16.558053

63. Zhao, S., Song, Y., Xu, L., Hu, H., Wang, J., Huang, F., & Shi, L. (2023). Self-Assembly Nanochaperone with Tunable Hydrophilic–Hydrophobic Surface for Controlled Protein Refolding. In Macromolecular Bioscience (Vol. 23, Issue 11). Wiley. https://doi.org/10.1002/mabi.202300205

64. Herrmann, T., Torres, R., Salgado, E. N., Berciu, C., Stoddard, D., Nicastro, D., Jenni, S., & Harrison, S. C. (2021). Functional refolding of the penetration protein on a non-enveloped virus. In Nature (Vol. 590, Issue 7847, pp. 666–670). Springer Science and Business Media LLC. https://doi.org/10.1038/s41586-020-03124-4

65. Michaux, C., Roussel, G., Lopes-Rodrigues, M., Matagne, A., & Perpète, E. A. (2016). Unravelling the mechanisms of a protein refolding process based on the association of detergents and co-solvents. In Journal of Peptide Science (Vol. 22, Issue 7, pp. 485–491). Wiley. https://doi.org/10.1002/psc.2893

66. Żwirowski, S., Kłosowska, A., Obuchowski, I., Nillegoda, N. B., Piróg, A., Ziętkiewicz, S., Bukau, B., Mogk, A., & Liberek, K. (2017). Hsp70 displaces small heat shock proteins from aggregates to initiate protein refolding. In The EMBO Journal (Vol. 36, Issue 6, pp. 783–796). Springer Science and Business Media LLC. https://doi.org/10.15252/embj.201593378

67. Sarker, A., Rathore, A. S., & Gupta, R. D. (2019). Evaluation of scFv protein recovery from E. coli by in vitro refolding and mild solubilization process. In Microbial Cell Factories (Vol. 18, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12934-019-1053-9

68. Yao, S., Li, W., Wu, Y., Martin, G., & Ashokkumar, M. (2023). The Impact of High-Intensity Ultrasound-Assisted Extraction on the Structural and Functional Properties of Hempseed Protein Isolate (HPI). In Foods (Vol. 12, Issue 2, p. 348). MDPI AG. https://doi.org/10.3390/foods12020348

69. Enoki, S., Saeki, K., Maki, K., & Kuwajima, K. (2004). Acid Denaturation and Refolding of Green Fluorescent Protein. In Biochemistry (Vol. 43, Issue 44, pp. 14238–14248). American Chemical Society (ACS). https://doi.org/10.1021/bi048733+

70. Heim, B., Handrick, R., Hartmann, M. D., & Kiefer, H. (2021). Refolding and characterization of two G protein-coupled receptors purified from E. coli inclusion bodies. In S. D'Auria (Ed.), PLOS ONE (Vol. 16, Issue 2, p. e0247689). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0247689

71. Jensen, P.K. *Monitoring protein refolding using diverse separation and spectroscopy techniques*. 1997.

72. Upadhyay, A. K., Singh, A., Mukherjee, K. J., & Panda, A. K. (2014). Refolding and purification of recombinant L-asparaginase from inclusion bodies of E. coli into active tetrameric protein. In Frontiers in Microbiology (Vol. 5). Frontiers Media SA. https://doi.org/10.3389/fmicb.2014.00486

73. Moghadam, M., et al., *Refolding process of cysteine-rich proteins:Chitinase as a model.* Rep Biochem Mol Biol, (Vol. 4, Issue 1, pp. 19-24).

74. Kolivand, S., Nazari, M., Modarressi, M. H., Najafabadi, M. R. H., Hemati, A., Ghafouri-Fard, S., & Motevaseli, E. (2020). Optimized protocol for soluble prokaryotic expression, purification and refolding of the human inhibin α subunit, a cysteine rich peptide chain. In Human Antibodies (Vol. 28, Issue 2, pp. 131–139). IOS Press. https://doi.org/10.3233/hab-190399

75. Enkhtuya, B., Ren, Y., Hu, Y., Chen, Y., Hu, J., Yu, X., Wang, W., & Fan, J. (2021). Selection of tobacco etch virus protease variants with enhanced oxidative stability for tag-removal in refolding of two disulfide-rich proteins. Research Square Platform LLC. https://doi.org/10.21203/rs.3.rs-174410/v1

76. Ma, F.-H., An, Y., Wang, J., Song, Y., Liu, Y., & Shi, L. (2017). Synthetic Nanochaperones Facilitate Refolding of Denatured Proteins. In ACS Nano (Vol. 11, Issue 10, pp. 10549–10557). American Chemical Society (ACS). https://doi.org/10.1021/acsnano.7b05947

77. Yoo, H., Nagornyak, E., Das, R., Wexler, A. D., & Pollack, G. H. (2014). Contraction-Induced Changes in Hydrogen Bonding of Muscle Hydration Water. In The Journal of Physical Chemistry

Letters (Vol. 5, Issue 6, pp. 947–952). American Chemical Society (ACS). https://doi.org/10.1021/jz5000879

78. Żwirowski, S., Kłosowska, A., Obuchowski, I., Nillegoda, N. B., Piróg, A., Ziętkiewicz, S., Bukau, B., Mogk, A., & Liberek, K. (2017). Hsp70 displaces small heat shock proteins from aggregates to initiate protein refolding. In The EMBO Journal (Vol. 36, Issue 6, pp. 783–796). Springer Science and Business Media LLC. https://doi.org/10.15252/embj.201593378

79. Obuchowski, I., Piróg, A., Stolarska, M., Tomiczek, B., & Liberek, K. (2019). Duplicate divergence of two bacterial small heat shock proteins reduces the demand for Hsp70 in refolding of substrates. In D. A. Garsin (Ed.), PLOS Genetics (Vol. 15, Issue 10, p. e1008479). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pgen.1008479

80. Kozell, A., Solomonov, A., Gaidarov, R., Benyamin, D., Rosenhek-Goldian, I., Greenblatt, H. M., Levy, Y., Amir, A., Raviv, U., & Shimanovich, U. (2023). Sound-mediated nucleation and growth of amyloid fibrils. Cold Spring Harbor Laboratory. https://doi.org/10.1101/2023.09.16.558053

81. Fitzpatrick, A. W. P., Park, S. T., & Zewail, A. H. (2013). Exceptional rigidity and biomechanics of amyloid revealed by 4D electron microscopy. In Proceedings of the National Academy of Sciences (Vol. 110, Issue 27, pp. 10976–10981). Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.1309690110

82. Balbirnie, M., Grothe, R., & Eisenberg, D. S. (2001). An amyloid-forming peptide from the yeast prion Sup35 reveals a dehydrated β-sheet structure for amyloid. In Proceedings of the National Academy of Sciences (Vol. 98, Issue 5, pp. 2375–2380). Proceedings of the National Academy of Sciences. https://doi.org/10.1073/pnas.041617698

Funds:

This research has been funded by the Committee of Science of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. BR21882447).

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Maksim Rebezov, V. M. Gorbatov Federal Research Center for Food Systems, Department of Scientific Research, 26 Talalikhin Str., Moscow, 109316, Russia; Ural State Agrarian University, Department of Biotechnology and Food, 42 Karl Liebknecht str., Yekaterinburg, 620075, Russia
Tel.: +79999002365
E-mail: rebezov@ya.ru
ORCID: https://orcid.org/0000-0003-0857-5143

***Bahytkul Assenova**, Shakarim University, Department of Food Production Technology and Biotechnology, 20A Glinka Str., Semey, 071412, Kazakhstan,
Tel.: +77076586117
E-mail: asenova.1958@mail.ru
ORCID: https://orcid.org/0000-0003-2996-8587

Albina Luneva, Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Department of Veterinary Medicine, 49 Timiryazevskaya Str., Moscow, 127434, Russia,
Tel.: +7918417-21-38
E-mail: albina.luneva@mail.ru
ORCID: https://orcid.org/0000-0002-4863-3590

Mars Khayrullin, K.G. Razumovsky Moscow State University of Technologies and Management (The First Cossack University), Department of Scientific Research, 73 Zemlyanoy Val, Moscow, 109004, Russia,
Tel.: +79049755219
E-Mail: 89049755219@ya.ru
ORCID: https://orcid.org/0000-0003-1697-7281

Rustem Zalilov, Nosov Magnitogorsk State Technical University, Department of Scientific Research, 38 Lenin Str., Magnitogorsk, 455000 Russia,
E-mail: zalilow74@yandex.ru
ORCID: https://orcid.org/0000-0001-9642-8930

Yury Lysenko, Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Department of Veterinary Medicine, 49 Timiryazevskaya Str., Moscow, 127434, Russia,
Tel.: +7961518-07-22
E-mail: yuraduban45@mail.ru
ORCID: https://orcid.org/0000-0002-2629-2334

Raisa Savkina, Plekhanov Russian University of Economics, Department of Restaurant Business, 36 Stremyanny per, 117997, Moscow, Russia,
E-mail: kafedrains@yandex.ru
ORCID: https://orcid.org/0009-0000-8460-1396

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 14.3.2024
Revised: 25.5.2024
Accepted: 30.5.2024
Published: 26.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 588-604
https://doi.org/10.5219/1971
ISSN: 1337-0960 online
www.potravinarstvo.com

Exploring natural colourants for enhanced sausage appeal: A review of sourcing, extraction methods, and applications, with emphasis on beetroot as an example

Anuarbek Suychinov, Aitbek Kakimov, Zhanibek Yessimbekov, Eleonora Okuskhanova, Dinara Akimova, Zhumatay Urazbayev

ABSTRACT

Incorporating natural colourants in sausage production offers an avenue to enhance product desirability while meeting consumer preferences for clean-label ingredients. This paper thoroughly examines the selection, formulation, processing, and quality control aspects involved in utilizing natural colourants in sausages, with particular attention to beetroot. Key subjects explored encompass a variety of natural colourant sources and types, diverse extraction techniques, factors influencing colour stability, regulatory considerations, and consumer perception. Furthermore, the paper delves into emerging trends and advancements in sausage manufacturing, such as plant-based alternatives, functional fortification, and sustainability initiatives. By strategically harnessing the potential of natural colourants, including beetroot, sausage producers can customize their offerings to align with consumer preferences, distinguish their products, and bolster their competitiveness in the market.

Keywords: Natural colourants, sausage production, beetroot, clean label ingredients, formulation, processing, quality control, extraction methods, colour stability, regulatory considerations, consumer perception, plant-based alternatives, functional fortification, sustainability initiatives, market competitiveness

INTRODUCTION

Natural colourants are widely employed in the food business to improve the visual appeal of goods. They can be used in place of or in addition to synthetic colourants. Natural colourants can be produced from botanical, mineral, or microbiological sources. They are prized for their capacity to produce vivid colours while satisfying consumer demands for minimally processed and clean-label materials. These colourants fall into two general categories: extracts and pigments. Carotenoids, anthocyanins, chlorophyll, and betalains are examples of pigments; paprika, beetroot, turmeric, annatto, and spirulina are extracts **[1]**. With their distinct qualities, these natural colourants are used in various food items to improve aesthetic appeal and satisfy customers' desire for natural ingredients. Spirulina and other naturally occurring food colouring sources in beverage model solutions. The study used UV-Vis Spectrophotometry and Colourimetry to test colour and stability and assess the storage conditions **[2]**. Other findings show that algae's antioxidant qualities allow them to be used as natural colourants and have various uses in different sectors. They emphasize how crucial it is to assess the patterns and key elements influencing the synthesis of algal pigments **[3]**.

Additionally, the antioxidant qualities of anthocyanins are naturally occurring pigments found in a wide variety of meals, fruits, and vegetables, particularly berries. It contributed to the trend of using antioxidants produced from natural products by highlighting the advantages of anthocyanins for health and their usage as colourants because of their antioxidant activity **[4]**. Considering the latest developments in pH-sensitive indicator films made

of natural colourants for intelligent food freshness monitoring, it covered several kinds of natural pigment markers and how they are used to check the freshness of different foods, such as curcumin and anthocyanins **[5]**.

The food sector is using more and more natural colourants from various sources in place of synthetic colourants. These natural pigments come in various hues and may be found in plants, fruits, vegetables, and minerals. Natural colourants, such as anthocyanins, carotenoids, betalains, and chlorophylls, have major environmental and biological sources that have been discovered. Furthermore, natural colourants have become a viable and healthy alternative that gives food systems intriguing technical and sensory features **[6]**.

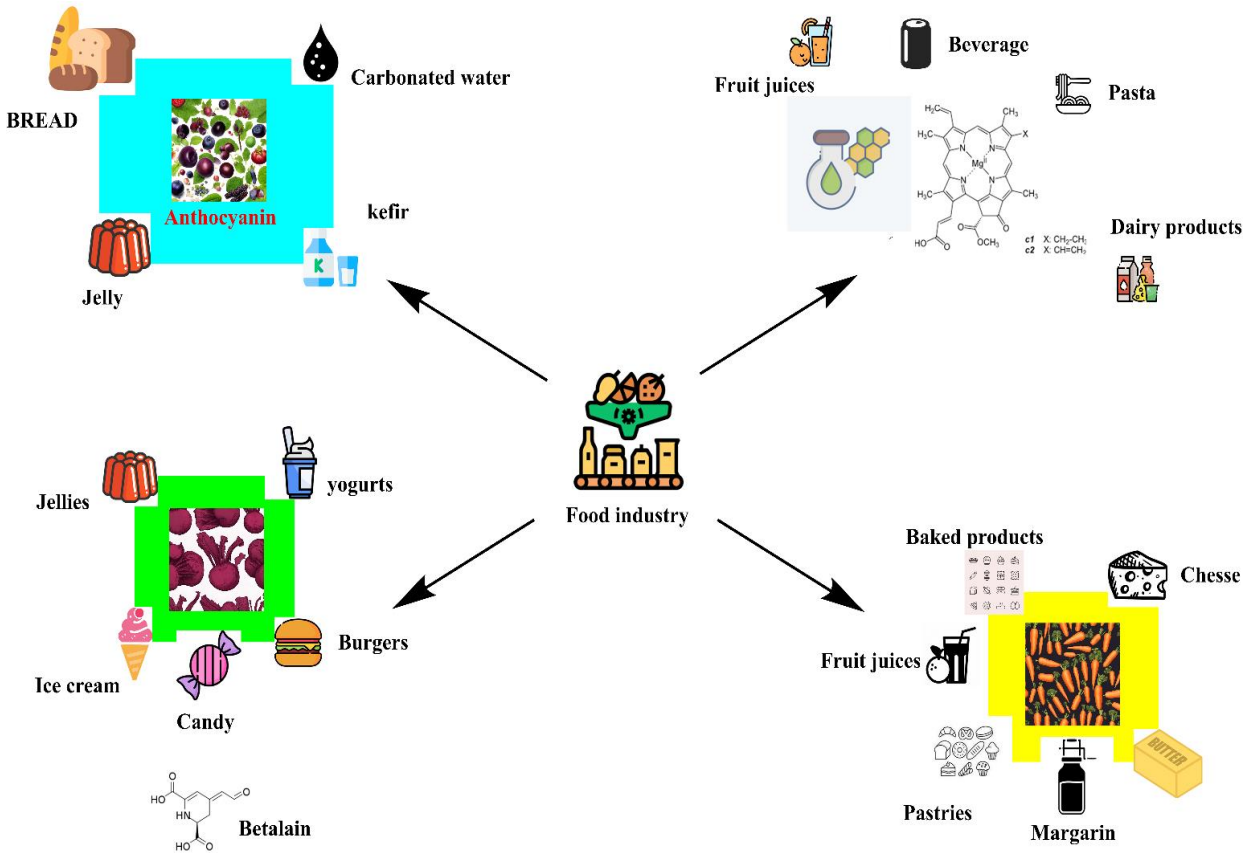


Figure 1 Scheme of the application of natural colourant in different food industries.

This has spurred research into novel natural hair dye supplies and environmentally friendly production and application technologies. It has also been highlighted how anthocyanins, ubiquitous in human diets and found in a wide variety of foods, fruits, and vegetables, have antioxidant qualities. In addition to being utilised as colourants, anthocyanins have strong antioxidant properties, which supports the trend toward using antioxidants sourced from natural sources **[7]**.

Fruits include a variety of colourant molecules, including anthocyanins, betalains, carotenoids, and chlorophylls, making them a dependable supply of food colourants. Furthermore, fruits have bioactive qualities, making natural food colouring substitutes desirable. However, there are issues with raw material sustainability and stability when extracting and applying natural colourants from fruits. It has been found that optimum stabilisation and extraction techniques can overcome these drawbacks. Recent developments have shown that natural fruit colourants may enhance the colour of various food products. But colour constancy is still difficult to maintain **[8]**, **[9]**.

Additionally, using natural colourants in intelligent packaging has been investigated to track the freshness of food items, with encouraging outcomes in terms of real-time food quality monitoring. Fruit natural colourants may have advantages, but it's vital to consider how they could affect your health. Certain naturally occurring colourants, including curcumin, anthocyanins, and catechins, have been shown to suppress the growth of different cell types, indicating their potential as drugs that prevent cancer through chemotherapy. Nonetheless, more investigation is required to validate these results and ascertain the ideal concentrations and modes of administration for natural colouring agents. The growing consumer desire for minimally processed and clean-label goods has increased the demand for natural food colourings. As a result, despite stability, sustainability, and affordability issues, interest in natural substitutes—like fruits—has surged again. To overcome these constraints,

creative alternatives are being explored, such as the creation of novel, safer, and more effective natural compounds derived from fruits **[8]**, **[10]**.

Natural colourants are categorised according to their chemical composition and place of origin. Pigments are the main agents responsible for the vivid colours found in fruits, vegetables, and other natural sources. The four main pigment categories frequently used as natural colourants are betalains, chlorophyll, anthocyanins, and carotenoids. The pigments known as carotenoids are found in many fruits, vegetables, and plants. They are the cause of the hues red, orange, and yellow. Carrots and sweet potatoes contain beta-carotene, known for its antioxidant qualities and vivid orange colour **[11]**.

Tomatoes, watermelons, and pink grapefruits are rich sources of lycopene, which gives them a rich red colour and heart health advantages. Green leafy vegetables, such as spinach and kale, contain lutein, which helps maintain eye health and gives these veggies their yellow hue. Water-soluble pigments called anthocyanins, which are part of the flavonoid group, are responsible for the red, purple, and blue hues found in various fruits, vegetables, and flowers. Anthocyanins, which give their vivid colours, are abundant in berries, including blueberries, strawberries, raspberries, and blackberries **[12]**.

Red wine and other products made from grapes are coloured by anthocyanins found in the skins of grapes, especially the purple and red kinds. Red cabbage's rich purple hue is caused by anthocyanins, frequently employed as a natural pH indicator. Green leafy vegetables and herbs are rich in chlorophyll, a green pigment that helps plants perform photosynthesis. Chlorophyll also acts as a natural colourant, giving green hues to many objects. Common sources of chlorophyll that give food items vivid green hues are spinach, parsley, and mint. Beets and certain cacti are the main sources of betalains, which include betacyanins and betaxanthins. Because of the betacyanins in their roots, beets provide vivid red and purple colours frequently utilised as natural food colouring ingredients. The prickly pear cactus's fruits, blooms, and stems generate betalain pigments, which come in red, purple, and yellow hues **[12]**.

These pigments – carotenoids, anthocyanins, chlorophyll, and betalains—contribute to various natural colours in fruits, vegetables, and plants. Their availability and distinct chemical properties make them valuable ingredients in food colouring applications, providing visual appeal and potential health benefits.

Fruits and vegetables colourant

Because of its bright red colour and possible health advantages, tomato juice—made from the Solanum lycopersicum plant—has attracted much attention in the food business as a natural colourant. Tomato juice is used in sausage manufacturing as a colouring agent and a source of bioactive ingredients, which are important in attracting consumers' attention **[13]**.

Investigating tomato juice's colour stability, nutritional qualities, flavour modification, and application techniques is part of science. Tomato juice's deep red hue results from its high quantity of carotenoid pigments, especially lycopene, which has strong antioxidant qualities. The striking red colour is mainly attributed to lycopene, which also shows remarkable stability when processed differently. However, lycopene and other colours in tomato juice can become unstable due to several circumstances such as pH levels, temperature swings, and exposure to oxygen during processing and storage **[13]**, **[14]**.

Tomato juice has several nutritional advantages since it contains essential vitamins, minerals, and phytochemicals. It also improves appearance. The main pigment in tomato juice, lycopene, has been linked to several health benefits, such as anti-inflammatory, antioxidant, and maybe anti-cancer qualities. Tomato juice is a great way to improve the appearance of sausage recipes while adding nutrients to the finished product and satisfying customer demands for healthier food alternatives. Tomato juice adds taste to sausages beyond its colour and nutritional value. It adds a hint of sweetness and acidity to balance the savoury flavours of the meat **[15]**, **[16]**.

Tomato juice may be more effective as a colourant and taste enhancer by using pre-treatment procedures (e.g., concentration, enzymatic treatment) and formulation alterations (e.g., pH modulation, antioxidant addition). Furthermore, thorough sensory research and consumer testing are essential to determine if tomato-enhanced sausage products are acceptable and to identify areas that require further optimisation **[16]**.

Complex taste profiles are produced by the interaction of tomato-derived chemicals with other components in the sausage matrix, which are impacted by various processing settings and ingredient proportions. Sausage producers may create goods with well-rounded and enticing flavour profiles by carefully adjusting formulation parameters and utilising tomato juice's synergistic effects with other flavour-enhancing ingredients. Tomato juice must be carefully included in sausage recipes while considering all processing factors to provide the best possible colour stability, taste retention, and nutritional integrity **[15]**, **[17]**, **[18]**.

Functional properties and chemical composition

Natural colourants offer several functional properties in food and beverages. Firstly, they provide vibrant hues, enhancing the aesthetic appeal of products and influencing how consumers perceive them. Additionally, many natural colourants contain antioxidant compounds like polyphenols and carotenoids, which protect against oxidative damage and contribute to overall health and wellness. Furthermore, certain natural colourants, especially those sourced from spices and herbs, can enhance flavour in food products, adding depth and complexity to the overall sensory experience. Natural colourants come in a variety of chemical forms, and each one gives food and drink goods special qualities:

Carotenoids, known for their antioxidant qualities, come in yellow, orange, and red hues. Anthocyanins have pH-dependent colours that range from red to purple to blue. They also have anti-inflammatory and antioxidant properties. Chlorophyll is the green pigment that gives many foods and drinks their distinctive hue **[19]**.

The water-soluble pigments called betalains, mostly present in beets and certain cacti, give reddish-violet and yellow-orange hues. There is growing interest in using Beta vulgaris variants as sources of nitrate for cured meats. Artificial nitrate and nitrite salts were traditionally used in meat curing for colour stability and taste improvement. However, interest in natural alternatives has increased due to the need for clean-label products. Beetroot, spinach beet, and Swiss chard are examples of beta vulgaris, which shows promise as a natural source of nitrate **[20]**.

The varying nitrate levels in these veggies result from farming techniques and fertiliser. Their promise as natural healing agents resides in their ability to perform functions similar to synthetic additions without posing health risks. The benefits of adding Beta vulgaris extracts to meat processing include less lipid oxidation, better colour stability, and increased sensory qualities. Utilising the potential of Beta vulgaris to produce meat products that are healthier and more natural is made easier by methods like direct addition or fermentation-induced nitrite production. Notwithstanding, obstacles continue to exist, such as guaranteeing uniform nitrate concentrations in Beta vulgaris cultivars and thoroughly evaluating the safety and quality consequences of meat processing. More studies must investigate their use in reformulated meat products that correspond with customer expectations for healthier, clean-label choices. Investigating Beta vulgaris as a natural supply of nitrate offers a viable route toward more **[21]**.

The successful application of natural colourants in food and beverage products requires understanding their functional properties and chemical composition. By utilizing these unique properties, formulators can meet consumer demand for natural and healthy ingredients by creating visually appealing and nutritionally enhanced products. The search results also revealed additional information about extracting natural colourants from different botanical sources using different methods, such as aqueous and ultrasound-assisted extraction. These methods offer alternative approaches for obtaining natural colourants with potential applications in food production and textile colouration **[22]**.

Different extraction techniques

Ultrasound-assisted Extraction (UAE): is a novel, environmentally friendly method that has been researched for its advantages in the extraction process, such as its high yields, quick extraction periods, and lack of need for high temperatures. Nevertheless, the non-standardization of the UAE factors makes comparisons more difficult and impedes advancing this topic's research. Standardizing the UAE process parameters can make it easier for the scientific community to compare the findings **[23]**.

Aqueous Extraction for Textile Colouration: This research examines whether it is possible to create natural textile colourants using a variety of botanical sources. The study's successful use of the aqueous dye extraction method prevents solvent toxicity and provides a new environmentally safe dye and a straightforward colouring technique **[24]**.

Extraction of Anthocyanins: This research's central focus was the extraction of anthocyanins from Aronia melanocarpa skin waste, aiming to utilise it as a sustainable source of natural colourants. Implementing an integrated extraction-adsorption process enhanced anthocyanin yields of superior quality compared to traditional batch methods, thereby increasing extraction yield and purity **[25]**.

Aqueous and Solvent Extraction for Colouration of Cellulosic Substrates: Natural dyes derived from plant sources were extracted using aqueous and solvent extraction techniques to colour cellulosic substrates. The recovered colourants demonstrated Excellent colouring capacity, which may eventually replace synthetic dyes as a sustainable method of clean manufacture **[26]**.

Several crucial processes are involved in the solvent extraction method used to separate natural colourants from botanical sources. First and foremost, the choice of solvent is crucial; frequent options include acetone, ethanol, methanol, hexane, and ethyl acetate. The selection of these solvents considers several aspects, such as availability, cost, polarity, toxicity, and regulatory compliance. After that, the plant material is prepared by drying, grinding, and screening it until the particle size is consistent. This formulation increases pigment extraction

efficiency and solvent penetration. The first step in the extraction process is to combine the prepared plant material with the selected solvent in the right vessel, like an ultrasonic bath, reflux device, or Soxhlet extractor. The pigments are transferred from the plant material to the solvent phase more easily by agitation and heating. Several extraction cycles may be carried out under different circumstances to guarantee the highest possible pigment recovery. Following extraction, solid plant material is separated from the solvent containing dissolved pigments using centrifugation or filtering. The pigment extract is concentrated by solvent evaporation, eliminating any remaining remnants **[27]**, **[28]**.

Additional purification procedures, such as chromatography or precipitation, can be utilised to separate certain pigments or eliminate contaminants. The process's characterisation and quality assurance are crucial components. Mass spectrometry, chromatography, and spectrophotometry are used to evaluate the extracted pigment concentrate's colour, purity, and chemical makeup. Quality control tests assess variables, including colour stability, solubility, and sensory characteristics, to ensure the extract is suitable for the planned uses. Research on the effects of different solvents on pigment yield and extraction efficiency has been conducted on a variety of sources, including red shrimp shell wastes, brown algae, *Nannochloropsis oculata*, and red fruit **[28]**, **[29]**.

Supercritical extraction technique

The ecologically friendly method of obtaining natural colourants from botanical sources is called supercritical fluid extraction (SFE). This technique uses supercritical fluids—like carbon dioxide (CO_2)—as the extraction solvent, and it operates under certain pressure and temperature parameters. Because of its favourable characteristics, such as low toxicity, nonflammability, and ease of removal from the extracted product, carbon dioxide is the recommended supercritical fluid. However, additional supercritical fluids such as nitrous oxide, propane, and ethane may be used based on the particular needs of the extraction process. Temperature and pressure changes above the critical point in the SFE process improve the supercritical fluid's solvent qualities, such as improved diffusivity and solubility. The plant material pulverised and dried to contain the appropriate pigments is put into an extraction vessel, usually a column or chamber made of stainless steel. After that, supercritical CO_2 is poured into the extraction vessel, which works as a solvent to dissolve the desired pigments while removing unwanted substances. The dissolved pigments are gathered in an expansion chamber or separator after being removed from the extraction vessel containing the supercritical CO_2. After leaving the extraction vessel, the supercritical CO_2 returns to its gaseous state due to a drop in temperature and pressure. This transformation precipitates the dissolved pigments and may be separated from the CO_2. To get a concentrated extract, the recovered pigments go through further processing to get rid of any remaining solvent residues **[30]**, **[31]**.

The optimisation of SFE parameters, such as temperature, pressure, CO_2 flow rate, and extraction time, is highlighted in studies using SFE for pigment extraction from a variety of sources, including brewer's grains, maise yellow powder, red pitaya fruit peel, and Malaysia tiger shrimp waste. This is done to achieve high pigment yields **[32]**.

SFE, in general, has several benefits over conventional solvent extraction techniques, such as increased environmental sustainability, less solvent residues, and greater selectivity. It is widely used to extract natural colourants and other bioactive components from plant sources in the food, pharmaceutical, and cosmetic sectors **[33]**.

Pressing and Maceration: Traditional techniques for removing natural colourants from plant sources include pressing and maceration. Plant materials are pressed to release their juices, which include various chemicals and natural colourants. Colourants from high-moisture fruits, vegetables, and seeds are frequently extracted using this technique. On the other hand, maceration involves immersing plant materials in a liquid solvent for some time to extract their soluble chemicals, including natural colourants. It works well for removing colour from plant materials, including roots, bark, and dried plants that are difficult to press or have a low moisture content **[34]**, **[35]**.

Enzyme-assisted extraction

An advanced technique for removing bioactive substances and natural colourants from botanical sources is enzyme-assisted extraction. This method releases the desired components from the plant material by breaking down the cell walls with the help of enzymes. Choosing the right enzymes, prepping the plant material, processing it enzymatically under controlled circumstances, and extracting and recovering the released chemicals are the usual steps. Enzyme-assisted extraction has advantages over chemical extraction methods, including sustainability, gentle extraction conditions, and selectivity. Research has indicated that the extraction yield of natural colourants and other bioactive chemicals from plant materials may be considerably increased by using enzyme-assisted extraction. In contrast to aqueous extraction alone, the inclusion of enzymes boosted the number of water-soluble compounds by 30% in a study on the extraction of water-soluble antiviral compounds from the

macroalga Solieria chordalis. A further investigation demonstrated the effective use of thermostable cellulase and immobilised β-glucosidase in the enzyme-assisted extraction and conversion of polydatin to resveratrol from Polygonum cuspidatum, highlighting the potential of this technique to extract certain bioactive chemicals. In conclusion, the gentle and selective extraction of natural colourants and other bioactive substances from botanical sources appears to be a promising use of enzyme-assisted extraction. Its benefits for sustainability, product quality, and selectivity make it a desirable choice for a range of uses in the food, medicine, and cosmetics sectors **[36]**.

Colour stability

Depending on the kind of pigment, the pH sensitivity of natural colourants considerably impacts their durability and colour manifestation. For example, the pigments called anthocyanins, which give fruits and vegetables red, purple, and blue hues, change colour in response to pH. Similarly, betalains, present in certain cacti and beets, demonstrate varying degrees of stability throughout various pH values. The green pigment in plants, called chlorophyll, is generally stable across a broad pH range but can change colour under extremely acidic or alkaline conditions. Changes in pH have less of an impact on carotenoids like lycopene and beta-carotene; in certain cases, acidic environments can increase their stability **[37]**.

The formulation pH, processing conditions, and packaging are some elements that affect natural colourants' pH sensitivity and colour stability. To achieve desired colour outputs and preserve stability in food and beverage items, it is important to comprehend these elements. Manufacturers may enhance the quality of their products and maximise the effectiveness of natural colourants by meticulously regulating the pH of the formulation and the processing conditions. Research has indicated that adding natural colourants to food goods can improve their colour permanence. For example, because purple carrot puree has a high amount of anthocyanins and total phenols, adding it to strawberry jam was found to increase the colour stability of the jam **[38]**.

A higher concentration of purple carrot puree led to a progressive increase in pH, viscosity, total anthocyanin, and total phenol levels, decreasing anthocyanin degradation throughout storage. Additionally, studies have demonstrated that natural colourants with high anthocyanin content may be produced by extraction process optimisation, making them appropriate for mass production in various sectors. To get a dark red extract from roselle calyces with a high anthocyanin concentration, stirring-assisted extraction (SAE) conditions were more successful than simple maceration and ultrasound-assisted extraction. These results emphasise how crucial it is to optimise extraction methods to maximise the potential of natural colourants for industrial applications **[39]**.

Exposure to light and oxygen

The durability of natural colourants in various goods can be greatly impacted by exposure to light and air. Natural colourants, especially those sourced from plants, may fade, degrade, or change in colour due to light exposure, particularly ultraviolet (UV) radiation. Colour-sensitive items must be protected by opaque or UV-blocking packaging since low-light barrier materials, such as clear glass or plastic containers, can worsen light-induced deterioration. Conversely, exposure to oxygen can trigger oxidation processes in natural colourants, resulting in colour changes, loss of brightness, or the development of off-flavours **[40]**.

Oxygen-impermeable packaging, such as vacuum-sealed or nitrogen-flushed packaging, is frequently used to reduce oxygen exposure and maintain colour stability. Important variables impacting light and oxygen exposure and their effects on natural colourants include selecting packing materials and formats, storage conditions, and processing techniques. The durability of natural colourants is greatly enhanced by appropriate storage conditions, which include temperature, humidity, and light exposure **[41]**.

Furthermore, natural colourants may degrade due to processing procedures such drying, grinding, and mixing that expose them to light and oxygen. Colour stability may be preserved by limiting exposure during processing and using safeguards like antioxidants or inert environments. Through comprehension of the effects of light and oxygen exposure on natural colourants and the implementation of suitable safeguards, producers may guarantee the stability and excellence of goods that are sensitive to colour throughout their shelf life **[42]**.

Temperature

Natural colourants are highly sensitive to temperature, influencing their molecular mobility, chemical reactions, and interactions in food and beverage items. For example, thermal degradation must be considered, especially for heat-sensitive pigments, such as chlorophyll and anthocyanins. Elevated temperatures may hasten its disintegration, leading to colour changes or fading. The anthocyanins compounds, which give red, purple, and blue colours, are especially prone to deterioration, resulting in unfavourable colour shifts **[43]**. Similarly, extended exposure to high temperatures can also impact chlorophyll, which gives a green hue, causing colour alterations or loss **[44]**. Furthermore, freezing temperatures can affect the durability of colour, particularly in frozen goods where the production of ice crystals can damage cell structures and cause colour migration or bleeding **[45]**.

Additionally, temperature affects natural colourants' oxidative stability, especially in goods that include lipids. Elevated temperatures can potentially hasten lipid oxidation processes, leading to peculiar tastes, smells, and colour alterations. Natural colourant stability is largely dependent on variables, including formulation design, storage temperatures, and processing conditions. Manufacturers can guarantee the stability and quality of colour-sensitive items for the duration of their shelf life by closely managing these variables **[46]**.

Advantages of natural colourant

Natural colourants have several benefits, and they align with current wellness and health trends. These advantages include several important areas:

Clean Label: To enhance their perception of being natural and healthful, natural colourants are obtained from botanical, animal, or mineral sources and are frequently extracted using straightforward procedures. Natural colourant-containing products can thus make clean-label claims, which indicate minimum processing and no artificial chemicals or additions **[47]**.

Nutritional Value: Rich concentrations of bioactive chemicals with possible health advantages may be found in several naturally occurring colourants, including anthocyanins, carotenoids, and chlorophyll. These substances may have antioxidant qualities and improve the health of the immune system and the eyes, among other bodily systems. Manufacturers can improve the nutritional profile of their products by adding natural colourants **[47]**, **[48]**.

Allergen-Free and Non-Toxic: Natural colourants are often considered less harmful than synthetic equivalents, especially for people with allergies or sensitivities to artificial additives. Their natural origin decreases the possibility of negative responses or intolerance in vulnerable people. Sustainability and Environmental Concerns: Plant extracts and mineral pigments are two examples of sustainable and renewable resources frequently used in creating natural colourants. Compared to synthetic colourants derived from petroleum, this sustainable method leaves a smaller environmental impact, which aligns with customer demands for environmentally friendly goods **[47]**, **[49]**.

Clean label

Food producers are refining their recipes to remove artificial ingredients and incorporate natural substitutes. Customers identify authenticity, well-being, and health with natural and healthful components. Food producers are emphasising the naturalness and purity of their goods by labelling them as "organic," "all-natural," and "non-GMO" to appeal to customers who are concerned about their health. Customers are looking for third-party certifications and verifications that vouch for the integrity and legitimacy of product claims as their interest in clean-label items rises. Consumers may feel reassured by certifications like USDA Organic, Non-GMO Project Verified, and Clean Label Project that goods fulfil strict requirements for processing, sourcing, and ingredient quality. As manufacturers take advantage of consumers' perceptions of the healthfulness and transparency of their products, clean label promises have emerged as a central theme in product marketing and branding tactics **[50]**.

The clean label movement is a burgeoning trend propelled by consumer demand and typified by a propensity for food and drink products with easily identifiable, uncomplicated ingredients that have undergone little processing. Consumers seeking products that align with their values of sustainability, wellness, health, and transparency are transforming the food industry in various ways. Consumers carefully read ingredient lists because they are pickier about products that contain ingredients they can recognise and comprehend. Consequently, manufacturers simplify ingredient labels and replace synthetic chemicals, preservatives, and artificial additives with well-known, natural components. Artificial additives are becoming less common due to the trend since they are considered unnecessary or even hazardous. These include flavourings, colours, sweeteners, and artificial preservatives **[51]**.

Sustainability and environmental factors

Through several important methods, the use of natural colourants greatly minimizes environmental impact and promotes sustainability in a variety of sectors. First, plants, fruits, vegetables, and minerals are common renewable sources of natural colourants. These sources can be restored by using sustainable agriculture methods, and the need for finite fossil fuel-based materials for synthetic colourants may be decreased. Second, compared to the creation of synthetic colourants, the production procedures involved in extracting natural colourants usually take less energy and produce less greenhouse gas emissions. This lower energy use and carbon footprint help lessen climate change's effects. The biodegradability of natural colourants is an additional factor **[52]**.

They minimise harm to ecosystems and lower the danger of environmental contamination since they may break down over time into organic molecules without leaving behind persistent contaminants. Furthermore, compared to manufacturing synthetic colourants, extracting natural colourants often uses fewer synthetic chemicals and

easier methods. As a result, there is less chemical pollution and less environmental damage brought on by the production and disposal of chemicals. Additionally, the production and preservation of these plants are aided by natural colourants frequently drawing their supply from a wide variety of plant species **[53]**, **[54]**.

This promotes ecological equilibrium, habitat preservation, and biodiversity protection. In addition, natural colourant extraction techniques often utilise less water than industrial methods for producing synthetic colourants. This reduces the environmental effect of water extraction and treatment while also aiding in conserving freshwater supplies. Finally, the natural colourant market encourages sustainable farming methods, including regenerative agriculture, agroforestry, and organic farming. This reduces dependency on artificial fertilisers and pesticides while promoting soil health, biodiversity, and ecosystem resilience **[55]**.

To improve visual appeal, offer uniqueness, and satisfy consumer desire for clean-label ingredients, natural colourants are frequently used in the meat and sausage sector. In this business, colouring meat products is one of the main uses for natural colourants Beetroot powder, annatto, and paprika extract are some ingredients used to preserve or improve the natural colour of meat products. They shield food against unintended discolouration when processed, stored, or cooked. Natural colourants like turmeric and paprika extract are employed in sausage compositions to provide colour and improve or balance the product's flavour **[56]**. As a result, producers may produce desired tastes without using flavour enhancers or artificial additives. Additionally, natural colourants assist meat and sausage producers meet clean label requirements. They increase the marketability of the product by substituting natural ingredients for synthetic ones, which appeals to customers who want ingredient lists that are clearer and simpler. Additionally, using natural colourants satisfies customer inclinations toward wellness and health. These colourants are seen as more healthful than artificial ones, which aligns with consumer aspirations for whole, minimally processed foods. Natural colourants have an aesthetic value that goes beyond their practical use in meat and sausage products. They pique consumers' curiosity and affect their purchase decisions by introducing vivid, enticing hues **[57]**, **[58]**.

Meat and sausage products

The meat and sausage sector, which provides a wide range of goods such as beef, pig, chicken, and other kinds of sausages, is an essential part of the world food market. Production in this sector happens in several steps, from packaging and processing to distribution and sale. Key variables driving the meat and sausage sector are evolving consumer demands, regulatory compliance, and ongoing technical improvements. The creation of fermented meat products, the utilization of secondary raw materials for sausage manufacturing, and the optimization of sausage recipes to increase economic efficiency are just a few of the facets of the meat and sausage sector that have been the subject of a recent study. These studies reflect the industry's continuous attempts to fulfil customer demand and enhance manufacturing procedures through innovation and optimization **[59]**. Study of the meat processing sector in certain areas, including Kazakhstan and Ukraine, has also been conducted. This study has focused on changes in the market, changes in consumer behaviour, and initiatives to improve the productivity of meat processing businesses. Other research papers and industry studies can offer more information on particular subjects within the meat and sausage business. These resources can offer in-depth insights into various facets of this dynamic sector. Several important factors impacting its operations and worldwide reach define the meat and sausage sector. First, it provides a wide selection of goods, such as processed, frozen, and fresh meats in addition to a range of sausages made with various tastes and components. This broad product line accommodates many customer tastes and culinary customs. Second, a considerable portion of the world's production and consumption of meat and sausage products comes from big players in continents, including North America, Europe, and Asia. Their existence highlights the industry's pervasive impact on global food markets. In addition, the sector works under strict regulations designed to protect public health and ensure food safety. The regulation and standardization of different manufacturing, distribution, and labelling areas maintain strict quality and safety standards **[60]**, **[61]**. Moreover, the business is driven by innovation because of continuous technical developments in meat processing and sausage preparation. These developments demonstrate the industry's dedication to ongoing improvement by boosting productivity, raising the calibre of products, and strengthening food safety protocols. Finally, consumer trends and preferences greatly influence industry marketing tactics and product innovation. Businesses respond to shifting customer needs by launching new goods, tastes, and packaging alternatives that suit changing dietary preferences and way-of-life decisions. Altogether, these salient features underscore the fluid character of the meat and sausage sector, which endeavours to satisfy heterogeneous consumer preferences while conforming to legal guidelines and harnessing technical advancements. Efforts to optimize production processes, address consumer preferences, and ensure product safety underscores the industry's commitment to innovation and sustainability **[62]**.

Additional research can be done to understand better particular facets of the meat and sausage sector, such as market trends or production methods. Slaughtering and dressing are essential in meat processing, turning animals

into edible meat products. While dressing includes the subsequent phases of corpse preparation and basic processing, slaughtering is the compassionate and effective killing of animals for sustenance. Before the actual killing procedure, animals are usually stunned during the slaughtering phase to make them insensible to pain. This compassionate procedure guarantees the least pain and conforms with legal and ethical requirements. After the animals are stunned, they are killed using techniques like throat-cutting or stunning, which is followed by exsanguination, which guarantees quick and efficient bleeding. Depending on the species, the dressing removes the animal's skin, hair, feathers, or outer covering after slaughter. Careful handling is required in this phase to avoid contamination and preserve product quality. The process of evisceration, which involves removing internal organs such as the kidneys, liver, and intestines, comes after skinning **[63]**.

To avoid spoiling and microbial infection, these organs must be removed immediately. Strict sanitary regulations and hygiene standards are necessary during the dressing and slaughtering procedures to guarantee the safety and quality of the meal. To limit the danger of contamination and achieve customer expectations for safe and healthy meat products, facilities must comply with industry standards and regulatory norms. The meat processing stages are crucial for setting the groundwork for later procedures like chilling, chopping, and packing, including slaughtering and dressing. Meat processors may create premium meat products by ensuring animals are treated humanely, keeping environments clean, and using the right handling methods **[64]**.

Beetroot

Beta vulgaris, or beetroot, contains water-soluble pigments called betalains, especially the reddish-violet betacyanins, which naturally colour meat products. These pigments enhance the colour of meat products, such as fermented beef sausage (sucuk) and emulsified pork sausage, making them more visually attractive and in line with customer expectations for natural additions. Beetroot colourant usage is part of a more significant movement in food processing toward natural, health-promoting additives and more straightforward labelling. Beetroot extract is added to beef sausages as a natural substitute for artificial nitrites, frequently used in meat products due to their ability to preserve the meat. Although synthetic nitrites help prevent the growth of pathogenic germs and preserve the distinctive red colour of cured meats, they have been connected to several health hazards, such as a higher chance of methemoglobinemia and several types of cancer. According to the relevant study, nitrite levels in beef sausage can be decreased using beetroot extract during manufacture, which may lessen the health hazards related to nitrite consumption. Beetroot extract is added to beef sausages as a natural substitute for artificial nitrites, frequently used in meat products due to their ability to preserve the meat. Although synthetic nitrites are useful in preventing the growth of dangerous germs and preserving the distinctive red colour of cured meats, they have been connected to several health problems, such as an elevated risk of certain cancers and methemoglobinemia. The study in question demonstrates that using beetroot extract in beef sausage production can decrease sausage nitrite levels, potentially reducing the health risks associated with nitrite consumption **[65]**.

It has been investigated if adding beetroot and radish powders to dry fermented sausages as natural sources of nitrites may improve the final product's safety and nutritional value. Studies have shown that although these powders may lower the amount of residual nitrite in sausages, adding them might negatively impact their colour and lipid oxidation. Furthermore, because beetroot and chestnut extracts have a high polyphenol content that offers antibacterial and antioxidant qualities, they have become viable substitutes for sodium nitrite. To enhance the health profile of meat products, it is important to consider customer perception and preferences during the reformulation process. Labelling must be precise and easy to read to inform customers of changes. As a naturally occurring source of nitrate, beetroot has gained popularity as a practical way to lower the nitrite content in meat products. It has been demonstrated that adding beetroot powder to dry-fermented sausages helps nitrites develop during the ripening phase, offering a workable method for reducing nitrites. But betalains—natural pigments that give beetroot its characteristic purple colour—may hurt the sausages' colour. Furthermore, research has shown that treatments containing 1% beetroot powder had TBARS values that were higher than the threshold for rancidity detection using sensory means after prolonged storage. Consequently, even though beetroot appears to have promise in lowering nitrite concentration, cautious assessment is required to minimise any possible negative effects on other quality attributes of meat products **[65]**.

A thorough literature review will yield important information about using beetroot powder and extract as natural colourants in meat products. It will also emphasise how they improve sensory perception, nutritional value, and competitiveness in the market. Food scientists and industry experts may make informed judgments by clarifying the processes underlying the sensory impacts of colourants generated from beetroot to address consumer requests for clean-label products with higher sensory quality. Beetroot powder's natural food colouring qualities can improve the visual attractiveness of the sausage without the need of artificial chemicals, making it a possible filler and colouring ingredient in chicken sausage preparation. Talain, a naturally occurring pigment found in beetroot powder, gives the sausage a red hue, increasing its commercial appeal. Furthermore, beetroot powder has

a high antioxidant capacity and is rich in phenolic acids, which can enhance the product's nutritional value and safety. Additionally, the study discovered that using beetroot powder instead of tapioca improved the chicken sausage's softness and ability to hold water. This implies that beetroot powder may also improve the sausage's texture and ability to hold moisture. However, there can be disadvantages to consider when making chicken sausages using beetroot powder. One disadvantage is adding beetroot powder might give the sausage an earthy flavour that some customers may not find appealing. The study also found that increased beetroot powder concentrations can alter the sausage's taste and perhaps lower its appeal overall. Moreover, geosmin, a substance in red beets that affects the sausage's sensory qualities, is responsible for the earthy flavour. When utilising beetroot, it's crucial to carefully weigh the intended colour increase against any potential taste impacts **[66]**.

The increasing need for natural food colouring in meat products highlights customer preferences for components with clear labels and apprehensions about artificial additions. In response, scientists have considered plant colourant substitutes for conventional synthetic dyes. Of these substitutes, beetroot extract is distinguished by its vivid red colour, which comes from betalains, which are also recognized for their antioxidant qualities. Beetroot extract and extract powder can improve sausage's nutritional profile and aesthetic appeal while satisfying consumer demands for clean-label products. The sausages' pH and moisture content are both greatly impacted by red beet, with increased amounts of both following. The sausages' colour characteristics were also significantly affected by the inclusion of red beet, which resulted in a drop in lightness and a significant rise in redness with a matching decrease in yellowness. This change in colour characteristics persisted throughout the storage period, suggesting that red beet continues to impact sausage colour. Despite the notable changes in colour features, the addition of red beet powder did not significantly affect other sensory attributes, including taste, tenderness, juiciness, and overall acceptability. Similarly, the addition of red beet did not affect the textural attributes of the sausages, such as their gumminess, cohesion, hardness, and springiness. But it's important to remember that although red beet helped the sausages' colour, further processing, including concentration and extraction as a juice, may be necessary to fully utilise its antioxidant properties in meat products **[67]**.

Case studies

According to the findings regarding the betalain content and antioxidant properties of concentrated beetroot extract used by researchers provide valuable insights into its potential as a natural colourant and functional ingredient in food products. While the betacyanin and betaxanthin content in the beetroot extract sample was slightly lower compared to previous studies, indicating potential variability due to processing methods, the total betalain pigment content remained substantial. This suggests that despite variations, beetroot extract can serve as a rich source of betalain pigments known for their antioxidant properties. The high antioxidant activity observed in our beetroot extract sample underscores its potential health-promoting benefits. The presence of phenolic compounds, particularly 4-Hydroxy benzoic acid, contributes to its antioxidant capacity, protecting against oxidative stress and potential health risks associated with free radicals. The natural colour provided by beetroot extract contributed to the sausage's enhanced sensory quality, leading to higher acceptability scores among panellists. In contrast, carmine, with its artificial dark pink colour, hurt panellists' perception of appearance and colour. This highlights the importance of natural colourants like beetroot extract in meeting consumer preferences for clean-label products with improved sensory characteristics. The observed differences in colour stability between sausages processed with fermentation and heat treatment methods underscore the importance of processing techniques in preserving the colour quality of beetroot-derived colourants. While both methods effectively enhanced the red colour of sausages, factors such as storage conditions may influence long-term colour retention. The potential of beetroot extract and powder as natural colourants and functional ingredients in meat products offer improved sensory attributes, oxidative stability, and consumer acceptance compared to traditional colourants. These results contribute to developing clean-label products with enhanced nutritional and sensory profiles, meeting the evolving demands of health-conscious consumers in the food industry **[68]**, **[69]**.

Aykın-Dinçer et al. conducted a study to examine the use of beetroot extract and extract powder as natural food colourants in sausages and compare their results with those of typical colourants like carmine. The study sought to determine how beetroot-derived colourants affected the end goods' quality qualities by evaluating criteria such as betalain concentration, total phenolic substance content, antioxidant capacity, and sensory attributes. For sausages to be formulated and accepted in the food sector, it is essential to comprehend how beetroot extract and powder affect colour stability, lipid oxidation, pH, moisture content, and sensory qualities. Natural ingredients used in food processing have become more popular due to customer desire for clean-label goods. Varieties of Beta vulgaris, such as spinach, chard, and beetroot, have come to light as possible nitrate sources to improve meat products' nutritional content and quality. Due to their high nitrate content, these vegetables provide a healthy substitute for the artificial nitrate/nitrite salts often used in meat curing. Nitrate and nitrite are essential for improving colour stability, slowing oxidative processes, and giving meat products their

distinct tastes during the age-old curing process. The investigation of *Beta vulgaris* extracts in meat processing seeks to improve meat products' nutritional profile and sensory qualities while extending their shelf life **[70]**.

In another study, researchers aim to evaluate the physical characteristics of natural colourants for potential integration into sausage formulations. Utilizing laboratory methodologies, parameters such as pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), and colourimetric properties (Lab*) were meticulously analysed. Results indicated a negligible impact of the natural colourants on the aforementioned parameters, suggesting their viability for food colouring applications. Furthermore, the study underscored the imperative of embracing natural colourants in food formulations, elucidating the potential health hazards posed by synthetic counterparts and extolling the virtues of natural alternatives. Conclusively, the investigation determined that the TDS values of the assessed natural colourants fell within permissible thresholds for food use, with the beetroot-derived colourant notably approximating the visual characteristics of commercial sausage hues. These findings furnish invaluable insights into the physical scrutiny of natural colourants, particularly within the context of sausage production, resonating with contemporary consumer preferences favouring natural and health-conscious food constituents **[71]**.

Furthermore, the studies explore the possibility of using radish and beetroot powders in fermented dry sausages instead of nitrite to reduce health and safety risks related to using sodium nitrite. This study explains how adding vegetable powders impacts important sausage characteristics such as moisture content, weight loss, and water activity by analysing six treatment variants during the ripening and storage phases. Interestingly, it is noticed that the beetroot powders and radish create nitrite, which is more noticeable in treatments with larger concentrations. Specifically, beetroot powder significantly affects colour and pigments, indicating that these powders can be used as viable substitutes for nitrite through a simple drying procedure. An analysis of the sausages' colour progression, nitrite and nitrate concentration, and pigments is conducted. Powdered beetroot and radish affect colour development during processing; differences in colour characteristics from the control treatment are apparent. Furthermore, during the manufacturing of sausages, nitrate is converted to nitrite, and the amounts of nitrite and nitrate vary noticeably throughout treatments **[72]**. The colour properties of beet juice and powdered beet juice are also evaluated in the investigations. The results indicate a preference for darker colours in both varieties, with the powdered form having a more prominent dark colouration, probably due to the concentration effect during the drying process. According to a chromaticity study, both the beet juice and the powdered beet juice showed a colour spectrum spanning from red to yellow. Humidity decreased due to dehydration, which made the colour characteristics more intense since the pigments were concentrated. The study also highlighted how betalains are easily degraded in the presence of light and oxygen, and their high water solubility makes them more likely to be lost during sanitation operations. It has been determined that maintaining pH stability is essential to maintaining the integrity of betalains. These observations provide insightful analysis of the stability and colour characteristics of beet-derived colourants, essential for guiding their use in food items **[73]**.

To reduce the health concerns connected to chemical additions in meat products, the usefulness of beetroot powder in substituting nitrite was also investigated in Turkish fermented beef sausage or sucuk. Four different sausage recipes were created with different amounts of beetroot powder and sodium nitrite. Beetroot powder significantly improved the samples' red colour (a* value) and successfully maintained the intended shade during storage. Surprisingly, residual nitrite levels did not substantially vary between samples at the end of the trial. Furthermore, there was a correlation between elevated lactic acid bacteria counts and greater amounts of beetroot powder. Throughout the storage period, the sensory assessment ratings of beetroot powder samples were similar to those of control samples. The results indicate that it is possible to preserve the quality of Turkish fermented sausage by reformulating it using beetroot powder instead of nitrite and storing it in vacuum-sealed bags at 4°C for up to 56 days. The study also emphasises how natural additions, such as beetroot powder, can take the place of artificial additives in meat products, thereby satisfying customer demands for safer and clean-label food alternatives. Its all-encompassing method, which includes taste analysis and quality assessment, offers insightful information on the suitability of beetroot powder as a nitrite substitute in meat products. The food sector may benefit from this research by creating clean-label goods free of artificial ingredients, and satisfying customer needs for natural and safe meat options. The study essentially confirms that beetroot powder might serve as a good substitute for nitrite in Turkish fermented sausage, offering a natural source of nitrate with antioxidant and colour-enhancing properties. These findings contribute to ongoing efforts in the food industry to develop safer and natural meat products without compromising quality or sensory **[74]**.

Future Perspectives

Beetroot powder's use in the chicken sausage as a colouring and filler creates opportunities for more study and advancement in the meat processing sector. Subsequent research endeavours may delve into refined blends and processing methods to improve the advantages of beetroot powder while minimizing

any possible disadvantages. Furthermore, examining the impact of varying concentrations of beetroot powder on shelf life, consistency, and nutritional value would offer a significant understanding of its use in manufacturing chicken sausages. Additionally, consumer research is required to determine preferences for the flavour and colour profile of chicken sausages that contain beetroot powder. This will help manufacturers create goods that meet the needs and expectations of their target market.

CONCLUSION

In conclusion, adding beetroot powder to chicken sausage as a colouring and filler presents exciting chances to improve the product's visual and sensory qualities. The study showed that beetroot powder enhances product quality by positively impacting softness, colour, and water-holding capacity. To guarantee customer acceptability, the sensory elements—specifically, the taste profile—must be carefully considered. Beetroot powder offers a natural and maybe advantageous component for creating chicken sausages, opening doors for creativity and uniqueness in the meat processing sector. Additional research and development work is necessary to fully realise the potential of beetroot powder and satisfy customer demand for meat products that are more enticing and healthier.

REFERENCES

1. Luzardo-Ocampo, I., Ramírez-Jiménez, A. K., Yañez, J., Mojica, L., & Luna-Vital, D. A. (2021). Technological Applications of Natural Colorants in Food Systems: A Review. In Foods (Vol. 10, Issue 3, p. 634). MDPI AG. https://doi.org/10.3390/foods10030634
2. Yu, D., Cheng, S., Li, Y., Su, W., & Tan, M. (2023). Recent advances on natural colorants-based intelligent colorimetric food freshness indicators: fabrication, multifunctional applications and optimization strategies. In Critical Reviews in Food Science and Nutrition (pp. 1–25). Informa UK Limited. https://doi.org/10.1080/10408398.2023.2252904
3. Kana, T. M., Geider, R. J., & Critchley, C. (1997). Regulation of photosynthetic pigments in micro-algae by multiple environmental factors: a dynamic balance hypothesis. In New Phytologist (Vol. 137, Issue 4, pp. 629–638). Wiley. https://doi.org/10.1046/j.1469-8137.1997.00857.x
4. Bendokas, V., Skemiene, K., Trumbeckaite, S., Stanys, V., Passamonti, S., Borutaite, V., & Liobikas, J. (2019). Anthocyanins: From plant pigments to health benefits at mitochondrial level. In Critical Reviews in Food Science and Nutrition (Vol. 60, Issue 19, pp. 3352–3365). Informa UK Limited. https://doi.org/10.1080/10408398.2019.1687421
5. Mohseni-Shahri, F. S., & Moeinpour, F. (2023). Development of a pH-sensing indicator for shrimp freshness monitoring: Curcumin and anthocyanin-loaded gelatin films. In Food Science & Nutrition (Vol. 11, Issue 7, pp. 3898–3910). Wiley. https://doi.org/10.1002/fsn3.3375
6. Jurić, S., Jurić, M., Król-Kilińska, Ż., Vlahoviček-Kahlina, K., Vinceković, M., Dragović-Uzelac, V., & Donsì, F. (2020). Sources, stability, encapsulation and application of natural pigments in foods. In Food Reviews International (Vol. 38, Issue 8, pp. 1735–1790). Informa UK Limited. https://doi.org/10.1080/87559129.2020.1837862
7. Cui, H., Xie, W., Hua, Z., Cao, L., Xiong, Z., Tang, Y., & Yuan, Z. (2022). Recent Advancements in Natural Plant Colourants Used for Hair Dye Applications: A Review. In Molecules (Vol. 27, Issue 22, p. 8062). MDPI AG. https://doi.org/10.3390/molecules27228062
8. Aeka, R. (2019). Effect of Extracting Conditions on Plant Extract Colors and Stability of Antioxidant Properties during in vitro Gastrointestinal Digestion.
9. Yusof, Z., Mahmood, N. Z., Othman, R., & Yaacob, J. S. (2023). Effect of Fertilization on Expression of Bioactive Carotenoid and Chlorophyll Pigments in Clinacanthus nutans Lindau for Potential Use as Functional Natural Colourants. In Sains Malaysiana (Vol. 52, Issue 4, pp. 1121–1132). Penerbit Universiti Kebangsaan Malaysia (UKM Press). https://doi.org/10.17576/jsm-2023-5204-07
10. Vega, E. N., Molina, A. K., Pereira, C., Dias, M. I., Heleno, S. A., Rodrigues, P., Fernandes, I. P., Barreiro, M. F., Stojković, D., Soković, M., Carocho, M., Barreira, J. C. M., Ferreira, I. C. F. R., & Barros, L. (2021). Anthocyanins from Rubus fruticosus L. and Morus nigra L. Applied as Food Colorants: A Natural Alternative. In Plants (Vol. 10, Issue 6, p. 1181). MDPI AG. https://doi.org/10.3390/plants10061181
11. Yusof, Z., Mahmood, N. Z., Othman, R., & Yaacob, J. S. (2023). Effect of Fertilization on Expression of Bioactive Carotenoid and Chlorophyll Pigments in Clinacanthus nutans Lindau for Potential Use as Functional Natural Colourants. In Sains Malaysiana (Vol. 52, Issue 4, pp. 1121–1132). Penerbit Universiti Kebangsaan Malaysia (UKM Press). https://doi.org/10.17576/jsm-2023-5204-07

12. Khoo, H.-E., Prasad, K. N., Kong, K.-W., Jiang, Y., & Ismail, A. (2011). Carotenoids and Their Isomers: Color Pigments in Fruits and Vegetables. In Molecules (Vol. 16, Issue 2, pp. 1710–1738). MDPI AG. https://doi.org/10.3390/molecules16021710
13. Popescu, G.-S., Ienciu, A., Pirvulescu, L., Radu, F., & Bordean, D. M. (2023). Improving the quality of unheated tomato juice by using plants rich in bioactive components. In SGEM International Multidisciplinary Scientific GeoConference EXPO Proceedings. 23rd SGEM International Multidisciplinary Scientific GeoConference 2023. STEF92 Technology. https://doi.org/10.5593/sgem2023v/6.2/s25.32
14. Prakasha, R., Pandey, H., & Vinay, G. M. (2024). Enhancing Nutritional Quality and Color Stability of Tomato Juice through Low-Intensity Pulsed Electric Field Treatment. In European Journal of Nutrition & Food Safety (Vol. 16, Issue 4, pp. 123–131). Sciencedomain International. https://doi.org/10.9734/ejnfs/2024/v16i41414
15. Nikolaev, D. V., Bozhkova, S. E., Andryushchenko, D. S., Margarita Vasilievna, Levina, T. Y., Goroshko, D. D., Smutnev, P. V., & Tyurin, I. Y. (2021). The effectiveness of the technology boiled sausage products with tomato ingredients. In The Agrarian Scientific Journal (Issue 3, pp. 58–61). FSBEU HE Saratov SAU. https://doi.org/10.28983/asj.y2021i3pp58-61
16. Kwon, R. S., Lee, G. Y., Lee, S., & Song, J. (2024). Antimicrobial properties of tomato juice and peptides against typhoidal Salmonella. In A. Oladeinde (Ed.), Microbiology Spectrum (Vol. 12, Issue 3). American Society for Microbiology. https://doi.org/10.1128/spectrum.03102-23
17. Kryzhova, Y., Slobodianiuk, N., & Moskalenko, I. (2023). Application of modern technologies to improve the quality of sausage products. In Animal Science and Food Technology (Vol. 14, Issue 1). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.1.2023.49
18. Cao, Z., Wu, J., Wu, R., & Liu, M. (2009). Development of the tomato assortment sausage. In Journal of Inner Mongolia Agricultural University (Issue 2, pp. 169–172). Inner Mongolia Agricultural University.
19. Cadariu, A. I., Cocan, I., Negrea, M., Alexa, E., Obistioiu, D., Hotea, I., Radulov, I., & Poiana, M.-A. (2022). Exploring the Potential of Tomato Processing Byproduct as a Natural Antioxidant in Reformulated Nitrite-Free Sausages. In Sustainability (Vol. 14, Issue 19, p. 11802). MDPI AG. https://doi.org/10.3390/su141911802
20. Petropoulos, S. A., Sampaio, S. L., Di Gioia, F., Tzortzakis, N., Rouphael, Y., Kyriacou, M. C., & Ferreira, I. (2019). Grown to be Blue—Antioxidant Properties and Health Effects of Colored Vegetables. Part I: Root Vegetables. In Antioxidants (Vol. 8, Issue 12, p. 617). MDPI AG. https://doi.org/10.3390/antiox8120617
21. Bahanla Oboulbiga, E., Parkouda, C., Dabiré, C., Guissou, A. W. D. B., Traore, K., Semde, Z., Douamba, Z., Sawadogo-Lingani, H., & Dicko, M. H. (2022). Storage stability of dried tomato slices during storage as affected by salt and lemon pretreatments. In International Journal of Food Properties (Vol. 25, Issue 1, pp. 450–462). Informa UK Limited. https://doi.org/10.1080/10942912.2022.2046051
22. Petropoulos, S. A., Sampaio, S. L., Di Gioia, F., Tzortzakis, N., Rouphael, Y., Kyriacou, M. C., & Ferreira, I. (2019). Grown to be Blue—Antioxidant Properties and Health Effects of Coloured Vegetables. Part I: Root Vegetables. In Antioxidants (Vol. 8, Issue 12, p. 617). MDPI AG. https://doi.org/10.3390/antiox8120617
23. Chetia, P., Priyadharshini, P., Manimegalai, S., Radha, P., Kumaran, K., & Tilak, M. (2023). Phytochemical analysis and extraction of natural dyes from mulberry leaves. In The Pharma Innovation (Vol. 12, Issue 8, pp. 1500–1504). AkiNik Publications. https://doi.org/10.22271/tpi.2023.v12.i8r.22228
24. Bouchez, A., Vauchel, P., Périno, S., & Dimitrov, K. (2023). Multi-Criteria Optimization including Environmental Impacts of a Microwave-Assisted Extraction of Polyphenols and Comparison with an Ultrasound-Assisted Extraction Process. In Foods (Vol. 12, Issue 9, p. 1750). MDPI AG. https://doi.org/10.3390/foods12091750
25. Manicketh, T. J., & Francis, M. S. (2020). Extraction of natural colorants fromAraucaria columnaris,Macaranga peltataandAverrhoa bilimbifor textile coloration. In International Journal of Clothing Science and Technology (Vol. 32, Issue 6, pp. 789–801). Emerald. https://doi.org/10.1108/ijcst-06-2019-0075
26. Wathon, M. H., Beaumont, N., Benohoud, M., Blackburn, R. S., & Rayner, C. M. (2019). Extraction of anthocyanins from Aronia melanocarpa skin waste as a sustainable source of natural colorants. In Coloration Technology (Vol. 135, Issue 1, pp. 5–16). Wiley. https://doi.org/10.1111/cote.12385
27. Morshed, M., Hridam Deb., Azad, S., Mst. Zakia Sultana., Ashaduzzaman., & Guha, A. (2016). Aqueous and Solvent Extraction of Natural Colorants from Tagetes Erecta L., Lawsonia Inermis, Rosa L for Coloration of Cellulosic Substrates. In American Journal of Polymer Science and Technology (Vol. 2, Issue 2, pp. 34–39). Science Publishing Group. https://doi.org/10.11648/j.ajpst.20160202.13
28. Fathordoobady, F., Abd Manap., Y. Z., Selamat, J., & Pratap-Singh, A. (2019). Development of supercritical fluid extraction for the recovery of betacyanins from red pitaya fruit (Hylocereus polyrhizus) peel: a source

of natural red pigment with potential antioxidant properties. In International Food Research Journal (Vol. 26, Issue 3, pp. 1023–1034). Universiti Putra Malaysia.

29. Abou Elmaaty, T., Sayed-Ahmed, K., Elsisi, H., & Magdi, M. (2022). Optimization of Extraction of Natural Antimicrobial Pigments Using Supercritical Fluids: A Review. In Processes (Vol. 10, Issue 10, p. 2111). MDPI AG. https://doi.org/10.3390/pr10102111
30. Tzima, S., Georgiopoulou, I., Louli, V., & Magoulas, K. (2023). Recent Advances in Supercritical CO2 Extraction of Pigments, Lipids and Bioactive Compounds from Microalgae. In Molecules (Vol. 28, Issue 3, p. 1410). MDPI AG. https://doi.org/10.3390/molecules28031410
31. Kitada, K., Machmudah, S., Sasaki, M., Goto, M., Nakashima, Y., Kumamoto, S., & Hasegawa, T. (2008). Supercritical CO2 extraction of pigment components with pharmaceutical importance from Chlorella vulgaris. In Journal of Chemical Technology & Biotechnology (Vol. 84, Issue 5, pp. 657–661). Wiley. https://doi.org/10.1002/jctb.2096
32. Jitrangsri, K., Chaidedgumjorn, A., & Satiraphan, M. (2020). Supercritical fluid extraction (SFE) optimization of trans-resveratrol from peanut kernels (Arachis hypogaea) by experimental design. In Journal of Food Science and Technology (Vol. 57, Issue 4, pp. 1486–1494). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-019-04184-9
33. Lion. Supercritical Fluid Extraction: Advantages And Disadvantages. Retrieved from https://www.angleroller.com/blog/supercritical-fluid-extraction-advantages-and-disadvantages.html.
34. Yusuf, M., Shabbir, M., & Mohammad, F. (2017). Natural Colorants: Historical, Processing and Sustainable Prospects. In Natural Products and Bioprospecting (Vol. 7, Issue 1, pp. 123–145). Springer Science and Business Media LLC. https://doi.org/10.1007/s13659-017-0119-9
35. R, G., M, P. E., A, R. K., S, S., & Krishna, K. R. (2023). Natural colour extraction from horticultural crops, advancements, and applications—a review. In Natural Product Research (pp. 1–19). Informa UK Limited. https://doi.org/10.1080/14786419.2023.2280796
36. Lombardelli, C., Benucci, I., Mazzocchi, C., & Esti, M. (2022). Green Enzymatic Recovery of Functional Bioactive Compounds from Unsold Vegetables: Storability and Potential Health Benefits. In Applied Sciences (Vol. 12, Issue 23, p. 12249). MDPI AG. https://doi.org/10.3390/app122312249
37. Iorizzo, M., Curaba, J., Pottorff, M., Ferruzzi, M. G., Simon, P., & Cavagnaro, P. F. (2020). Carrot Anthocyanins Genetics and Genomics: Status and Perspectives to Improve Its Application for the Food Colorant Industry. In Genes (Vol. 11, Issue 8, p. 906). MDPI AG. https://doi.org/10.3390/genes11080906
38. Abdel-Hady, M. M., Gamila, Y. A., & Afaf, M. A. (2014). Color stability of strawberry jam fortified by purple carrot puree. In Egyptian Journal of Agricultural Research (Vol. 92, Issue 1, pp. 323–336). Agricultural Research Center, Ministry of Agriculture and Land Reclamation.
39. Gérard, V., Ay, E., Morlet-Savary, F., Graff, B., Galopin, C., Ogren, T., Mutilangi, W., & Lalevée, J. (2019). Thermal and Photochemical Stability of Anthocyanins from Black Carrot, Grape Juice, and Purple Sweet Potato in Model Beverages in the Presence of Ascorbic Acid. In Journal of Agricultural and Food Chemistry (Vol. 67, Issue 19, pp. 5647–5660). American Chemical Society (ACS). https://doi.org/10.1021/acs.jafc.9b01672
40. Rodriguez, R. G., Sagum, R. S., & Trinidad, T. P. (2023). Lipote fruit anthocyanin-rich extracts as food colourants: extraction optimization and stability of natural and copigmented anthocyanins. In Food Research (Vol. 7, Issue 1, pp. 280–291). Rynnye Lyan Resources. https://doi.org/10.26656/fr.2017.7(1).1003
41. Rodriguez, R. G., Sagum, R. S., & Trinidad, T. P. (2023). Lipote fruit anthocyanin-rich extracts as food colourants: extraction optimization and stability of natural and copigmented anthocyanins. In Food Research (Vol. 7, Issue 1, pp. 280–291). Rynnye Lyan Resources. https://doi.org/10.26656/fr.2017.7(1).1003
42. Goyudianto, B. A., Meliana, C., Meliana, C., Muliani, D., J, J., Sadeli, Y. E., & Ratnasari, N. R. P. (2021). The Stability of Phycocyanin, Phycoerythrin, and Astaxanthin from Algae Towards Temperature, pH, Light, and Oxygen as a Commercial Natural Food Colorant. In Indonesian Journal of Life Sciences (Vol. 3, Issue 2, pp. 28–42). Indonesia International Institute for Life Sciences. https://doi.org/10.54250/ijls.v3i2.126
43. Šaravanja, N., Knežević, N., Sabolović, M. B., & Brnčić, S. R. (2016). Influence of pH and Temperature on the Stability of Natural Food Colourant. Book of abstracts of EuroFoodChem XVIII. Madrid. CHP-P-223-x.
44. Goyudianto, B. A., Meliana, C., Meliana, C., Muliani, D., J, J., Sadeli, Y. E., & Ratnasari, N. R. P. (2021). The Stability of Phycocyanin, Phycoerythrin, and Astaxanthin from Algae Towards Temperature, pH, Light, and Oxygen as a Commercial Natural Food Colorant. In Indonesian Journal of Life Sciences (Vol. 3, Issue 2, pp. 28–42). Indonesia International Institute for Life Sciences. https://doi.org/10.54250/ijls.v3i2.126
45. Chang, Y., Liu, Y., Bai, Y., Teng, S., Guo, Y., Dou, H., & Ye, K. (2022). Effect of Different Degrees of Deep Freezing on the Quality of Snowflake Beef during Storage. In Foods (Vol. 11, Issue 15, p. 2175). MDPI AG. https://doi.org/10.3390/foods11152175

46. Fernández-López, J. A., Angosto, J. M., Giménez, P. J., & León, G. (2013). Thermal Stability of Selected Natural Red Extracts Used as Food Colorants. In Plant Foods for Human Nutrition (Vol. 68, Issue 1, pp. 11–17). Springer Science and Business Media LLC. https://doi.org/10.1007/s11130-013-0337-1

47. Renita, A. A., Gajaria, T. K., Sathish, S., Kumar, J. A., Lakshmi, D. S., Kujawa, J., & Kujawski, W. (2023). Progress and Prospective of the Industrial Development and Applications of Eco-Friendly Colorants: An Insight into Environmental Impact and Sustainability Issues. In Foods (Vol. 12, Issue 7, p. 1521). MDPI AG. https://doi.org/10.3390/foods12071521

48. Remini, H., Dahmoune, F., Sahraoui, Y., Madani, K., Kapranov, V. N., & Kiselev, E. F. (2018). Recent advances on stability of anthocyanins. In RUDN Journal of Agronomy and Animal Industries (Vol. 13, Issue 4, pp. 257–286). Peoples' Friendship University of Russia. https://doi.org/10.22363/2312-797x-2018-13-4-257-286

49. Vega, E. N., Ciudad-Mulero, M., Fernández-Ruiz, V., Barros, L., & Morales, P. (2023). Natural Sources of Food Colourants as Potential Substitutes for Artificial Additives. In Foods (Vol. 12, Issue 22, p. 4102). MDPI AG. https://doi.org/10.3390/foods12224102

50. Fuentes, R., Lanero, A., & Luis Vázquez, J. (2021). Are Official Certifications A Relevant Aspect In The Consumption Of Natural Wines? A Prospective Study Among Spanish Producers/Consumers. In Trakia Journal of Sciences (Vol. 19, Issue Suppl.1, pp. 393–398). Trakia University. https://doi.org/10.15547/tjs.2021.s.01.057

51. Business, Agricultural and Food Sciences. (2016). A Clear Label Strategy for Food Additives.

52. Renita, A. A., Gajaria, T. K., Sathish, S., Kumar, J. A., Lakshmi, D. S., Kujawa, J., & Kujawski, W. (2023). Progress and Prospective of the Industrial Development and Applications of Eco-Friendly Colorants: An Insight into Environmental Impact and Sustainability Issues. In Foods (Vol. 12, Issue 7, p. 1521). MDPI AG. https://doi.org/10.3390/foods12071521

53. Dewi, G., & Kollanoor Johny, A. (2022). Lactobacillus in Food Animal Production—A Forerunner for Clean Label Prospects in Animal-Derived Products. In Frontiers in Sustainable Food Systems (Vol. 6). Frontiers Media SA. https://doi.org/10.3389/fsufs.2022.831195

54. Mertoglu-Elmas, G. (2017). The Effect of Colourants on the Content of Heavy Metals in Recycled Corrugated Board Papers. In BioResources (Vol. 12, Issue 2). BioResources. https://doi.org/10.15376/biores.12.2.2690-2698

55. Agudelo Patiño, T., Poveda-Giraldo, J. A., Salas Moreno, M. H., Rengifo Mosquera, G., & Cardona Alzate, C. A. (2023). Potential for Sustainable Production of Natural Colorants in the Tropical Forest: A Biorefinery Case of Annatto Seeds. In Sustainability (Vol. 15, Issue 4, p. 3079). MDPI AG. https://doi.org/10.3390/su15043079

56. Renita, A. A., Gajaria, T. K., Sathish, S., Kumar, J. A., Lakshmi, D. S., Kujawa, J., & Kujawski, W. (2023). Progress and Prospective of the Industrial Development and Applications of Eco-Friendly Colourants: An Insight into Environmental Impact and Sustainability Issues. In Foods (Vol. 12, Issue 7, p. 1521). MDPI AG. https://doi.org/10.3390/foods12071521

57. Ali, S., Yan, Q., Irfan, M., & Fahad, S. (2023). Relating biogas technology and environmental impact assessment: a roadmap towards clean energy for environmental sustainability. In Environmental Science and Pollution Research (Vol. 30, Issue 28, pp. 72832–72853). Springer Science and Business Media LLC. https://doi.org/10.1007/s11356-023-27553-6

58. Bellucci, E. R. B., Barretto, T. L., Rodriguez, J. M. L., Bis-Souza, C. V., Barba, F. J., & Barretto, A. C. da S. (2021). Natural colourants improved the physicochemical and sensorial properties of frozen Brazilian sausage (linguiça) with reduced nitrite. In Scientia Agricola (Vol. 78, Issue 3). FapUNIFESP (SciELO). https://doi.org/10.1590/1678-992x-2019-0211

59. Cobos, Á., & Díaz, O. (2014). Chemical Composition of Meat and Meat Products. In Handbook of Food Chemistry (pp. 1–32). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-41609-5_6-1

60. Nurakhova, B. Zh., Burakhanova, A. K., & Baizhaxynova, G. K. (2023). Production of meat and products of its processing in Kazakhstan: improving the quality and assortment. In Problems of AgriMarket (Issue 1, pp. 151–160). Kazakh Research Institute of Economy of Agro-Industrial Complex and Rural Development. https://doi.org/10.46666/2023-1.2708-9991.17

61. Bal-Prylypko, L., Nikolaienko, M., Cherednichenko, O., & Stepasiuk, L. (2022). Trends of the Development of the Meat Processing Industry of Ukraine and Practical Approaches to the Optimization the Recipe of Sausage Products. In Ekonomika APK (Vol. 29, Issue 5, pp. 10–19). Ekonomika APK. https://doi.org/10.32317/2221-1055.202205010

62. Spada, E., De Cianni, R., Di Vita, G., & Mancuso, T. (2024). Balancing Freshness and Sustainability: Charting a Course for Meat Industry Innovation and Consumer Acceptance. In Foods (Vol. 13, Issue 7, p. 1092). MDPI AG. https://doi.org/10.3390/foods13071092
63. Gómez, I., Janardhanan, R., Ibañez, F. C., & Beriain, M. J. (2020). The Effects of Processing and Preservation Technologies on Meat Quality: Sensory and Nutritional Aspects. In Foods (Vol. 9, Issue 10, p. 1416). MDPI AG. https://doi.org/10.3390/foods9101416
64. Zhang, J., Wei, Z., Lu, T., Qi, X., Xie, L., Vincenzetti, S., Polidori, P., Li, L., & Liu, G. (2023). The Research Field of Meat Preservation: A Scientometric and Visualization Analysis Based on the Web of Science. In Foods (Vol. 12, Issue 23, p. 4239). MDPI AG. https://doi.org/10.3390/foods12234239
65. Guya, M. E., & Neme, G. (2016). Camel slaughtering practices and meat production in Eastern Ethiopia. In Science, Technology and Arts Research Journal (Vol. 4, Issue 3, p. 123). African Journals Online (AJOL). https://doi.org/10.4314/star.v4i3.19
66. Yaghoubi, M., Alirezalu, K., Mazloomi, S. M., Marcinkowska-Lesiak, M., Azadmard-Damirchi, S., Peighambardoust, S. H., Hesari, J., Rastgoo, A., Phimolsiripol, Y., & Mousavi Khaneghah, A. (2024). Enhancing beef sausage packaging with calcium alginate active film infused with nisin and ε-polylysine nanoparticles and beetroot extract. In LWT (Vol. 191, p. 115665). Elsevier BV. https://doi.org/10.1016/j.lwt.2023.115665
67. Sirini, N., Munekata, P. E. S., Lorenzo, J. M., Stegmayer, M. Á., Pateiro, M., Pérez-Álvarez, J. Á., Sepúlveda, N., Sosa-Morales, M. E., Teixeira, A., Fernández-López, J., Frizzo, L., & Rosmini, M. (2022). Development of Healthier and Functional Dry Fermented Sausages: Present and Future. In Foods (Vol. 11, Issue 8, p. 1128). MDPI AG. https://doi.org/10.3390/foods11081128
68. Swastike, W., Suryanto, E., Rusman, R., Hanim, C., Jamhari, J., Erwanto, Y., & Jumari, J. (2020). The Substitution Effects of Tapioca Starch and Beetroot Powder as Filler On The Physical and Sensory Characteristics Of Chicken Sausage. In Jurnal Ilmu dan Teknologi Hasil Ternak (Vol. 15, Issue 2, pp. 97–107). Brawijaya University. https://doi.org/10.21776/ub.jitek.2020.015.02.5
69. Jin, S.-K., Choi, J.-S., Moon, S.-S., Jeong, J.-Y., & Kim, G.-D. (2014). The Assessment of Red Beet as a Natural Colourant, and Evaluation of Quality Properties of Emulsified Pork Sausage Containing Red Beet Powder during Cold Storage. In Korean Journal for Food Science of Animal Resources (Vol. 34, Issue 4, pp. 472–481). Korean Society for Food Science of Animal Resources. https://doi.org/10.5851/kosfa.2014.34.4.472
70. Nisa, A., Saeed, K., Hina, S., Zahra, N., Mazhar, S., Kalim, I., & Syed, Q. (2015). Nutritional, Antioxidant, Microbiological and Toxicological Studies on Red Dye Extracted from Red Beet Roots (Beta vulgaris). In Research Journal of Chemical Sciences (Vol. 5, Issue 4, pp. 1–6). International Science Congress Association.
71. Gong, S., Jiao, C., Guo, L., & Jiang, Y. (2023). Beetroot (Beta vulgaris) Extract against Salmonella Typhimurium via Apoptosis-Like Death and Its Potential for Application in Cooked Pork. In International Journal of Molecular Sciences (Vol. 24, Issue 18, p. 14217). MDPI AG. https://doi.org/10.3390/ijms241814217
72. Aykın-Dinçer, E., Güngör, K. K., Çağlar, E., & Erbaş, M. (2020). The use of beetroot extract and extract powder in sausages as natural food colourant. In International Journal of Food Engineering (Vol. 17, Issue 1, pp. 75–82). Walter de Gruyter GmbH. https://doi.org/10.1515/ijfe-2019-0052
73. Khoiriyah, R. A. R., Al Awwaly, K. U., & Susilo, A. (2023). Physical Analysis of Rosella Flower, Beetroot, and Red Fruit for Sausage Colorant. In International Journal of Current Science Research and Review (Vol. 06, Issue 09). Everant Journals. https://doi.org/10.47191/ijcsrr/v6-i9-16
74. Munekata, P. E. S., Pateiro, M., Domínguez, R., Pollonio, M. A. R., Sepúlveda, N., Andres, S. C., Reyes, J., Santos, E. M., & Lorenzo, J. M. (2021). Beta vulgaris as a Natural Nitrate Source for Meat Products: A Review. In Foods (Vol. 10, Issue 9, p. 2094). MDPI AG. https://doi.org/10.3390/foods10092094

Funds:

This research was funded by Ministry of Agriculture of Kazakhstan, grant number IRN BR BR24892775.

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Anuarbek Suychinov**, Kazakh Research Institute of Processing and Food Industry (Semey Branch), 29 Bairursynov Street, 071410, Semey, Kazakhstan,
Tel.: +7 7222 770026
E-mail: asuychinov@gmail.com
ORCID: https://orcid.org/0000-0003-4862-3293

Aitbek Kakimov, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: bibi.53@mail.ru
ORCID: https://orcid.org/0000-0002-9607-1684

Zhanibek Yessimbekov, Kazakh Research Institute of Processing and Food Industry (Semey Branch), 29 Bairursynov Street, 071410, Semey, Kazakhstan,
Tel.: +7 7222 770026
E-mail: zyessimbekov@gmail.com
ORCID: https://orcid.org/0000-0002-8556-9954

Eleonora Okuskhanova. Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: eleonora-okushan@mail.ru
ORCID: https://orcid.org/0000-0001-5139-9291

Dinara Akimova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan; Kazakh Research Institute of Processing and Food Industry (Semey Branch), 29 Bairursynov Street, 071410, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
Tel.: +7 7222 770026
E-mail: akdilife@mail.ru
ORCID: https://orcid.org/0000-0001-8922-2854

Zhumatay Urazbayev, Kazakh Research Institute of Processing and Food Industry, Gagarin Ave., 238 "G", 050060, Almaty, Kazakhstan,
Tel.: +7 7222 770026
E-mail: sb.info@rpf.kz
ORCID: https://orcid.org/0000-0003-1898-0564

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 1.6.2024
Revised: 20.6.2024
Accepted: 24.6.2024
Published: 26.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 605-618
https://doi.org/10.5219/1987
ISSN: 1337-0960 online
www.potravinarstvo.com

Assessment of the physicochemical profile of gluten-free flour and pasta products

Fariza Sagyntay, Auelbek Iztaev, Assel Boranbaeva, Baltash Tarabayev, Begzada Soltybayeva, Nurbibi Mashanova, Viera Šottníková, Anar Kabylda

ABSTRACT

The production of food products that do not contain gluten is being actively developed since it is not recommended for certain medical reasons, such as celiac disease. Therefore, developing high-quality and highly nutritional gluten-free (GF) pasta products is crucial. A shortage of domestic gluten-free food products characterises the consumer market of Kazakhstan. Buckwheat, rice, and corn flour are widely used to make gluten-free pasta. The results of the study showed that buckwheat flour contains significantly higher amounts of protein (11.9%), ash (1.54%), iron (2.47%), calcium (38.53%), magnesium (56.11%), phosphorus (267.55) and lower carbohydrate content (67.99%) compared to rice and corn flour. Moreover, pasta made from buckwheat flour also showed higher protein 9.39%, Ca (28.80 mg/100g), Mg (48.28 mg/100g), Fe (2.28 mg/100g), Na (5.50 mg/100g), P (196.45 mg/100g) content. Also, amino acids, such as lysine, tyrosine, alanine, valine, etc., were elevated in buckwheat flour-based pasta. Taken together, these data hint that buckwheat has the potential to become a nutrient-rich GF paste ahead of corn and rice. However, further research is needed to determine the cooking qualities and consumer acceptability.

Keywords: gluten-free pasta, flour, celiac, buckwheat, rice, corn

INTRODUCTION

Currently, the production of special foods, including products that do not contain certain ingredients whose presence in food is not recommended for certain medical reasons (allergens, certain types of proteins, oligosaccharides, polysaccharides, etc.). is being actively developed **[1]**. Celiac disease is an immune-mediated enteropathy arising from consuming prolamins of wheat, rye, and barley. A lifelong adherence to a gluten-free diet is known to be the only therapeutic option for individuals with celiac disease **[2]**. The consumer market of Kazakhstan is characterised by a shortage of domestic specialised food products, including gluten-free products, which determines the need to expand their range and develop recipes and innovative production technologies **[3]**, **[4]**. The food engineering of gluten-free flour products is described by two main directions: products based on natural gluten-free raw materials, mainly of plant origin (gluten-free cereals, pseudo-cereals, legumes, nuts, etc.). The biocatalytic direction focused on removing or modifying gluten in gluten-containing raw materials **[5]**. The degree of complexity in the production of gluten-free products is closely related to the role of gluten in the technological system, which is a structure-forming agent. Obtaining high-quality gluten-free products requires the search for ingredients capable of replacing them. Until now, recipes of gluten-free pasta products based on amaranth, corn, rice, chickpea and buckwheat flours are proposed **[6]**, **[7]**, **[8]**. Rice and corn are more popular raw materials in gluten-free pasta production. Meanwhile, buckwheat, amaranth, quinoa, teff and oats are becoming increasingly popular in production as they improve the nutritional content of products **[9]**. The production of pasta products of high quality and high nutritional content is one of the basic conditions for improving nutrition in general. Nevertheless, there is a shortage of established GF paste recipes based on non-

conventional raw materials. To improve the nutritional value of GF pasta products, this study investigated the physicochemical composition of corn, rice, and buckwheat flour and pasta products based on them. To the best of our knowledge, this is the first study proposing pasta production from rice-corn or buckwheat flour for the Kazakhstan market. The results of the study showed that there is a significant difference between aforementioned raw materials and pasta products based on them in terms of proximate composition, including amino acid profile.

Scientific Hypothesis

The physicochemical profiles of different GF raw materials differ significantly, and pasta made from GF flour also varies significantly in nutrient content.

MATERIAL AND METHODOLOGY

Samples

The research objects were samples of buckwheat, rice, and corn flour, corn starch derived from local markets. Xanthan gum and egg white are used as additives.

Chemicals

Distilled water (Tandem distribution LPP, Kazakhstan), hydrochloric acid (purity ≥37%), and sulphuric acid (purity ≥94%) were purchased from Topan LLP, Kazakhstan; sodium hydroxide (R&S Alita, Kazakhstan), boric acid (purity ≥99%) (Alchemica, Kazakhstan), rectified ethyl alcohol (purity ≥95%) (Alfa Organic Malt Distillery, LLP, Kazakstan), and lanthanum nitrate 6-aqueous (LabStar LLC, Russia). All chemicals were of analytical grade quality.

Instruments

Laboratory scales M-ETP2 FLAT (Mercury WP Tech Group Co. Ltd, China), electric drying cabinet SESH-ZM (Ukraine), Kjeldahl flasks (100, 250, and 500 cm^3) (Steklopribor Co. Ltd, Kazakhstan), burettes with a capacity of 25 or 50 cm^3 (Steklopribor Co. Ltd, Kazakhstan), droplet eliminator version KO-60 (Ningbo Greetmed Medical Instruments Co., Ltd., China), electric muffle furnace (Nabertherm, Germany), atomic absorption spectrometer (Agilent, China), graphite cuvettes (Spectrolab, USA), hollow cathode lamps (Agilent, China). More information on the different instruments and chemicals used in the various experiments are given in **[10]**, **[11]**, **[12]**, **[13]**, **[14]**, **[15]**, **[16]**, **[18]**, **[19]**, **[20]**, **[21]**, **[22]**, **[23]**.

Laboratory Methods

The tests of proximate chemical composition were conducted for flour as well as for different GF pasta. The protein, fat, total ash, starch, and testable acidity were analysed according to the GOST 10846-91 **[10]**, GOST 29033-91 **[11]**, GOST P 51411-99 **[12]**, GOST 10845-98 **[13]**, GOST 27493-87 **[14]**, respectively. In addition, the mass fraction of carbohydrates was detected using the permanganatometric method. The GOST 13496.3-92 **[15]** was used to determine the moisture content and pH. Mineral contents were analysed according to the GOST 32343-2013 (ISO 6869-2000) **[16, 17]** by the atomic absorption spectroscopy (AAS) method, which is the quantitative analysis based on the properties of atoms to absorb light at a certain wavelength (resonance absorption). Vitamin A content was examined based on GOST P54635-2011 **[18]**. Besides, the hydro-soluble vitamins were determined using GOST P 50929-96 (M-04-41-2005) **[19]**. The energy value of pasta products was calculated based on the actual content of proteins, fats, and carbohydrates.

Determination of total arsenic (Ars) was conducted by atomic absorption spectrometry with hydride generation with preliminary mineralisation of the sample under pressure using the GOST 30178-96 **[20]**. Inversion-voltamperometric methods were used for determining the content of cadmium (Cd), lead (Pb), and mercury (Hg) according to GOST EN 14083-2013 **[21]**. This method is based on the ability of elements to electrochemically precipitate on the indicator electrode from the analysed solution at a given potential of the limiting diffusion current, and then dissolve in the process of anodic polarisation at a certain potential characteristic of each element.

The amino acid profile of developed formulations was analysed to maximize the biological value of pasta products. The amino acid profile of raw materials was studied under GOST 31480-2012 (M-04-38-2011) **[22]**. The amino acid composition of pasta was determined in accordance with GOST P 55569-2013 (M-04-38-2009) **[23]**.

Description of the Experiment

Sample preparation: The flour samples were obtained from the local market. Subsequently, 50 g were extracted from each sample manually. Then, samples were dried in air or a desiccator. The thoroughly mixed material was placed in a clean and dry test tube for further experiments.

Number of samples analyzed: 5 samples (3 samples of flour, 2 samples of pasta).

Number of experiment replication: 3.

Design of the experiment: In the first phase, we obtained non-conventional GF raw materials and determined the proximal composition of rice, corn, and buckwheat flour. In the next phase, we generated two different GF pasta using rice, corn, and buckwheat flour as raw materials. The recipe of the developed pasta samples is given in Table 1. The dough was made with room temperature water (cold mixing, 30 °C). After kneading, all the samples were rounded, placed in polythene bags, and kept at room temperature for 1 hour. Then, the samples were rolled using a regular rolling pin to form pasta straws 1 mm wide and 50 mm long. After, the samples were photographed, labelled, described, and placed in a drying cabinet at 40 °C for 4 hours so that the final moisture content was not lower than 13%. Finally, the proximal composition of developed GF pasta samples was detected.

Statistical Analysis

All data were computed on one-way analysis of variance (ANOVA) or independent sample t-test, followed by the least significant difference (LSD) test to calculate significant differences between the samples ($p \leq 0.05$) using SPSS software (version 25.0, IBM Corporation, New York, USA). Outcomes were expressed as triplicate analyses' mean ± standard deviation (SD) values.

Table 1 Recipe of pasta samples based on GF flour.

Recipe No.1	Buckwheat flour	70%
	Corn starch	30%
	Egg white	9.4%
	Xanthan gum	5%
	Salt	1%
	Water	100%
Recipe No.2	Corn flour	60%
	Rice flour	40%
	Xanthan gum	5%
	Salt	1%
	Water	70%

RESULTS AND DISCUSSION

Study of the chemical composition of rice, maise, buckwheat flour

The quality of food products, including gluten-free pasta, is largely determined by the quality of raw materials. As can be seen in Table 2.

Table 2 Physicochemical composition of rice, maise, buckwheat flour.

Physicochemical parameters	Rice	Corn	Buckwheat
Protein, %	^{B}7.77 ±0.01	^{B}8.0 ±0.00	^{A}11.9 ±0.05
Fat, %	^{A}1.16 ±0.03	^{B}1.71 ±0.003	^{A}1.12 ±0.005
Carbonhydrates, %	^{A}76.62 ±0.18	^{B}71.91 ±0.04	^{C}67.99 ±0.4
Ash, %	^{A}0.92 ±0.005	^{B}0.85 ±0.00	^{C}1.54 ±0.01
Starch, %	^{A}74.38 ±0.01	^{B}70.23 ±0.08	^{C}67.1 ±0.05
Titratable acidity, °T	^{A}1.5 ±0.006	^{B}6.31 ±0.008	^{C}8.4 ±0.06
Energy, kcal	^{A}329.18 ±0.4	^{B}316.95 ±0.03	^{C}314.45 ±0.2
Vitamin A, mg/100mg	ND	0.03 ±0.006	ND
Iron, mg/100g	^{A}0.27 ±0.005	^{C}1.70 ±0.008	^{B}2.47 ±0.005
Calcium, mg/100g	^{A}8.12 ±0.01	^{B}19.65 ±0.07	^{C}38.53 ±0.01
Magnesium, mg/100g	^{A}41.52 ±0.01	^{B}26.99 ±0,005	^{C}56.11 ±0.05
Copper, mg/100g	^{A}0.111 ±0.006	^{B}0.044 ±0.003	ND
Sodium, mg/100g	ND	8.50 ±0.2	ND
Phosphorus, mg/100g	^{A}87.6 ±0.1	^{B}96.74.±0.1	^{C}267.5 ±1.2
Pb	0.001 ±0.0003	0.0016 ±0.0002	0.002 ±0.0006
Cd	0.0007 ±0.0003	0.0033 ±0.0006	ND
As	ND	ND	ND
Hg	ND	ND	ND

Note: ND, not detected; $^{A, B, C}$ mean $p < 0.01$.

The buckwheat flour contains a significantly higher amount of protein (11.9%), ash (1.54%), TA (8.4 °T), iron (2.47%), calcium (38.53%), magnesium (56.11%), phosphorus (267.55) and lower carbohydrate content (67.99%) compared to rice and corn flour (p <0.01). A recent study reported that the flour from Fagopyrum esculentum and *Fagopyrum tataricum* buckwheat varieties also exhibited higher protein content (11.81-14.90%), ash (2.58-2.85%) and lower total carbohydrate (61.69-67.83%) compared to hard wheat flour **[24]**. It was shown that protein and ash content in buckwheat flour fractions increased in the order from internal to external fractions **[25]**. Rice flour differs significantly from others in carbohydrate content (76.62%). Similar amounts of total carbohydrates were found in Thai rice varieties, although this may depend on variety and processing **[26]**. It is reported that the longer the hydrolysis lasts, the higher the carbohydrate content of white rice flour **[27]**. The presented values indicate that a higher fat content is found in corn flour (1.71%). The lipid content of treated and untreated white and yellow maise varieties differed significantly **[28]**.

Table 3 Amino acid profile analysis of different GF flour.

Amino acids	Rice	Corn	Buckwheat
Arginine	^{A}0.945 ±0.002	^{B}0.873 ±0.004	^{C}1.346 ±0.00
Lysine	^{A}0.250 ±0.008	^{B}0.392 ±0.005	^{C}1.686 ±0.005
Tyrosine	^{A}0.288 ±0.005	^{B}0.285 ±0.002	^{C}0.303 ±0.002
Phenylalanine	^{A}0.620 ±0.01	^{A}0.602 ±0.001	^{B}0.646 ±0.001
Histidine	^{A}0.290 ±0.00	^{B}0.288 ±0.001	^{C}0.478 ±0.001
Leucine+Isoleucine	^{A}0.629 ±0.00	^{B}0.787 ±0.005	^{C}1.117 ±0.002
Methionine	^{A}0.260 ±0.01	^{B}0.252 ±0.005	^{C}0.538 ±0.001
Valine	^{A}0.502 ±0.003	^{B}0.602 ±0.005	^{C}0.999 ±0.001
Proline	^{A}1.677 ±0.005	^{B}1.362 ±0.00	^{C}0.704 ±0.005
Threonine	^{A}0.419 ±0.05	^{B}0.472 ±0.005	^{C}0.750 ±0.05
Serine	^{A}0.603 ±0.1	^{B}0.708 ±0.03	^{C}0.675 ±0.06
Alanine	^{A}0.394 ±0.08	^{B}0.522 ±0.001	^{C}1.150 ±0.2
Glycine	^{A}0.419 ±0.8	^{B}0.446 ±0.05	^{C}0.750 ±0.05

Note: ND, not detected; $^{A, B, C}$ mean p <0.01.

It is well known that starch provides unique functionality in GF pasta processing. The starch content was the lowest in buckwheat flour, consistent with the previous study **[29]**. The starch content of buckwheat grain varies between 60-70% **[30]**. Vitali et al. (2010) reported that the total starch content of buckwheat, corn, and rice flour was 54.15%, 60.25%, and 64.84%, respectively **[31]**. Rice has been shown to contain higher amounts of amylose, a constituent of starch **[32]**.

Moreover, rice and corn flour lagged behind buckwheat flour in iron, calcium, and magnesium content. In a previous study, enhanced Ca and Mg were found in white buckwheat flour **[33]**. Another paper emphasizes that buckwheat is used as a "smart food" because it is high in nutrients and minerals such as Ca (110 mg/100g), Mg (231-390 mg/100g), and P (330-347 mg/100g) **[34]**.

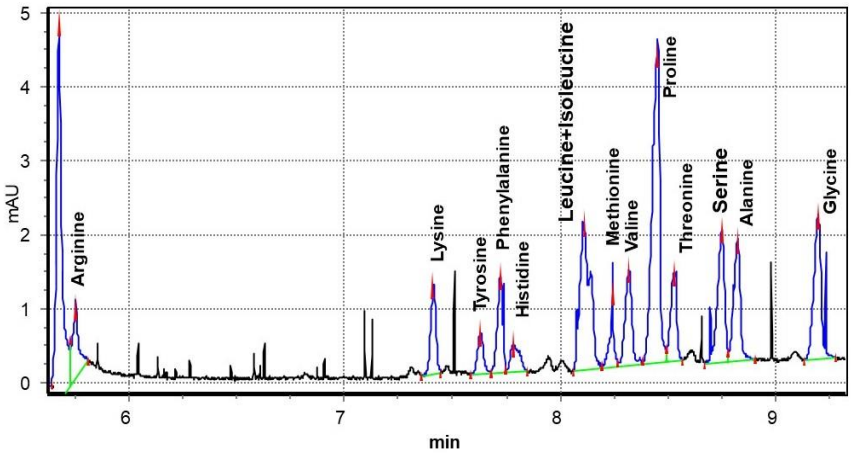
Safety is a crucial aspect of the food products industry. Metals are widespread and at certain concentrations, including some heavy metals, lead to health issues. Regarding toxic trace elements, Pb (lead) and Cd (cadmium) were detected in rice and corn flour, while As (arsenic) and Hg (mercury) were not identified in all GF flour samples. Mercury is particularly dangerous because of its highly toxic effect and its ability to accumulate in the body **[35]**. Analysis of the results showed their safety and compliance with TR TS 021/2011 requirements. Of interest is that rice-based foods have been shown to contain significantly more toxic elements **[36]**, which may be due to contaminated soil.

Table 4 Water-soluble vitamin contents of different GF flour.

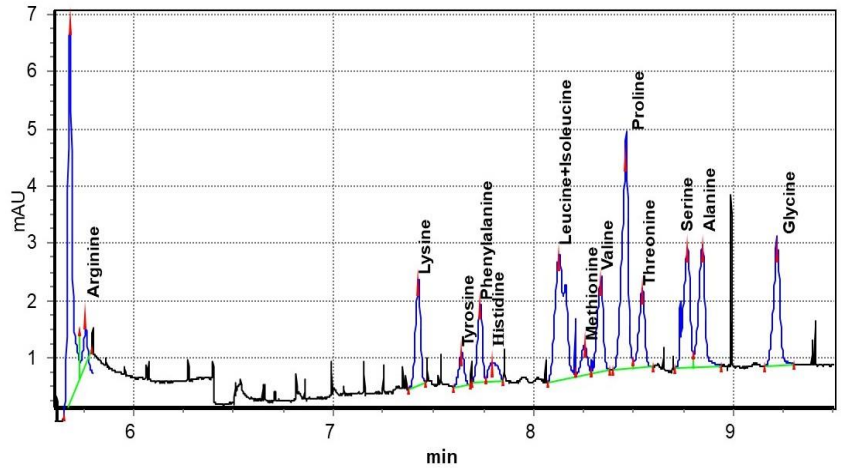
Contents	Rice	Corn	Buckwheat
B1 (thiamine chloride)	^{A}0.16±0.003	^{B}0.11±0.003	^{C}0.14±0.008
B2 (riboflavin)	^{A}0.13±0.003	^{B}0.07±0.004	^{C}0.19±0.005
B6 (pyridoxine)	^{A}0.08±0.02	^{B}0.07±0.003	-
B3 (nicotinic acid)	^{A}0.23±0.003	^{B}0.57±0.008	^{C}0.29±0.008
B5 (pantothenic acid)	0.03±0.002	0.04±0.003	0.03±0.001

Note: ND, not detected; $^{A, B, C}$ mean p <0.01.

A)



B)



C)

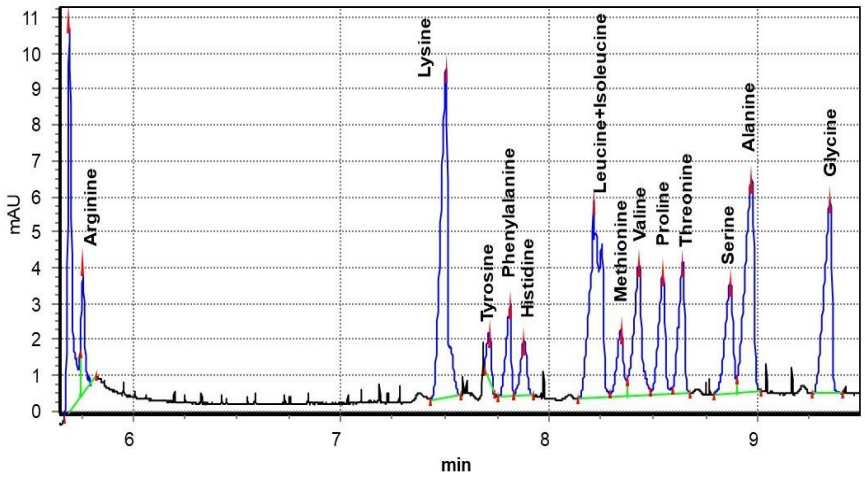
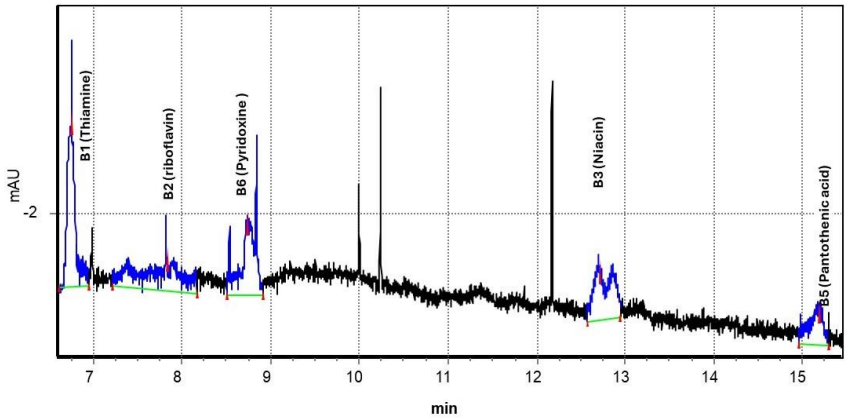
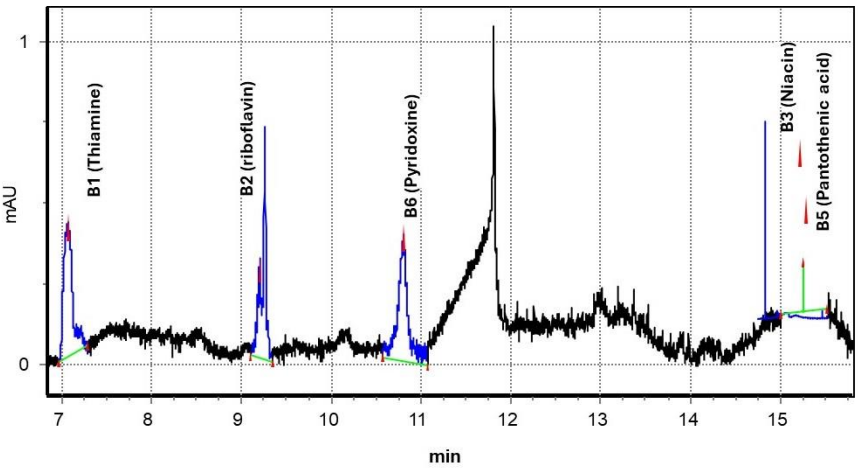


Figure 1 Chromatograms of amino acid composition of a) rice flour, b) corn flour, and c) buckwheat flour; the x-axis is a retention time (min), and the y-axis represents abundance.

A)



B)



C)

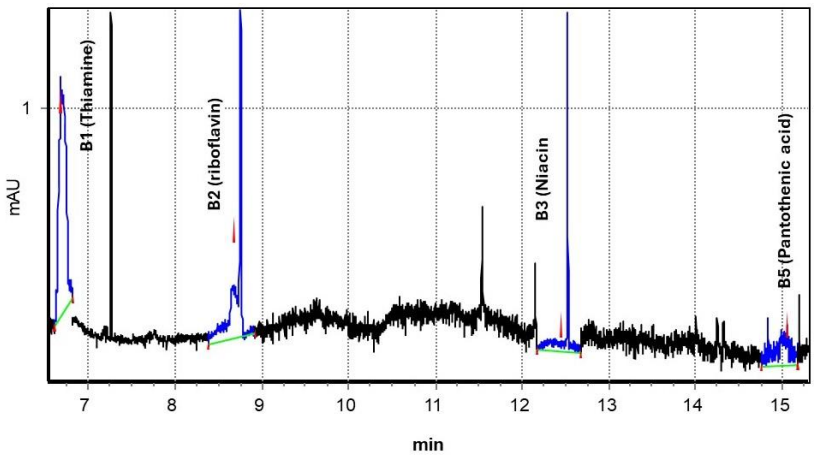


Figure 2 Chromatograms of water-soluble vitamin composition of a) rice flour, b) corn flour, and c) buckwheat flour; the x-axis is a retention time (min), and the y-axis represents abundance.

In terms of amino acid composition, the buckwheat flour was rich in indispensable amino acids, including lysine, histidine, leucine+isoleucine, phenylalanine, methionine, threonine, valine compared to other flour (p <0.01) (Table 3, Figure 1). The results suggest that buckwheat significantly exceeds other cereal crops' nutritional value and protein content. Saeed et al. revealed that lysine (1.03-11 g/kg), isoleucine (0.88-1.19 g/kg) and leucine (1.01-1.05 g/kg) in buckwheat cultivars flour [37]. Notably, it has been demonstrated that egg-white protein-fortified pasta increases nutritional quality **[38]**. It should be noted that amino acid profiles may vary according to soil, cultivar, and climate.

Furthermore, it has been observed that patients with celiac disease are deficient in vitamins **[39]**. Our research outcomes also demonstrated that rice flout contains greater content of B1 (0.16 mg/100g) and B6 (0.008 mg/100g), while corn flour was rich in B3 (0.57 mg/100 g) and buckwheat in B2 (0.19 mg/100g) (Table 4, Figure 2). In the previous study, buckwheat, rice, and corn flour did not significantly differ in B1 content, while buckwheat was rich in B2 (0.22 mg/100 g) **[40]**.

Study of the chemical composition of rice, maise, buckwheat flour-based pasta

The obtained results of experiments on the determination of proximal chemical content in the selected pasta samples indicate that the protein content in the buckwheat and corn starch-based pasta demonstrated significantly greater (p <0.05) protein content 9.39%, Ca (28.80 mg/100g), Mg (48.28 mg/100g), iron (2.28 mg/100g), Na (5.50 mg/100g), P (196.45 mg/100g) compared to rice and corn flour-based GF pasta (Table 5, Figure 5). Besides, the recipe No. 2 exhibited lower starch (1.63%) and higher moisture content (6,47%). The pasta made of buckwheat flour also showed similar protein content in a previous study (9.9%) **[41]**. In addition, noodles prepared with buckwheat, corn, and potato starches contained more potassium, phosphorus, and magnesium, while mineral content decreased with increasing amounts of starch **[42]**. The pasta made of rice and corn flour showed higher total starch (69.2%) in our study. A previously published study also reported that rice-based GF pasta exhibited greater total starch (89.3%) content **[41]**. Arcangelis et al. reported that gelatinization of buckwheat, rice, and corn flours using 0.1% propylene glycol alginate and 0.5% fatty acid monoglycerides proved to be the best combination to produce a gluten-free paste with good nutritional and culinary properties **[41]**.

Therefore, it was suggested that other pseud o-grain-based flours, such as amaranth, can be added to the rice-corn pasta to increase its protein content. For example, rice-based GF pasta enriched with amaranth flour or soybean flour showed increased protein content **[43]**, **[44]**. Also, the corn pasta prepared with 70% corn flour and 30% broad bean flour resulted in increased protein content **[45]**.

Table 5 Physicochemical composition of the two different manufactured GF pasta.

Physicochemical parameters	Recipe No.1 (rice +corn flour)	Recipe No.2 (buckwheat+corn strach)
Protein, %	^{B}7.91 ±0.1	^{A}9.39 ±0.3
Fat, %	^{A}1.62 ±0.09	^{B}1.07 ±0.1
Carbonhydrates, %	^{A}73.80 ±0.2	^{B}68.61 ±0.3
Ash, %	^{B}1.40 ±0.01	^{A}1.63 ±0.07
Starch, %	^{A}69.2 ±0.6	^{B}66.5 ±0.5
Titratable acidity, °T	^{A}4.31 ±0.08	^{B}3.13 ±0.1
pH	^{B}5.17 ±0.04	^{A}5.67 ±0.02
Moisture, %	-	6.47 ±0.005
Energy	^{A}321.06 ±0.2	^{B}308.24 ±0.05
Vitamin A, mg/100mg	^{A}0.018 ±0.001	^{B}0.026 ±0.001
Iron, mg/100g	^{B}1.18 ±0.09	^{A}2.28 ±0.02
Calcium, mg/100g	^{B}13.72 ±0.1	^{A}28.80 ±0.3
Magnesium, mg/100g	^{B}30.3 ±0.1	^{A}48.25 ±0.05
Copper, mg/100g	^{A}0.08 ±0.001	^{B}0.02 ±0.003
Sodium, mg/100g	^{B}4.07 ±0.06	^{A}5.50 ±0.09
Phosphorus, mg/100g	^{B}90.98 ±0.3	^{A}196.48 ±0.2

Note: ND, not detected; *A, B, C* mean p <0.05.

The presence of essential amino acids may determine the biological value of proteins, and the amino acid content of GF pasta may depend on different raw materials. Our results indicate that pasta products based on buckwheat flour and corn starch showed elevated levels of almost all amino acids except histidine, methionine, and proline (Table 6). The essential amino acids, including phenylalanine (6.93-7.27%), leucine (7.23-8.52%), and lysine (5.83-6.03%) were detected in buckwheat noodles **[46]**. A previous study on buckwheat cookies

showed a predominance of alanine, lysine, isoleucine, leucine, glutamic acid, glycine, cysteine content **[37]**. Essential amino acids are of particular importance, among which lysine is significant. Lysine is an essential amino acid for cellular growth and repair **[47]**. Phenylalanine and tyrosine are precursors of monoamine neurotransmitters in the brain **[48]**. Messia et al. showed that pasta made from 100% buckwheat had higher levels of His, Lys, Met than other GF pasta, while the maize-based pasta showed higher levels of Leu **[49]**.

Table 6 Amino acid profile analysis of different recipe-base pasta.

Amino acids	Recipe No.1	Recipe No.2
Arginine	^{B}1.139 ±0.001	^{A}2.037 ±0.001
Lysine	^{B}0.235 ±0.005	^{A}0.354 ±0.006
Tyrosine	^{B}0.339 ±0.001	^{A}0.349 ±0.001
Phenylalanine	^{B}0.632 ±0.002	^{A}0.644 ±0.003
Histidine	^{A}0.265 ±0.001	^{B}0.238 ±0.002
Leucine+Isoleucine	^{B}0.606 ±0.005	^{A}0.671 ±0.002
Methionine	^{A}0.262 ±0.005	^{B}0.231 ±0.002
Valine	^{B}0.486 ±0.001	^{A}0.571 ±0.001
Proline	^{A}1.624 ±0.100	^{B}1.441 ±0.2
Threonine	^{B}0.413 ±0.001	^{A}0.424 ±0.001
Serine	^{B}0.535 ±0.002	^{A}0.572 ±0.002
Alanine	^{B}0.340 ±0.001	^{A}0.465 ±0.03
Glycine	^{B}0.388 ±0.001	^{A}0.498 ±0.001

Note: ND, not detected; $^{A, B, C}$ mean p <0.01.

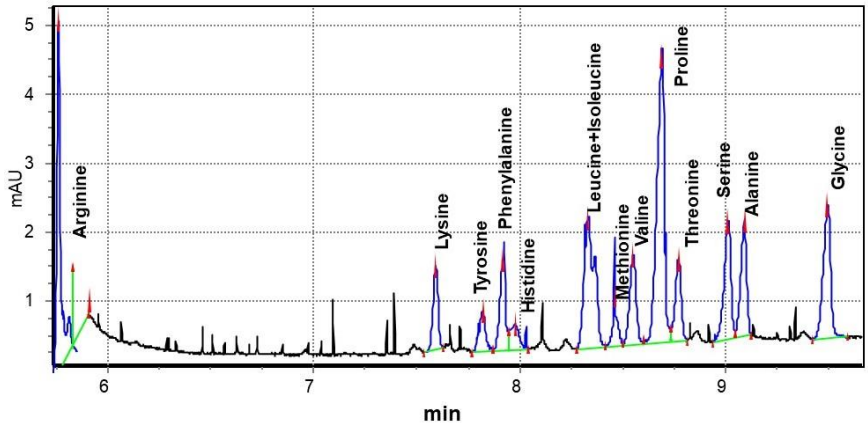
GF products have been shown to contain lower amounts of riboflavin, thiamine, and niacin compared to their wheat counterparts **[50]**. Regarding vitamins, the buckwheat-corn starch pasta demonstrated higher B2, B6, and B3, while B1 and B5 were detected only in rice-corn-based samples (Table 7). It is well known that B vitamins turn food into energy and are essential for the body's metabolism. For instance, vitamin B1 plays a critical role in energy metabolism and muscle contraction, while the role of both B1 and B6 vitamins in the transmission of nerve signals is well-documented **[51]**. B5 is involved in acetyl-CoA, fat, and protein synthesis, whereas B3 regulates intracellular calcium release **[52]**. Therefore, vitamin content is the most important issue in developing gluten-free pasta.

Table 7 Water-soluble vitamin contents of different recipe-based pasta.

Contents	Recipe No.1	Recipe No.2
B1 (thiamine chloride)	0.14 ±0.005	-
B2 (riboflavin)	^{B}0.14 ±0.01	^{A}0.35 ±0.1
B6 (pyridoxine)	^{B}0.047 ±0.001	^{A}0.072 ±0.001
B3 (pantothenic acid)	^{B}0.15 ±0.005	^{A}0.18 ±0.01
B5 (nicotinic acid)	0.031 ±0.005	-

Note: ND, not detected; $^{A, B, C}$ mean p <0.01.

A)



B)

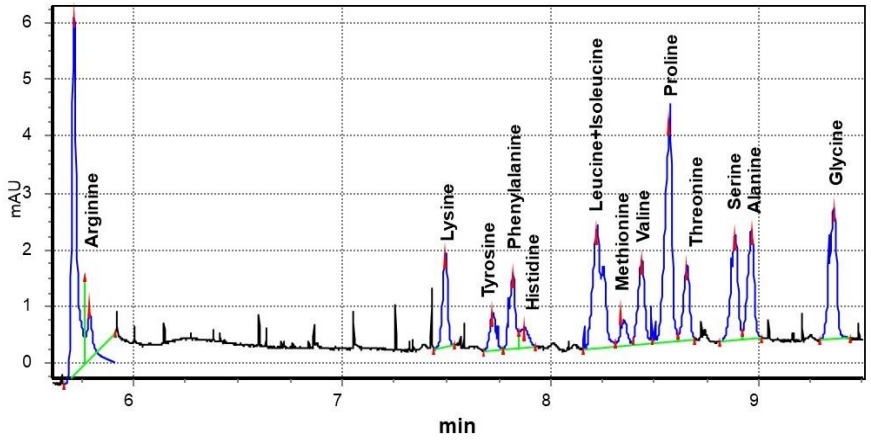
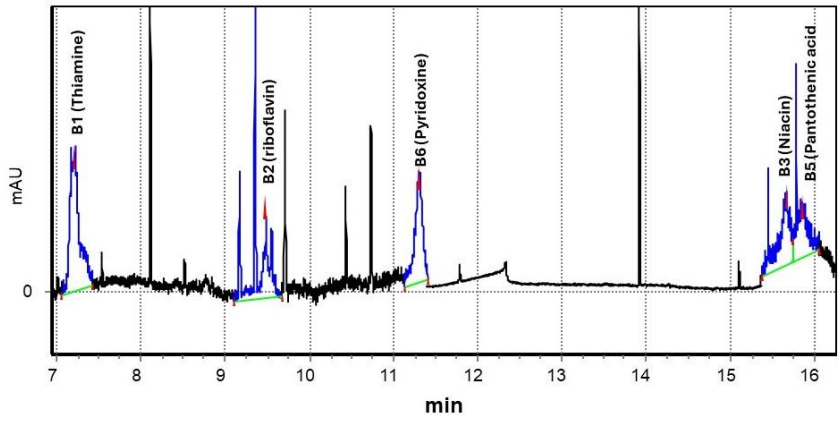


Figure 3 Chromatograms of amino acid composition of pasta A) No.1 recipe; B) No.2 recipe.

A)



B)

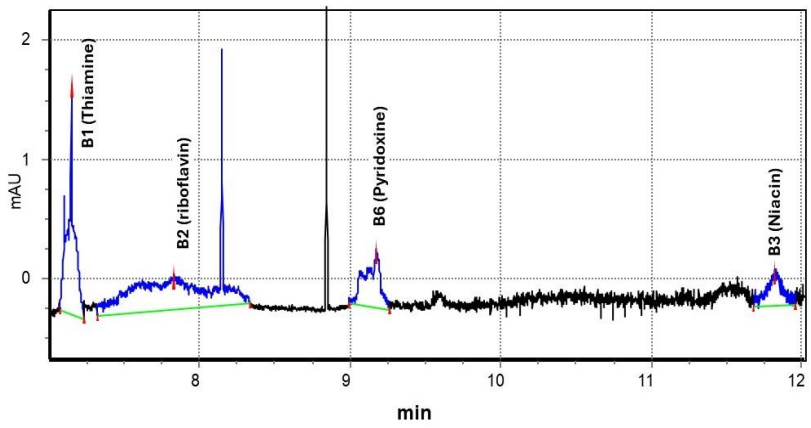


Figure 4 Chromatograms of water-soluble vitamin composition of a) No.1 recipe; b) No.2 recipe pasta.

Figure 5 Ready pasta made from rice-corn flour (left) and from buckwheat flour (right).

CONCLUSION

The results of the study showed that buckwheat flour contains significantly higher amounts of protein (11.9%), ash (1.54%), iron (2.47%), calcium (38.53%), magnesium (56.11%), phosphorus (267.55) and lower carbohydrate content (67.99%) compared to rice and corn flour. Moreover, pasta made from buckwheat flour also showed higher protein 9.39%, Ca (28.80 mg/100g), Mg (48.28 mg/100g), Fe (2.28 mg/100g. Regarding amino acid composition, higher contents of arginine, threonine, lysine, leucine-isoleucine, methionine, phenylalanine, were observed in buckwheat flour. Meanwhile, ready gluten free pasta made from buckwheat flour also showed higher contents of protein, ash, iron, calcium, magnesium, vitamin A, vitamins B2, B3, B6, and essential amino acids including leucine+isoleucine, lysine, phenylalanine, threonine, and valine. Taken together, these data hint that buckwheat has the potential to become a nutrient-rich gluten free paste, surpassing corn and rice. However, further research is needed to determine culinary quality and consumer acceptability.

REFERENCES

1. Wu, X., Qian, L., Liu, K., Wu, J., & Shan, Z. (2021). Gastrointestinal microbiome and gluten in celiac disease. In Annals of Medicine, (Vol. 53, Issue 1, pp. 1797–1805). Informa UK Limited. https://doi.org/10.1080/07853890.2021.1990392
2. Aljada, B., Zohni, A., & El-Matary, W. (2021). The Gluten-Free Diet for Celiac Disease and Beyond. In Nutrients (Vol. 13, Issue 11, p. 3993). MDPI AG. https://doi.org/10.3390/nu13113993
3. Kabylda, A., Sagyntay, F., Iztaev, A., & Muslimov, N. (2022). Investigation of the influence of non-traditional raw materials on the rheological properties of dough in the production of gluten-free pasta. In Journal of Hygienic Engineering and Design (Vol. 40, pp. 36-40). Consulting and Training Center – KEY.
4. Omaraliyeva, A., Botbayeva, Z., Agedilova, M., Abilova, M., & Zhanaidarova, A. (2021). Determining the optimal parameters of ultra-high-frequency treatment of chickpeas for the production of gluten-free flour. In Eastern-European Journal of Enterprise Technologies (Vol. 5, Issue 11 (113), pp. 51–60). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2021.241877
5. O'Shea, N., Arendt, E., & Gallagher, E. (2014). State of the Art in Gluten-Free Research. In Journal of Food Science (Vol. 79, Issue 6). Wiley. https://doi.org/10.1111/1750-3841.12479
6. Lux (née Bantleon), T., Spillmann, F., Reimold, F., Erdös, A., Lochny, A., & Flöter, E. (2023). Physical quality of gluten-free doughs and fresh pasta made of amaranth. In Food Science & Nutrition (Vol. 11, Issue 6, pp. 3213–3223). Wiley. https://doi.org/10.1002/fsn3.3301
7. Suo, X., Dall'Asta, M., Giuberti, G., Minucciani, M., Wang, Z., & Vittadini, E. (2022). The effect of chickpea flour and its addition levels on quality and in vitro starch digestibility of corn–rice-based gluten-free pasta. In International Journal of Food Sciences and Nutrition (Vol. 73, Issue 5, pp. 600–609). Informa UK Limited. https://doi.org/10.1080/09637486.2022.2040008
8. Bolarinwa, I. F., & Oyesiji, O. O. (2021). Gluten free rice-soy pasta: proximate composition, textural properties and sensory attributes. In Heliyon (Vol. 7, Issue 1, p. e06052). Elsevier BV. https://doi.org/10.1016/j.heliyon.2021.e06052
9. Gao, Y., Janes, M. E., Chaiya, B., Brennan, M. A., Brennan, C. S., & Prinyawiwatkul, W. (2017). Gluten-free bakery and pasta products: prevalence and quality improvement. In International Journal of Food Science & Technology (Vol. 53, Issue 1, pp. 19–32). Wiley. https://doi.org/10.1111/ijfs.13505
10. GOST 10846-91 Grain and its products. Method for determination of protein.
11. GOST 29033-91 Grain and its products. The method fat determination.
12. GOST P 51411-99 Grain and its products. Determination of ash content (total ash).
13. GOST 10845-98 Grain and its products. Method for determination of starch.
14. GOST 27493-87 Flour and bran. Method for determination of acidity by bollworm.
15. GOST 13496.3-92 Compound feeds, compound feed raw materials. Methods of moisture determination
16. GOST 32343-2013 (ISO 6869-2000) Fodder, mixed fodder. Determination of calcium, copper, iron, magnesium, manganese, potassium, sodium and zinc content by atomic absorption spectrometry method.
17. International Organization for Standardization. (2018). Occupational health and safety management systems—Requirements with guidance for use (ISO Standard No. 45001:2018).
18. GOST P 54635-2011 Functional food products. Method for determination of vitamin A.
19. GOST R 50929-96 Premixes. Methods of determination of B vitamins. M-04-41-2005 Methodology for measuring the mass fraction of free forms of water-soluble vitamins in samples of premixes, vitamin supplements, concentrates and mixtures by capillary electrophoresis using the capillary electrophoresis system "Kapel-105".

20. GOST 30178-96 Raw materials and food products. Atomic absorption method for determination of toxic elements.
21. GOST EN 14083-2013 Food products. Determination of trace elements. Determination of lead, cadmium, chromium and molybdenum by atomic absorption spectrometry with atomization in graphite furnace with preliminary mineralization of the sample at elevated pressure.
22. GOST 31480-2012 M-04-38- 2011 Compound feeds, compound feed raw materials. Determination of amino acids content (lysine, methionine, threonine, cystine and tryptophan) by capillary electrophoresis method.
23. GOST P 55569-2013 M-04-38-2009 Feeds, mixed fodder, mixed fodder raw materials. Determination of proteinogenic amino acids by capillary electrophoresis method.
24. Hussein, A. M. S., El-Aal, H. A. A., Morsy, N. M., & Hassona, M. M. (2024). Chemical, rheological, and sensorial properties of Baladi bread supplemented with buckwheat flour produced in Egypt. In Scientific Reports (Vol. 14, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-023-48686-1
25. Van Hung, P., Maeda, T., Tsumori, R., & Morita, N. (2007). Characteristics of fractionated flours from whole buckwheat grain using a gradual milling system and their application for noodle making. In Journal of the Science of Food and Agriculture (Vol. 87, Issue 15, pp. 2823–2829). Wiley. https://doi.org/10.1002/jsfa.3018
26. Kraithong, S., Lee, S., & Rawdkuen, S. (2018). Physicochemical and functional properties of Thai organic rice flour. In Journal of Cereal Science (Vol. 79, pp. 259–266). Elsevier BV. https://doi.org/10.1016/j.jcs.2017.10.015
27. Zulfisa, Z., Renatalia Fika, Ainun Naim, & Ezy Wirna. (2022). Effect of variations in hydrolysis time on carbohydrate levels of white rice flour (Oryza sativa L.) Use of the schoorl luff method. In Jurnal Ilmiah Kedokteran dan Kesehatan (Vol. 1, Issue 2, pp. 104–112). Politeknik Pratama Purwokerto. https://doi.org/10.55606/klinik.v1i2.733
28. Dongmo, H., Tambo, S. T., Teboukeu, G. B., Mboukap, A. N., Fotso, B. S., Tekam Djuidje, M. C., & Klang, J. M. (2020). Effect of process and variety on physico-chemical and rheological properties of two corn flour varieties (Atp and Kassaï). In Journal of Agriculture and Food Research (Vol. 2, p. 100075). Elsevier BV. https://doi.org/10.1016/j.jafr.2020.100075
29. Torbica, A., Hadnađev, M., & Dapčević Hadnađev, T. (2012). Rice and buckwheat flour characterisation and its relation to cookie quality. In Food Research International (Vol. 48, Issue 1, pp. 277–283). Elsevier BV. https://doi.org/10.1016/j.foodres.2012.05.001
30. Sofi, S. A., Ahmed, N., Farooq, A., Rafiq, S., Zargar, S. M., Kamran, F., Dar, T. A., Mir, S. A., Dar, B. N., & Mousavi Khaneghah, A. (2022). Nutritional and bioactive characteristics of buckwheat, and its potential for developing gluten-free products: An updated overview. In Food Science & Nutrition (Vol. 11, Issue 5, pp. 2256–2276). Wiley. https://doi.org/10.1002/fsn3.3166
31. Vitali, D., Amidžić Klarić, D., & Vedrina Dragojević, I. (2010). Nutritional and functional properties of certain gluten-free raw materials. In Czech Journal of Food Sciences (Vol. 28, Issue 6, pp. 495–505). Czech Academy of Agricultural Sciences. https://doi.org/10.17221/253/2009-cjfs
32. Marti, A., & Pagani, M. A. (2013). What can play the role of gluten in gluten free pasta? In Trends in Food Science & Technology (Vol. 31, Issue 1, pp. 63–71). Elsevier BV. https://doi.org/10.1016/j.tifs.2013.03.001
33. Veličković, A., Petrović, S., Savić, S., & Petronijević, Z. (2018). Composition of gluten-free flours with special reference to mineral substances. In XXIII Savetovanje o biotehnologiji (pp. 518–523). Univerzitet u Kragujevcu, Agronomski fakultet u Čačku.
34. Jha, R., Zhang, K., He, Y., Mendler-Drienyovszki, N., Magyar-Tábori, K., Quinet, M., Germ, M., Kreft, I., Meglič, V., Ikeda, K., Chapman, M. A., Janovská, D., Podolska, G., Woo, S.-H., Bruno, S., Georgiev, M. I., Chrungoo, N., Betekhtin, A., & Zhou, M. (2024). Global nutritional challenges and opportunities: Buckwheat, a potential bridge between nutrient deficiency and food security. In Trends in Food Science & Technology (Vol. 145, p. 104365). Elsevier BV. https://doi.org/10.1016/j.tifs.2024.104365
35. Park, J.-D., & Zheng, W. (2012). Human Exposure and Health Effects of Inorganic and Elemental Mercury. In Journal of Preventive Medicine & Public Health (Vol. 45, Issue 6, pp. 344–352). Korean Society for Preventive Medicine. https://doi.org/10.3961/jpmph.2012.45.6.344
36. Punshon, T., & Jackson, B. P. (2018). Essential micronutrient and toxic trace element concentrations in gluten containing and gluten-free foods. In Food Chemistry (Vol. 252, pp. 258–264). Elsevier BV. https://doi.org/10.1016/j.foodchem.2018.01.120
37. Saeed, F., Afzaal, M., Ikram, A., Imran, A., Hussain, S., Mohamed, A. A., Alamri, M. S., & Hussain, M. (2021). Exploring the amino acid composition and vitamin-B profile of buckwheat varieties. In Journal of Food Processing and Preservation (Vol. 45, Issue 9). Hindawi Limited. https://doi.org/10.1111/jfpp.15743

38. Rachman, A., Brennan, M. A., Morton, J., Toricco, D., & Breannan S. C. (2023). In-vitro digestibility, protein digestibility corrected amino acid, and sensory properties of banana-cassava gluten-free pasta with soy protein isolate and egg white protein addition. In Food Science and Human Wellness (Vol. 12, Issue 2, pp. 520-527). https://doi.org/10.1016/j.fshw.2022.07.054
39. Vici, G., Belli, L., Biondi, M., & Polzonetti, V. (2016). Gluten free diet and nutrient deficiencies: A review. In Clinical nutrition (Vol. 35, Issue 6, pp. 1236–1241). https://doi.org/10.1016/j.clnu.2016.05.002
40. Rybicka, I., & Gliszczynska-Swiglo, A. (2017). Gluten-Free Flours from Different Raw Materials as the Source of Vitamin B1, B2, B3 and B6. In Journal of nutritional science and vitaminology (Vol. 63, Issue 2, pp. 125–132). https://doi.org/10.3177/jnsv.63.125
41. Arcangelis, E.D., Cuomo, F., Trivisonno, M.C., Marconi, E., & Messia, M.C. (2020). Gelatinization and pasta making conditions for buckwheat gluten-free pasta. In Journal of Cereal Science (Vol. 95, p. 103073). https://doi.org/10.1016/j.jcs.2020.103073
42. Yalcin, S. (2020). Quality characteristics, mineral contents and phenolic compounds of gluten free buckwheat noodles. In Journal of Food Science and Technology (Vol. 58, Issue 7, pp. 2661–2669). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-020-04772-0
43. Cabrera-Chávez, F., Calderón de la Barca, A. M., Islas-Rubio, A. R., Marti, A., Marengo, M., Pagani, M. A., Bonomi, F., & Iametti, S. (2012). Molecular rearrangements in extrusion processes for the production of amaranth-enriched, gluten-free rice pasta. In LWT (Vol. 47, Issue 2, pp. 421–426). Elsevier BV. https://doi.org/10.1016/j.lwt.2012.01.040
44. Bolarinwa, I. F., & Oyesiji, O. O. (2021). Gluten free rice-soy pasta: proximate composition, textural properties and sensory attributes. In Heliyon (Vol. 7, Issue 1, p. e06052). Elsevier BV. https://doi.org/10.1016/j.heliyon.2021.e06052
45. Giménez, M. A., Drago, S. R., Bassett, M. N., Lobo, M. O., & Sammán, N. C. (2016). Nutritional improvement of corn pasta-like product with broad bean (Vicia faba) and quinoa (Chenopodium quinoa). In Food Chemistry (Vol. 199, pp. 150–156). Elsevier BV. https://doi.org/10.1016/j.foodchem.2015.11.065
46. Thanushree, M. P., Sudha, M. L., Martin, A., Vanitha, T., & Kasar, C. (2022). Enhancing the nutritional and quality profiles of buckwheat noodles: Studies on the effects of methods of milling and improvers. In LWT (Vol. 160, p. 113286). Elsevier BV. https://doi.org/10.1016/j.lwt.2022.113286
47. Matthews, D. E. (2020). Review of Lysine Metabolism with a Focus on Humans. In The Journal of Nutrition (Vol. 150, pp. 2548S-2555S). Elsevier BV. https://doi.org/10.1093/jn/nxaa224
48. Fernstrom, J. D., & Fernstrom, M. H. (2007). Tyrosine, Phenylalanine, and Catecholamine Synthesis and Function in the Brain2. In The Journal of Nutrition (Vol. 137, Issue 6, pp. 1539S-1547S). Elsevier BV. https://doi.org/10.1093/jn/137.6.1539s
49. Messia, M. C., Cuomo, F., Quiquero, M., Verardo, V., & Marconi, E. (2023). Assessment of Nutritional Value and Maillard Reaction in Different Gluten-Free Pasta. In Foods (Vol. 12, Issue 6, p. 1221). MDPI AG. https://doi.org/10.3390/foods12061221
50. Thompson, T. (1999). Thiamin, Riboflavin, and Niacin Contents of the Gluten-Free Diet. In Journal of the American Dietetic Association (Vol. 99, Issue 7, pp. 858–862). Elsevier BV. https://doi.org/10.1016/s0002-8223(99)00205-9
51. Paez-Hurtado, A. M., Calderon-Ospina, C. A., & Nava-Mesa, M. O. (2022). Mechanisms of action of vitamin B1 (thiamine), B6 (pyridoxine), and B12 (cobalamin) in pain: a narrative review. In Nutritional Neuroscience (Vol. 26, Issue 3, pp. 235–253). Informa UK Limited. https://doi.org/10.1080/1028415x.2022.2034242
52. Tardy, A.-L., Pouteau, E., Marquez, D., Yilmaz, C., & Scholey, A. (2020). Vitamins and Minerals for Energy, Fatigue and Cognition: A Narrative Review of the Biochemical and Clinical Evidence. In Nutrients (Vol. 12, Issue 1, p. 228). MDPI AG. https://doi.org/10.3390/nu12010228

Funds:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

*Fariza Sagyntay, Almaty Technological University, Faculty of Food Technology, Tole bi 100, 050012, Almaty, Kazakhstan,
Tel.: +7 707 271 97 00
E-mail: farizasagintaeva@gmail.com
ORCID: https://orcid.org/0000-0002-6805-8888

Auelbek Iztaev, Almaty Technological University, Faculty of Food Technology, Tole bi 100, 050012, Almaty, Kazakhstan,
Tel.: +7 700 216 225
E-mail: auelbekking@mail.ru
ORCID: https://orcid.org/0000-0002-7385--482X

Assel Boranbaeva, Taraz Regional University named after M.Kh.Dulaty, Suleimanova 7, 080000, Taraz, Kazakhstan,
Tel.: +7 702 817 9253
E-mail: aselboor@mail.ru
ORCID: https://orcid.org/0000-0002-9696-5303

Baltash Tarabayev, S.Seifullin Kazakh Agro-Technical Research University, Department of Technology of Food and Processing Production, Zhenis Ave. 62, Astana 010011, Kazakhstan,
Tel.: +7 771 255 70 96
E-mail:tarabaev5009@gmail.com
ORCID: https://orcid.org/0000-0002-5783-3973

Begzada Soltybayeva, Taraz Regional University named after M.Kh.Dulaty, Suleimanova 7, 080000, Taraz, Kazakhstan,
Tel.: +7 705519 21 75
E-mail: soltibaeva@mail.ru
ORCID: https://orcid.org/0000-0002-3260-4429

Nurbibi Mashanova, S.Seifullin Kazakh Agro-Technical Research University, Department of Technology of Food and Processing Production, Zhenis Ave. 62, Astana 010011, Kazakhstan,
Tel.: +7 705 739 81 03
E-mail: nurmashanova@gmail.com
ORCID: https://orcid.org/0000-0001-8664-5173

Viera Šottníková, Mendel University in Brno, Faculty of Agronomy, Department of Food Technology, Zemědělská 1, 613 00, Brno, Czech Republic,
Tel.: +420545133068
E-mail: viera.sottnikova@mendelu.cz
ORCID: https://orcid.org/0000-0002-5963-7116

Anar Kabylda, AF LPP "Kazakh Research Institute of Processing and Food Industry", Al-Farabi 47, 010000, Astana, Kazakhstan,
Tel.: +7 775 900 15 07
E-mail: anara121579@gmail.com
ORCID: https://orcid.org/0000-0002-7876-9368

Corresponding author: *

potravinárstvo®

OPEN ACCESS

Received: 2.6.2024
Revised: 18.6.2024
Accepted: 19.6.2024
Published: 26.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 619-632
https://doi.org/10.5219/1989
ISSN: 1337-0960 online
www.potravinarstvo.com

Evaluation of beef carcass quality using the muscle eye area *M. longissimus dorsi*

Olha Kruk, Anatolii Ugnivenko, Tetiana Antoniuk, Oleksandr Kolisnyk, Nataliia Slobodyanyuk, Dmytro Nosevych, Mykola Gruntkovskyi, Tatyana Naumenko

ABSTRACT

In Japan, Korea, the USA, and Australia, the area of the "muscle eye" (cross-section of the *M. longissimus dorsi* during the division of the half-carcass between the 12th and 13th rib into the front and rear parts) is used to assess the quality of cattle carcasses. The correlation between this feature and the slaughter and quality characteristics of carcasses in 20-22-month-old crossbred bulls (Ukrainian Black-and-White Dairy × Holstein) has been studied. The area of the "muscle eye" in bulls was determined before slaughter by an ultrasound analyzer Emperor 860. After slaughter, it was calculated by the length and depth of the "muscle eye". The colour of muscle and adipose tissue, carcass conformation, development, and subcutaneous fat thickness have been examined. The correlation between the size of the "muscle eye" and carcasses' quantitative and qualitative characteristics has been determined. The area of the "muscle eye" has a positive relationship (r = 0.612; p >0.999) with live weight after fasting, carcass weight (r = 0.598; p >0.999), flesh weight (r = 0.498; p >0.99), including the highest (r = 0.745; p >0.999), and first grade (r = 0.662; p >0.99), the amount of adipose tissue (r = 0.491; p >0.99) and tendons and ligaments (r = 0.435; p >0.99). With its increase, there is a tendency to an inverse relationship with the content of second-grade flesh in the carcass (r = 0.303), carcass conformation (r = 0.147), fat-irrigation thickness (r = 0.125), and marbling (r = 0.340). The area of the "muscle eye" is inversely correlated with the development of subcutaneous fat (r = -0.389; p >0.95) and the saturated colour of muscle tissue (r = -0.309). The correlation coefficients between the area of the "muscle eye" determined by ultrasound and quantitative and qualitative characteristics of carcasses are significantly higher than those obtained by post-slaughter calculation of the area of the muscle eye by measuring the length and depth. The data's practical significance is obtaining knowledge that allows beef to be sorted based on the correlation between the muscle eye area and the quality of carcasses and beef.

Keywords: marbling of beef, carcass weight, carcass yield, meat colour, fat colour, fatness, carcass conformation

INTRODUCTION

The evaluation of cattle carcasses in Ukraine and in developed countries is different. According to DSTU 4673-2006 "Cattle for slaughter. Technical specifications" **[1]**, beef is evaluated by pre-slaughter live weight, fatness, and carcass weight. In the European Union, beef carcasses are classified according to the EUROP system **[2]**. According to this system, the conformation (meatiness) of carcasses (grades E, U, R, O, P) and the development of adipose tissue (5 main grades) are determined. DSTU 4673:2006 **[1]** and the EUROP system **[2]** do not take into account the area of the "muscle eye" (the cross-section of the *M. longissimus dorsi* between the 12th and 13th rib during carcass cutting). The above-mentioned regulations only assess the quantitative characteristics of beef, but not its nutritional quality. In the world, the production of meat from cattle is adapted to the consumer's taste. It is selected for its sensory characteristics- the most important signs of nutritional value

- tenderness, juiciness, taste, and aroma. To establish the quality grade of carcasses and make a decision on the use of a particular cut by the consumer, the area of the "muscle eye" is considered the main feature by the standards of Japan **[3]**, Korea **[4]**, the United States **[5]** and Australia **[6]**. Therefore, the justification for the need to include the area of the "muscle eye" in Ukraine's regulatory documents on the classification of cattle carcasses is relevant to the livestock economy.

Analysis of recent research and publications. In Ukraine, beef is produced from dairy, combined, and beef cattle breeds and crossbreeds obtained from their crossbreeding. The largest share of cattle is Ukrainian Black-and-White and its crossbreeds with Holstein. A significant diversity of breeds is associated with differences in the quality and quantity of beef yield **[7]**, so there is a need to assess the characteristics of meat of each genetic group of animals. The features of quantitative and qualitative characteristics of meat from cattle with different areas of the "muscle eye" are not sufficiently disclosed. The area of the "muscle eye" significantly depends on the direction of breed productivity **[8]** and the breed of animals **[9]**. Thus, in 22-month-old bulls of the Ukrainian beef breed, the size is 133.5 cm^2, which is 30% more (p >0.999) than in their peers of the Ukrainian Black-and-White dairy breed **[8]**. In 30-month-old Hanvoo bulls, the average area of the "muscle eye" is 87.4 cm^2 **[10]**.

It has been proved **[8]** that increasing the slaughter age of Ukrainian beef bulls from 20 to 22 months had virtually no effect on the area of the "muscle eye". The cross-section of *M. longissimus dorsi* in bulls tends to increase in diameter when they reach a live weight of 500 kg, after which its growth slows down. The area of the "muscle eye" depends on the growth rate of bulls from birth to slaughter. Thus, its increase by 100 g helps to improve the cross-sectional area by 9%. Thus, increasing the growth rate of young animals, which improves protein deposition in muscle tissue, is one of the main ways to influence the muscle eye area within a breed.

A positive correlation between the area of the "muscle eye" and the pre-slaughter live weight and carcass weight has been found **[10]** in Hanvoo beef bulls. According to our data **[8]**, the area of the "muscle eye" in cattle of the Ukrainian meat breed directly correlates with slaughter weight (r = 0.614; p >0.95) and carcass yield (r = 0.653; p >0.95). This indicates its connection with the growth of muscle tissue. The data on the correlation between the area of the "muscle eye" and quantitative features of beef in specialized meat animals cannot be used as a basis for interpreting them for dairy cattle. Therefore, the study aimed to evaluate the correlation between the area of the muscle eye and quantitative features of beef yield after slaughter and qualitative features of carcasses in crossbred bulls from Ukrainian Black-and-White dairy cows and Holstein bulls.

Scientific Hypothesis

Previous studies on Ukrainian beef bulls have shown that better development of the "muscle eye" area directly correlates with slaughter weight and muscle tissue content in the carcass, particularly in the highest and first grades. No positive correlation has been confirmed with beef quality features, including sensory and processing properties and chemical composition. It is assumed that the "muscle eye" area in animals correlates with other quality features of the carcass – marbling, conformation, development, and thickness of the subcutaneous fat, which affect certain sensory and technological properties of beef. The relationship between the area of the "muscle eye" and the quantitative and qualitative features of beef in crossbred animals from the Ukrainian Black-and-White dairy and Holstein breeds may differ from the general features of their correlation in beef cattle.

MATERIAL AND METHODOLOGY

Samples

The study was conducted at the Zhuravushka farm in Brovary district, Kyiv region. 26 crossbred bulls from Ukrainian Black-and-White dairy (UBW) cows and Holstein (H) bulls were used in the study. From birth to 4 months of age, the bulls were housed in groups and fed dairy feed. During the dairy period, they were fed 547 kg of milk and 182 kg of skim milk. The animals had free access to hay and concentrated feed. The bulls were raised and fattened at the fattening site until they reached 20 to 22 months. The feed needs of the animals were met by the farm's feed base. At the site, bulls were kept in groups based on their age. The animals had free access to concentrated, roughage, juicy feed, and in summer to green fodder and mineral fertilisers, which were fed from self-made troughs by the rations developed on the farm.

During the eye animals lifetime, muscle eye area was determined using Emperor 860 according to the recommendations given in the international methodology of ICAR **[11]**. Animals at the slaughterhouse, after fasting, were fixed in a split, and the hair was cut in the study area to a hair length of no more than 1.5 cm. Before the ultrasound scanning, a gel was applied to the measurement site to ensure maximum contact between the transducer and the skin. To measure the area of the "muscle eye", the device was placed in the area of the *M. longissimus dorsi* between the 12^{th} and 13^{th} ribs. After the ultrasound examination, the bulls were slaughtered in the slaughterhouse of the Zhuravushka farm in the village of Kalynivka. Before slaughter, before and after a 24-hour fast, the live weight of the bulls was determined by weighing. Fasting was carried out with free access to

water. The slaughter of animals was carried out by the European Regulation No. 1099/2009 "On the protection of animals at the time of killing" **[12]**. After the slaughtering process was completed, paired bull carcasses were weighed and visually assessed for conformation and fatness according to the EUROP (2008) system **[2]**. The conformation of carcasses was evaluated on a scale of five grades (from 1 = thin to 5 = very fat), 15 subgrades from E+ (very high muscle development) to P- (very low muscle development). For statistical analysis, a numerical transformation was performed on a scale from 1 (corresponding to P-) to 15 (corresponding to E+). The carcasses were cooled and stored at 2 °C for 24 hours. Chilled half-carcasses were cut into quarters between the 12^{th} and 13^{th} rib. The thickness of subcutaneous fat was assessed in the area of the 12^{th}-13^{th} rib using a ruler, and the length and depth of the "muscle eye" were measured on the cross-section of the *M. longissimus dorsi*, at the point where the carcass was divided into front and back parts, according to the scheme shown in Figure 1. The uniformity of colour of muscle and adipose tissue was determined on a scale from 1 to 7 according to the methods described in JMGA **[3]**.

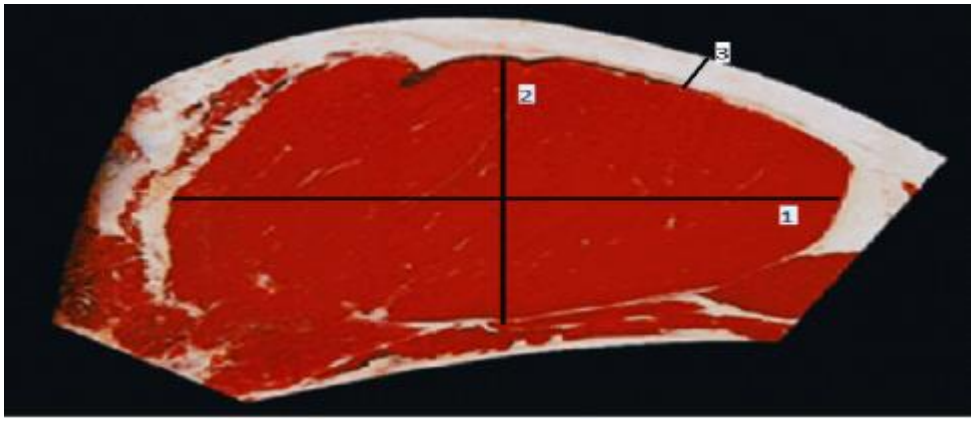


Figure 1 Length (1), depth (2) of the "muscle eye", (3) thickness of subcutaneous fat.

The area of the "muscle eye" was calculated according to (formula 1) under the order of the Ministry of Agriculture of Ukraine No. 290 of 06 August 2004 **[13]**:

$$S = 1 \times 2 \times 0.8 \quad (1)$$

Where:
S is the area of the "muscle eye", cm^2; 1 is the length of the "muscle eye", cm; 2 – depth of the "muscle eye", cm; 0.8 – coefficient.

According to the EUROP (2008) system **[2]**, the subcutaneous fat coverage of carcasses was assessed visually and divided into five grades (from 1 = thin to 5 = very fat). The marbling of meat was determined using a 12-point scale according to the JMGA (2000) **[3]**. All qualitative characteristics of the carcasses were evaluated by the "Rules for Slaughter Veterinary Examination of Animals and Veterinary and Sanitary Examination of Meat and Meat Products" (2002) **[14]**.

Chemicals

Gel for ultrasound examination (Himlaborreactiv LLC, Ukraine).

Animals, Plants and Biological Materials

Carcasses of crossbred bulls from Ukrainian Black-and-White dairy cows and Holstein bulls belonging to the Zhuravushka farm in Brovary district, Kyiv region.

Instruments

Static scales 4BDU-15X-P (Axis, Ukraine).
Weighing unit >0.5 kg, weighing range from 10 to 1,500 kg.
Weighing bulls monthly and before slaughter.
Machine for fixing animals.
Ruler.

Laboratory Methods

Scales from 1 to 7 are used to determine muscle (Figure 2), adipose tissue (Figure 3), marbling (Figure 4), conformation (Figure 5), and subcutaneous fat (Figure 6).

Figure 2 Scales for assessing muscle tissue colour according to the methodology (JMGA) **[3]**.

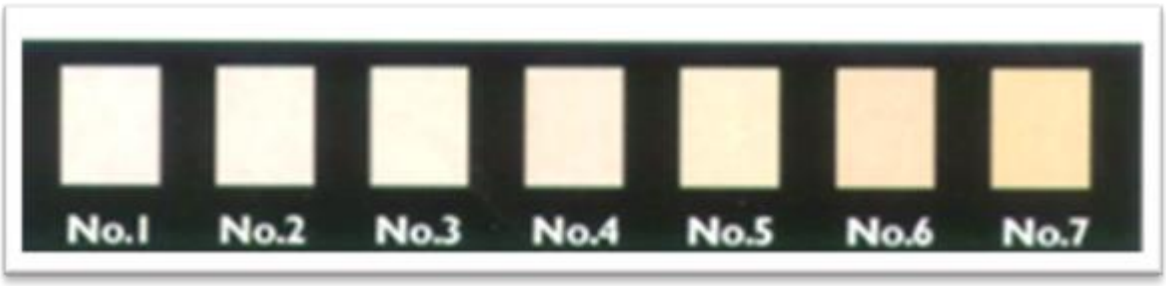


Figure 3 Scales for assessing the colour of adipose tissue according to the methodology (JMGA) **[3]**.

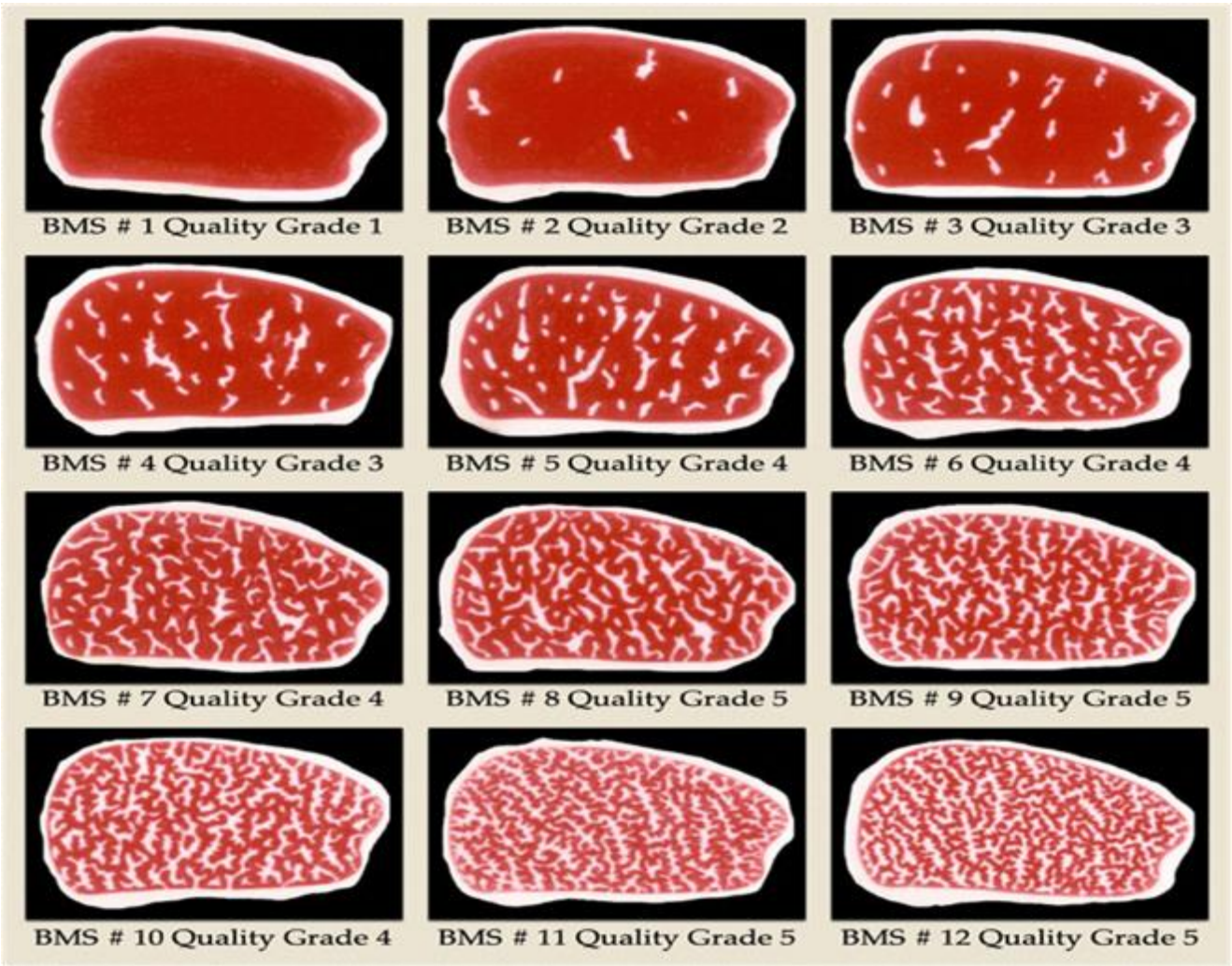
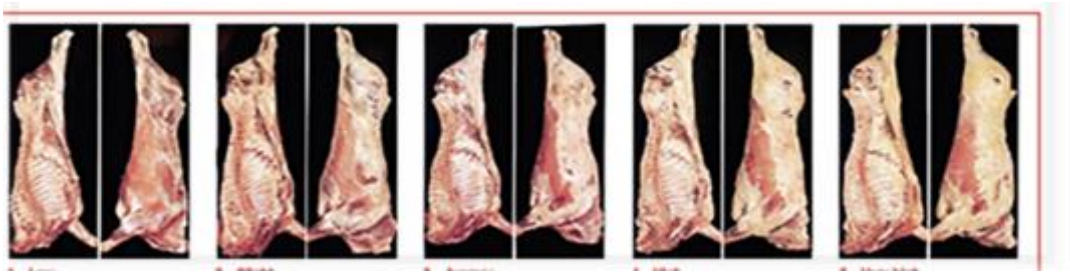


Figure 4 Scales for assessing the marbling of *M. longissimus dorsi* (JMGA) **[3]**.

(E) (U) (R) (O) (P)

Figure 5 Scales for assessing the conformation of carcasses (EUROP) **[2]**.



1 2 3 4 5

Figure 6 Scales for assessing the development of subcutaneous fat according to the EUROP system **[2]**.

Portable ultrasound scanner Emperor 860 (manufactured by Shenzhen Emperor Electronic Technology, China).

Description of the Experiment

Sample preparation: There are European Regulation No. 1099/2009 of 24 September 2009 "On the protection of animals at the time of killing" **[12]** and the Rules for Slaughter and Pre-Slaughter Veterinary Examination of Animals and Veterinary and Sanitary Examination of Meat and Meat Products (2002) **[14]**.

Animal preparation: Before slaughter, animals were fasted for 24 hours with free access to water. After slaughter, the conformation of the carcasses and the development of subcutaneous adipose tissue were visually examined. After 24 hours of storage at 2 °C in a refrigerator, the thickness of the subcutaneous fat, muscle marbling, and the length and depth of the "muscle eye" were determined.

Number of samples analyzed: in the experiment, one sample from each animal was used to analyse the qualitative characteristics of 26 carcasses of *M. longissimus dorsi* of the left half of them.

Number of repeated analyses: The value of the qualitative feature of the area of the "muscle eye" in the carcasses of slaughtered bulls was determined once during the animals' lives and once after slaughter.

Number of experiment replication: Each study was carried out five times, and the number of samples was three, resulting in fifteen repeated analyses.

Design of the experiment: In the first stage, 26 crossbred bulls were kept in a group from birth to 4 months of age. Then, the animals were fed with feed produced on the farm at the fattening site. In the second stage, the bulls were slaughtered in the slaughterhouse (Kalynivka village) by the requirements of European Regulation No. 1099/2009 **[12]**. After slaughtering the bulls, the degree of coverage of carcasses with subcutaneous fat was determined, the conformation (muscularity) of carcasses and the thickness of subcutaneous fat, the colour of muscle and adipose tissue, and the marbling of *M. longissimus dorsi* were assessed. At the last stage, the correlation between the area of the "muscle eye" and the quality properties of the carcasses was studied.

Statistical Analysis

The data obtained were statistically processed using Microsoft Excel 2016 and XLSTAT. The studied indicators were evaluated by correlation coefficients calculated according to the appropriate methods **[15]**.

RESULTS AND DISCUSSION

The live weight of bulls after 24 hours of fasting and carcass weight are the main signs of meat productivity, positively and significantly correlated with the area of the "muscle eye" of *M. longissimus dorsi* (Table 1). This indicates that the process of muscle tissue formation is closely correlated with the growth of animals since an increase in their live weight mainly determines the development of muscles. When using the evaluation of the area of the "muscle eye" by ultrasound during the life of 21-month-old animals, a significantly higher positive probable correlation was established. Studies **[16]** also found that the area of the "muscle eye" of *M. longissimus dorsi* significantly correlated with the slaughter weight of experimental animals, depending on their genotype. There was no correlation between the area of the "muscle eye" and the slaughter yield (carcass).

Table 1 Correlation coefficients between the area of the "muscle eye" and slaughter signs of commercial bulls.

Slaughter age	Sign			
	n	live weight after fasting	carcass weight	carcass yield
From 20 to 22 months	26	0.612***	0.598***	0.018
including at 21 months	21	0.613**	0.611**	0.107
including at 21 months for the use of ultrasound	11	0.916***	0.912***	-0.045

Notes: **)p >0.99; ***)p >0.999.

The nutritional value of beef is significantly influenced by muscle and adipose tissue. Connective and bone tissues – no. In our study, a positive significant relationship between the area of the "muscle eye" and the content of muscle tissue in the carcass, in particular of the highest and first grades, was proved (Table 2).

Table 2 Correlation coefficients between the area of the "muscle eye" and the absolute values of the morphological composition of carcasses of crossbred bulls.

Feature	Age		
	20 to 22 months (n = 26)	including at 21 months (n = 21)	including at 21 months (n = 11) for the use of ultrasound
Muscle tissue	0.498**	0.550**	0.931***
in particular of the highest grade	0.745***	0.727***	0.831***
-//- first grade	0.662***	0.718***	0.926***
-//- second grade	-0.303	-0.227	0.958***
Fatty tissue	0.491**	0.579**	0.437
Tendons and ligaments	0.435*	0.593**	-0.023
Bones	0.093	-0.014	0.758**

Notes: *)p >0.95; **) p >0.99; ***) p >0.999.

A significant positive correlation was previously established in animals between the area of the "muscle eye" and the content of premium muscle tissue **[17]**, valuable cuts **[18]**, and the proportion of flesh **[16]**.

A direct significant correlation was found between the area of the "muscle eye" and carcass weight, the amount of muscle tissue, in particular of the highest and first grades, can be explained by the fact that the *M. longissimus dorsi* muscle is located mainly in the thoracic and lumbar regions of the carcass. These are the most valuable cuts, and their muscle tissue makes up a significant proportion. The data presented in Table 2 and obtained in studies **[16]**, and **[17]** indicate that the area of the "muscle eye" of *M. longissimus dorsi* can be used to predict the amount of muscle tissue in carcasses and its belonging to the highest and first grades, which indicates a higher yield of valuable cuts for which the consumer pays the highest price. The conclusion that it is possible to use the data on the area of the "muscle eye" to predict the amount of beef produced and its belonging to a certain grade was also pointed out by other authors **[19]**.

A positive correlation was found between the area of the muscle eye and the total fat content of the carcass. A similar correlation between the area of the "muscle eye" and the fat content of the carcass was also found in other studies **[20]**. There was also a direct correlation between the cross-sectional area of *M. longissimus dorsi* and the content of tendons and ligaments in the carcass. There is practically no correlation between the number of bones in the carcasses of slaughtered animals and the area of the "muscle eye". This can be explained by the fact that

the growth of muscle tissue (including *M. longissimus dorsi*) and adipose tissue in the ontogeny of cattle is relatively faster bone growth is slower, and the correlation between the area of the "muscle eye" of the muscle under study and the bone content is much smaller. There is an inverse correlation between the cross-sectional area of *M. longissimus dorsi* and the amount of second-grade beef contains a significant amount of fat not separated from the muscle during deboning.

Statistical analysis was used in the following aspects of the research described above:

- comparative analysis: the quality of beef carcasses was compared between different groups of animals according to the indicators of the "muscle eye" zone, and the average size and structure of muscle fibers were compared.
- correlation analysis: relationships between various quality parameters of the beef carcass and indicators of the "muscle eye" zone, such as muscle mass, fat content, and moisture, were established.
- analysis of changes over time: studies are conducted in dynamics, and changes in the quality of the beef carcass and its "muscle eye" zone over time (for example, changes in the diet and physical activity of animals) have been studied.
- factor analysis: the influence of various factors on the quality of beef carcass was established through the analysis of the "muscle eye" zone.

The purposeful use of statistical analysis in this context was the collection of objective data, their analysis to identify dependencies and regularities, as well as support decision-making in the field of beef production with maximum quality indicators.

Evaluating the correlation between the area of the “muscle eye” and the quality characteristics of carcasses by international standards established a tendency for correlation with carcass conformation, the thickness of subcutaneous fat, and the colour of muscle and adipose tissue (Table 3).

Table 3 Correlations between the area of the “muscle eye” and the quality features of bull carcasses.

Age	Features					
	carcass conformation	**development of subcutaneous fat**	**thickness of subcutaneous fat**	**marbling of beef**	**colour of muscle tissue**	**colour of adipose tissue**
From 20 to 22 months (n = 26)	-0.147	-0.389*	-0.125	-0.340	-0.309	0.126
In particular. at 21 months (n = 21)	-0.185	-0.382	-0.183	-0.441*	-0.369	0.041
In particular. at 21 months for the use of ultrasound (n = 11)	0.018	-0.202	-0.033	0.136	-0.311	-0.100

Note: *) $p > 0.95$.

There was a correlation ($p > 0.95$) between the area of the “muscle eye” and the development of subcutaneous fat. This indicates that *M. longissimus dorsi* develops better with poorer carcass fatness. In their research **[20]**, they also proved that carcass yield and the proportion of edible parts of muscle tissue decrease in the presence of a significant amount of subcutaneous fat. That is, better development of subcutaneous fat and carcass fatness, in particular, antagonizes the area of the “muscle eye” and simultaneously reduces the amount of valuable edible parts in the carcass. Studies **[38]** have also found a significant correlation between the area of the muscle eye and carcass fatness, depending on the genotype of the animals. In addition, we **[21]** proved that the better development of adipose tissue under the skin did not correlate with the sensory characteristics of cooked beef and beef broth, as well as water retention, penetration, and marbling. Animals with better subcutaneous adipose tissue development consume more feed for growth **[22]**. This reduces the fat content in the middle of the muscles **[23]** and does not improve beef quality **[24]**. The development of adipose tissue depends on inbreeding **[22]** and positively affects the expression of meat forms **[25]**. Since subcutaneous fat has a low commercial value, it is considered **[26]** a waste, and technological aspects of diesel fuel production from it are developed **[27]**.

In the processing industry, fat from cattle has no high nutritional value. Therefore, the consumer's healthy diet aims to replace fatty raw materials with lean ones. The biological value of meat and its health properties for humans are improved by rosemary extract **[28]**, iodine compounds **[29]**, protein-wheat texture **[30]**, organic nitrite based on chard powder with the bacterial culture **[31]**, sea salt and natural dye betanin and beet juice **[32]**, a sourdough starter based on a combination of *Staphylococcus carnosus, L. plantarum, L. rhamnosus,* and *L. paracasei* (SC 2) **[33]**, a complex food supplement based on animal and plant raw materials in an amount of 0.5 to 1.5% in the dry form **[34]**, and fine grinding **[35]**. According to our data, there is a tendency for a correlation between the area of the "muscle eye" and the conformation of carcasses, which is slightly correlated with the development of fat and its thickness, since fatter carcasses are visually assessed as meatier. A study **[36]** also found a strong positive correlation between conformation score, carcass weight, and percentage of collapsed muscle tissue.

An inverse correlation exists between the area of the "muscle eye" and marbling (inclusions of adipose tissue in muscle bundles). The marbling of beef is the main factor determining its good sensory characteristics **[39]**. No factor has a more favourable effect on beef's flavour than marbling **[40]**. Cuts of meat with greater marbling have a better flavour. Since the area of the "muscle eye" directly correlates with the quantitative characteristics of beef, it can be effectively used in assessing the quality of cattle carcasses. The USDA **[5]**, EUROP **[2]**, JMGA **[3]**, and MSA **[6]** methods, it is supplemented by the severity of meat marbling. The composition of beef is influenced by the breed **[41]**, the content of intramuscular fat and its fatty acid composition, the sex and age of the animal at the time of slaughter, and the feeding and housing systems.

There is an inverse correlation between the area of the "muscle eye" and the thickness of the subcutaneous fat. The subcutaneous fat's thickness affects beef's quality by protecting the muscles from drying out in the cold storage room during carcass cooling **[42]**. To preserve carcasses, the quantity and quality of the fat should be optimal. Thus, in 24-month-old bulls of British and British crossbreds, it was found **[43]** that a uniform thickness of adipose tissue at the level of 6.0 mm is the standard of carcass and meat product quality for consumers, providing adequate beef yield with high protein content and the amount of edible muscle tissue with low-fat content.

There is an inverse correlation between the area of the "muscle cell" and the colour of the muscle tissue. With an increase in the area of the "muscle eye", the colour of the muscle tissue was more saturated. This significantly impacts consumer choice **[44]**, as the colour of meat is used **[45]** as an indicator of its freshness and healthiness. The higher colour saturation of beef is caused by feeding cattle on pastures, and its discolouration is caused by fattening on concentrated feed **[46]**.

A tendency to a straightforward, non-significant relationship between the area of the "muscle eye" of *M. longissimus dorsi* and the colour of adipose tissue was found, which was explained **[47]** by the significant content of green fodder, silage, hay, and haylage, which are rich in carotene and relatively low in concentrated fodder in the diet of bulls. The subcutaneous fat of cattle fed on concentrated feed without green feed was yellow **[48]**.

Thus, the sign of carcass quality is the area of the "muscle eye" in crossbred bulls from Ukrainian Black-and-White dairy cows and Holstein bulls aged 20 to 22 months positively correlates with pre-slaughter and slaughter weight, the content of muscle tissue in the carcass, including the highest and first grades, the amount of adipose tissue and tendons and ligaments in the carcass. There was no correlation between the cross-sectional area of *M. longissimus dorsi* and the bone content in the carcasses. Determining the correlation between the area of the "muscle eye" and the quality characteristics of carcasses evaluated by international standards, a negative correlation was found with the development of subcutaneous fat and marbling of muscle tissue. There is a tendency for both a weak inverse correlation in bulls aged 20 to 22 months and a straightforward correlation between the area of the "muscle eye" of *M. longissimus dorsi* and the thickness of the subcutaneous fat, conformation, and colour of muscle tissue. This indicates that in bulls aged 20 to 22 months, the growth rate of the longest muscle does not depend on the carcasses' meatiness, the subcutaneous fat's thickness, and the beef's colour. Comparison of our results with the data from the literature on the correlations between the area of the "muscle eye" of *M. longissimus dorsi* and signs of slaughter and the quality properties of carcasses of animals of different breeds show that in most cases they coincide. This indicates that it is possible to use the area of the "muscle eye" to predict the composition of beef carcasses.

The results of the ultrasound examination on live animals and the determination of the "muscle eye" area on the carcass were used to verify the accuracy of its assessment. According to the recommendations of ICAR **[37]**, the difference between the scan results and the carcass assessment shall be minimal, and the correlation coefficients between them shall be at least 0.8. Our studies on bulls show that the assessment of the area of the "muscle eye" by ultrasound during life is a reliable criterion with high repeatability after slaughter. According to our data, the average difference between the prediction of the muscle eye area using ultrasound and the post-

slaughter assessment is 0.5 cm^2 at 21 months. The correlation coefficients between the two methods of determining the area of the "muscle eye" are 0.973. This suggests the possibility of using the ultrasound method to predict the composition of carcasses during the life of animals and determine their suitability for slaughter. In studies by other authors **[49]**, it was also found that ultrasound scans performed immediately before slaughter are also more effective in predicting the subcutaneous fat depot, including intramuscular fat.

Thus, with the same carcass weight and adipose tissue content, an increase in the cross-sectional area of the longissimus indicates an increase in the yield of muscle tissue in cuts, including the highest and first grades, and a greater number of steaks for which the consumer pays the highest price when selling. These are the attributes that slaughterhouse producers and processing companies are focused on. Consumers are interested in beef's nutritional value and sensory characteristics. Therefore, it is necessary to determine the relationship between the quality characteristics of beef and the area of the "muscle eye" in cattle of the main breeds of Ukraine.

Prospects for further scientific research are to study the relationship between the area of the "muscle eye" and the characteristics of beef that affect consumer demand, in particular, sensory characteristics of meat, safety indicators and dietary properties for a balanced diet. Studies on the correlation between the area of the "muscle eye" of *M. longissimus dorsi* and quantitative and qualitative features of beef shall be tested in samples from other cattle breeds common in Ukraine and carcasses of other categories, depending on the sex and age of the animals. It is also necessary to study the possibility of integrating the established correlations between the area of the "muscle eye" and quantitative and qualitative features of beef into the management methods of cattle breeding, which will allow us to obtain carcasses with the desired characteristics.

CONCLUSION

The development of the area of the "muscle eye" in the carcasses of crossbred bulls from Ukrainian Black-and-White dairy cows and Holstein bulls at the age of 20 to 22 months can predict the content of only quantitative features - carcass weight, number of cuts of the highest and first grade, content of adipose tissue and tendons and ligaments, but not qualitative features - sensory and physical characteristics and chemical composition of meat. A straightforward correlation exists between the area of the "muscle eye" and pre-slaughter live weight ($r = 0.612$; $p > 0.999$), carcass weight ($r = 0.598$; $p > 0.999$), the amount of muscle tissue ($r = 0.498$; $p > 0.99$), including the highest ($r = 0.745$; $p > 0.999$) and first grade ($r = 0.662$; $p > 0.99$), the content of adipose tissue in the carcass ($r=0.491$; $p > 0.99$). There is a tendency for an inverse correlation between the area of the "muscle eye" and the amount of second-grade muscle tissue ($r = -0.303$), the thickness of the subcutaneous fat ($r = -0.125$), marbling ($r = -0.340$), and the colour of the muscle tissue ($r = -0.309$). The area of the "muscle eye" correlates inversely with the development of subcutaneous fat ($r = -0.389$; $p > 0.95$), and directly with the number of tendons and ligaments ($r = 0.435$; $p > 0.95$). In the future, studies shall be conducted to determine the correlation between the area of the "muscle eye" of *M. longissimus dorsi* and quantitative and qualitative features of beef and management factors for growing and fattening animals of other cattle breeds common in Ukraine to establish a compromise between the quality features of carcasses and beef.

REFERENCES

1. DSTU 4673:2006. Cattle for slaughter. General specifications. Quality management systems – Requirements.
2. Commission Regulation (EC). (2008). Commission Regulation (EC) No 1249/2008 of 10 December 2008 laying down detailed rules on the implementation of the Community scales for the classification of beef, pig and sheep carcasses and the reporting of prices thereof.
3. JMGA. (2000). Beef carcass grading standard. Japan Meat Grading Association. Tokyo, Japan.
4. Ministry for Food, Agriculture, Forestry, and Fisheries (MFAFF). (2007). Processing standard for meat products act, Grading, fabrication, and cutting of beef carcass. Seoul, Korea: Ministry for Food, Agriculture, Forest and Fisheries. P. 82.
5. USDA. (2001). United States Standards for Grades of Feeder Cattle.
6. MSA. (2015). Meat Standards Australia.
7. Christensen, M., Ertbjerg, P., Failla, S., Sañudo, C., Richardson, R. I., Nute, G. R., Olleta, J. L., Panea, B., Albertí, P., Juárez, M., Hocquette, J. F., & Williams, J. L. (2011). Relationship between collagen characteristics, lipid content and raw and cooked texture of meat from young bulls of fifteen European breeds. In Meat Science (Vol. 87, Issue 1, pp. 61–65). Elsevier. https://doi.org/10.1016/j.meatsci.2010.09.003
8. Ugnivenko, A., Getya, A., Nosevych, D., Antoniuk, T., Kruk, O., Slobodyanyuk, N., Ivaniuta, A., Omelian, A., Gryshchenko, S., & Israelian V. (2022). The study of "muscle eye" in bulls of Ukrainian black-spotted dairy-meat breed as a factor in improving the properties of meat products. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16., pp. 519–529). HACCP Consulting. https://doi.org/10.5219/1762

9. Pimentel-Concepción, M., Jaborek, J. R., Schweihofer, J. P., Garmyn, A. J., McKendree, M. G. S., Bradford, B. J., Hentschl, A., & Buskirk, D. D. (2024). Growth performance, carcass traits, and feeder calf value of beef × Holstein and Holstein feedlot steers. In Applied Animal Science (Vol. 40, Issue 1, pp. 56–68). Elsevier. https://doi.org/10.15232/aas.2023-02454
10. Bhuiyan, M. S. A., Kim, H. J., Lee, S. H., Cho, S. H., Yang, B. S., Kim, S. D., & Lee, S. H. (2017). Genetic parameters of carcass and meat quality traits in different muscles (longissimus dorsi and semimembranosus) of Hanwoo (Korean cattle). In Journal of Animal Science (Vol. 95., Issue 8., P. 3359–3369). American Society of Animal Science. https://doi.org/10.2527/jas.2017.1493
11. Zheplinska, M., Mushtruk, M., Shablii, L., Shynkaruk, V., Slobodyanyuk, N., Rudyk, Y., Chumachenko, I., Marchyshyna, Y., Omelian, A., & Kharsika, I. (2022). Development and shelf-life assessment of soft-drink with honey. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 114–126). HACCP Consulting. https://doi.org/10.5219/1738
12. Council Regulation (EC) No. 1099/2009. On the protection of animals at the time of death.
13. Order No. 290 of 06 August 2004. Rules of pre-slaughter veterinary inspection of animals and veterinary-sanitary examination of meat and meat products.
14. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/1711
15. Chi, N. T. K., Hue, P. T., Hanh, T. Q., & Ngoan, L. D. (2023). Carcass characteristics and meat quality of crossbred (Brahman× Lai Sind) and (Red Angus× Lai Sind) bulls kept in small-scale farms. In Online Journal of Animal and Feed Research (Vol. 13, Issue 3, pp. 153–161). Scienceline. https://doi.org/10.51227/ojafr.2023.24
16. Pogorzelska–Przybyłe, P., Nogalski, Z., & Wielgosz–Groth, Z. (2014). Prediction of the carcass value of young Holstein-Friesian bulls based on live body measurements. In Annals of Animal Science (Vol. 14, Issue 2, pp. 429–439). Sciendo. https://doi.org/10.2478/aoas-2014-0004
17. Batorska, M., Wiecek, J., Kunowska-Slosarz, M., Puppel, K., Slosarz, J., Golebiewski, M., & Balcerak, M. (2018). The effect of carcass weight on chemical characteristics and fatty acid composition of Longissimus dorsi and Semimembranosus muscles of European wild boar (Sus scrofa scrofa) meat. In Canadian Journal of Animal Science (Vol. 98, Issue 3, P. 557–564). Canadian Science Publishing. https://doi.org/10.1139/cjas-2017-0090
18. Vinay, V., Piña, C., Peraza-Mercado, G., & Hernández, B. (2014). Using ultrasound for the estimation of carcass characteristics in crossbreed (Bos taurus/Bos indicus) bulls. In Research Opinions in Animal and Veterinary Sciences (Vol. 4, Issue 12, pp. 639–643). CABI. http://www.roavs.com/pdf-files/Issue-12-2014/639-643.pdf
19. Oler, A., Głowinska, B., & Młynek, K. (2015). Slaughter and carcass characteristics, chemical composition and physical properties of longissimus lumborum muscle of heifers as related to marbling class. In Archives Animal Breeding (Vol. 58, Issue 1, pp. 145–150). FBN. https://doi.org/10.5194/aab-58-145-2015
20. Kruk, O., Ugnivenko, A., Antoniuk, T., Kolisnyk, O., Nosevych, D., Drachuk, I., Kolesnikova, O., Zhurenko, V., Shtonda, O., & Vakulenko, V. (2023). Quality of bull beef of the Ukrainian black and white dairy breed in dependence on the development of subcutaneous adipose tissue. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 997–1008). HACCP Consulting. https://doi.org/10.5219/1917
21. Ugnivenko, A., Kos, N., Nosevych, D., Mushtruk, M., Slobodyanyuk, N., Zasukha, Y., Otchenashko, V., Chumachenko, I., Gryshchenko, S., & Snizhko, O. (2022). The yield of adipose tissue and by-products in the course of the slaughter of inbred and outbred bulls of the Ukrainian beef breed. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 307–319). HACCP Consulting. https://doi.org/10.5219/1758.
22. Nogalski, Z., Wronski, M., Wielgosz-Groth, Z., Purwin, C., Sobczuk-Szul, M., Mochol, M., & Pogorzelska-Przybyłek, P. (2013). The effect of carcass conformation class (EUROP system) on the slaughter quality of young crossbred beef bulls and Holstein-Friesians. In Annals of Animal Science (Vol. 13, Issue 1, pp. 121–131). Sciendo. https://doi.org/10.2478/v10220-012-0064-9
23. Aalhus, J. L., Janz, J. A. M., Tong, A. K. W., Jones, S. D. M., & Robertson, W. M. (2001). The influence of chilling rate and fat cover on beef quality. In Canadian Journal of Animal Science (Vol. 81, Issue 3, pp. 321–330). Canadian Science Publishing. https://doi.org/10.4141/A00-084
24. Ugnivenko, A., Kruk, O., Nosevych, D., Antoniuk, T., Kryzhova, Y., Gruntovskyi, M., Prokopenko, N., Yemtcev, V., Kharsika, I., & Nesterenko, N. (2023). The expressiveness of meat forms of cattle depending on the content of adipose tissue under the skin and between the muscles. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 997–1008). HACCP Consulting. https://doi.org/10.5219/1869

25. Li, X., Fu, X., Yang, G., & Du, M. (2020). Enhancing intramuscular fat development via targeting fibro-adipogenic progenitor cells in meat animals. In Animal (Vol. 14, Issue 2, pp. 312 – 321). Elsevier. https://doi.org/10.1017/S175173111900209X
26. Mushtruk, M., Mushtruk, N., Slobodyanyuk, N., Vasyliv, V., & Zheplinska, M. (2024). Enhanced energy independence: converting animal fat into biodiesel. In International Journal of Environmental Studies (Vol. 81, Issue 1, pp. 1–11). Taylor & Francis. https://doi.org/10.1080/00207233.2024.2314860
27. Riabovol, M. V., & Bal-Prylypko, L. V. (2021). Justification and development of sausage technology with health properties. In Animal Science and Food Technology (Vol. 12, Issue 1, pp. 39–47). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.01.039
28. Nikolaenko, M. S. (2021). Optimization of the technology of production of functional semi-finished meat products covered in pastry. In Animal Science and Food Technology (Vol. 12, Issue 1, pp. 19–27). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal/2021.01.019
29. Filin, S., Bal-Prylypko, L., Nikolaenko, M., Holembovska, N., & Kushnir, Yu. (2023). Development of technology for plant-based minced semi-finished products. In Animal Science and Food Technology (Vol. 14, Issue 2, pp. 100–112). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.2.2023.100
30. Kryzhova, Yu., Slobodenyuk, N., & Moskalenko, I. (2023). Application of modern technologies to improve the quality of sausage products. In Animal Science and Food Technology (Vol. 14, Issue 1, pp. 49–64). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.1.2023.49
31. Bal-Prylypko, L., Nikolaenko, M., Kanishchev, O., Beyko, L., & Holembovska, N. (2023). Improving the technology for the production of raw dried beef products. In Animal Science and Food Technology (Vol. 14, Issue 4, pp. 26–39). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.4.2023.26
32. Bal-Prylypko, L., Danylenko, S., Mykhailova, O., Nedorizanyuk, L., Bovkun, A., Slobodyanyuk, N., Omelian, A., & Ivaniuta, A. (2024). Influence ofstarter cultures on microbiological and physical-chemical parameters of dry-cured products. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 18, pp. 313–330). HACCP Consulting. https://doi.org/10.5219/1960
33. Bober, A., Liashenko, M., Protsenko, L., Slobodyanyuk, N., Matseiko, L., Yashchuk, N., Gunko, S., & Mushtruk, M. (2020). Biochemical composition of the hops and quality of the finished beer. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 307–317). HACCP Consulting. https://doi.org/10.5219/1311
34. Zheplinska, M., Mushtruk, M., Vasyliv, V., Sarana, V., Gudzenko, M., Slobodyanyuk, N., Kuts, A., Tkachenko, S., & Mukoid, R. (2021). The influence of cavitation effects on the purification processes of beet sugar production juices. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 18–25). HACCP Consulting. https://doi.org/10.5219/1494
35. Muizniece, I., & Kairisa, D. (2021). Slaughter Results, Meat Chemical Composition and pH of Aberdinangus, Hereford and Limousin bulls. In Rural Sustainability Research (Vol. 46, Issue 341, pp. 13–21). Sciendo. https://doi.org/10.2478/plua-2021-0013
36. Kelava Ugarković, N., Kućar, J., Prpić, Z., Ivanković, A., & Konjačić, M. (2024). The rib-eye area in cattle determined by different methods. In Zbornik sažetaka 59. hrvatskog i 19. međunarodnog Simpozija agronoma (pp. 181–181). CroRIS.
37. Heggli, A., Alvseike, O., Bjerke, F., Gangsei, L. E., Kongsro, J., Røe, M., & Vinje, H. (2023). Carcase grading reflects the variation in beef yield – a multivariate method for exploring the relationship between beef yield and carcase traits. In Animal (Vol. 17, Issue 6, pp. 100854–100863). Elsevier BV. https://doi.org/10.1016/j.animal.2023.100854
38. Sakowski, T., Grodkowski, G., Gołębiewski, M., Slósarz, J., Kostusiak, P., Solarczyk, P., & Puppel, K. (2022). Genetic and environmental determinants of beef quality – A Review. In Frontiers in Veterinary Science (Vol. 9, pp. 819605–819613). Frontiers Media S.A. https://doi.org/10.3389/fvets.2022.819605
39. O'Quinn, T. G., Legako, J. F., Woerner, D. R., Kerth, C. R., Nair, M. N., Brooks, J. C., Lancaster J. M., & Miller, R. K. (2024). A current review of US beef flavor II: Managing beef flavor. In Meat Science (Vol. 209, p. 109403). Elsevier BV. https://doi.org/10.1016/j.meatsci.2023.109403
40. Hoa, V. B., Song, D. H., Seol, K. H., Kang, S. M., Kim, H. W., Bae, I. S., Kim, E. S., Park, Y. S., & Cho, S. H. (2023). A Comparative Study on the Carcass and Meat Chemical Composition, and Lipid-Metabolism-Related Gene Expression in Korean Hanwoo and Brindle Chikso Cattle. In Current Issues in Molecular Biology (Vol. 45, Issue 4, pp. 3279–3290). MDPI. https://doi.org/10.3390/cimb45040214
41. Malheiros, J. M., Balsassini, W. A., Dias, V. A. D., Silva, J. A. I. V., Curi, R. A., & Chardulo, L. A. L. (2015). Chemical and sensory meat characteristics of Nellore cattle (Bos indicus) finished with different

levels of backfat thickness in the longissimus thoracic muscle. In Boletim de Indústria Animal (Vol. 72, Issue 4, pp. 341–348). Biblioteca Virtual em Medicina Veterinária e Zootecnia. https://doi.org/10.17523/bia.v72n4p341

42. Boito, B., Kuss, F., Menezes, L. F. G. D., Lisbinski, E., Paris, M. D., & Cullmann, J. R. (2017). Influence of subcutaneous fat thickness on the carcass characteristics and meat quality of beef cattle. In Ciência Rural (Vol. 48, Issue 01, p. e20170333). SciELO. https://doi.org/10.1590/0103-8478cr20170333

43. Hughes, J., Clarke, F., Li, Y., Purslow, P., & Warner, R. (2019). Differences in light scattering between pale and dark beef longissimus thoracis muscles are primarily caused by differences in the myofilament lattice, myofibril and muscle fibre transverse spacings. In Meat Science (Vol. 149, pp. 96–106). Elsevier BV. https://doi.org/10.1016/j.meatsci.2018.11.006

44. Liu, T., Wu, J-P., Lei, Z-M., Zhang, M., Gong, X-Y., Cheng, S-R., Liang, Y., & Wang, J-F. (2020). Fatty Acid Profile of Muscles from Crossbred Angus-Simmental, Wagyu-Simmental, and Chinese Simmental Cattles. In Food Science of Animal Resources (Vol. 40, Issue 4, pp. 563–577). Korean Society for Food Science of Animal Resources. https://doi.org/10.5851/kosfa.2020.e33

45. Salim, A. P. A. A., Ferrari, R. G., Monteiro, M. L. G., & Mano, S. B. (2022). Effect of different feeding systems on color of longissimus muscle from Bos cattle: A systematic review and meta-analysis. In Meat Science (Vol. 192, p. 108871). Elsevier BV. https://doi.org/10.1016/j.meatsci.2022.108871

46. Kruk, O., & Ugnivenko, A. (2024). Relationship between the chemical composition, physical, technological, and sensory properties of beef and the colour of muscle tissue. In Animal Science and Food Technology (Vol. 15, Issue 1, pp. 42–54). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.1.2024.42

47. Clinquart, A., Ellies-Oury, M. P., Hocquette, J. F., Guillier, L., Santé-Lhoutellier, V., & Prache, S. (2022). On-farm and processing factors affecting bovine carcass and meat quality. In Animal (Vol. 16, Supplement 1, pp. 100426–100438). Elsevier BV. https://doi.org/10.1016/j.animal.2021.100426

48. Randhawa, I. A. S., McGowan, M. R., Porto-Neto, L. R., Hayes, B. J., & Lyons, R. E. (2021). Comparison of Genetic Merit for Weight and Meat Traits between the Polled and Horned Cattle in Multiple Beef Breeds. In Animals (Vol. 11, Issue 3, p. 870). MDPI. https://doi.org/10.3390/ani11030870

49. Beriain, M. J., Insausti, K., Valera, M., Indurain, G., Purroy, A., Carr, T. R., & Horcada, A. (2021). Effectiveness of using ultrasound readings to predict carcass traits and sensory quality in young bulls. In Computers and Electronics in Agriculture (Vol. 183, pp. 106060). Elsevier BV. https://doi.org/10.1016/j.compag.2021.106060

Funds:

The study was conducted within the framework of the academic research work “To substantiate systems of production and monitoring of safe animal husbandry products” funded by the state budget of Ukraine (state registration number: 0121U110192).

Acknowledgements:

We would like to thank you to Dr. for Anatolii Ugnivenko.

Conflict of Interest:

The authors have no conflicts of interest.

Ethical Statement:

According to Protocol No. 10 of 18.04.2020 at the meeting of the Ethics Commission of the Faculty of Livestock Raising and Water Bioresources, National University of Life and Environmental Sciences of Ukraine, Act No. 3 and 4 were signed during the experimental research, i.e. in the process of the slaughter of cattle "all the

Contact Address:

Olha Kruk, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Milk and Meat Production Technologies, Heroiv Oborony St., 15, 03041, Kyiv, Ukraine,
E-mail: olgakruk2016@ukr.net
ORCID: https://orcid.org/0000-0001-9975-8994

Anatolii Ugnivenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Milk and Meat Production Technologies, Heroiv Oborony St., 15, 03041, Kyiv, Ukraine,
E-mail:ugnivenko@nubip.edu.ua
ORCID: https://orcid.org/0000-0001-6278-8399

Tetiana Antoniuk, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Milk and Meat Production Technologies, Heroiv Oborony St., 15, 03041, Kyiv, Ukraine,
E-mail: antoniuk_t@nubip.edu.ua
ORCID: https://orcid.org/0000-0001-5045-5546

Oleksandr Kolisnyk, Agrofirm Svitanok, Marksa K. St., 11, 63209, Kharkiv region, Novovodolaz district, Novoselivka village, Ukraine,
E-mail: agro_svitanok@ukr.net
ORCID: https://orcid.org/0000-0002-0602-3575

*__Nataliia Slobodyanyuk__, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products Department of technology of meat, fish and marine products, Polkovnyka Potiekhina Str., 16, 03040, Kyiv, Ukraine,
E-mail: slob2210@ukr.net
ORCID: https://orcid.org/0000-0002-7724-2919

Dmytro Nosevych, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Milk and Meat Production Technologies, Heroiv Oborony St., 15, 03041, Kyiv, Ukraine,
E-mail: dknosevvich@i.ua
ORCID: https://orcid.org/0000-0003-2495-2084

Tatyana Naumenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technologies and Quality Management of Products of Agricultural Products, Department of Standardization and Certifying of Agricultural Products, Heroiv Oborony Str. 15, 03041, Kyiv, Ukraine,
E-mail: tetianarozbytska@nubip.edu.ua
ORCID: https://orcid.org/0000-0003-0098-927X

Mykola Gruntkovskyi, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Technologies in Poultry, Pig and Sheep Breeding, Heroiv Oborony str., 15, Kyiv, 03041, Ukraine,
E-mail: kolya_gr26@ukr.net
ORCID: https://orcid.org/0000-0002-6969-2987

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 2.12.2023
Revised: 10.5.2024
Accepted: 2.6.2024
Published: 26.6.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 633-653
https://doi.org/10.5219/1942
ISSN: 1337-0960 online
www.potravinarstvo.com

Development and quality cum nutritional assessment based on physical properties for corn extruded snacks enriched with protein and carbohydrates: A remedy to malnutrition for society

Muzammal Shafiq, Muhammad Waseem, Yaqoob Majeed, Muhammad Arslan Khalid, Tayyaba Nadeem, Maksim Rebezov, Mars Khairullin, Orynzhamal Sarsembenova

ABSTRACT

Malnutrition is one of the century's most pressing challenges. If malnutrition is not addressed early, people may suffer from non-communicable diseases. A proper, nutritious diet is necessary to overcome diseases like malnutrition. The technology like extrusion can develop rich fortified food products by retaining high nutrition content. In this study, extrusion technology was used to develop protein and carbohydrate snacks with proper nutrition based on the physical properties of commodities by combining different ratios of corn grits, apple pomace, and mung beans. The objectives of the study focused on the development of a food product based on engineering aspects and the quality cum nutritional evaluation of the finished product that can be used as a diet to combat malnutrition. Physical properties like color, rehydration ratio, porosity, bulk density, water solubility index, texture, sensory evaluation, hardness, and crispiness were studied in detail. The energy content of protein and carbohydrates was measured for nutritional assessment using Food Data Central as a standard, provided by the United States of America Department of Agriculture. Results reveal significant variations among treatments, with the addition of apple pomace impacting bulk density, water solubility index, and color attributes. Mung bean supplementation demonstrates a direct correlation with increased hardness and influences porosity. The rehydration ratio is positively affected by apple pomace. Sensory evaluation underscores the substantial impact on color, texture, crispiness, taste, and overall acceptability, providing valuable insights for snack formulation.

Keywords: extrusion, corn extruded snacks, malnutrition, extrudates, nutrient-enriched snacks

INTRODUCTION

The human diet has evolved from raw eating to processed foods **[1]**. Lack of access to healthy food results in one common and alarming condition called malnutrition. Among the young population, 22.1% suffer from mental disorders due to a lack of proper nutrients in the diet **[2]**. Proper nutrition intake helps decrease the effects of different diseases and prevent associated risks **[3]**. The body cannot regulate its functioning if nutrients are not supplied through a proper diet **[4]**. For adults, healthy food includes at least 400 g of fruits and vegetables, less than 10% intake of free sugar, and 30% intake of fats in a whole diet per day **[5]**. Protein, vitamins, carbohydrates, fats, minerals, and water are 6 essential nutrients. All dietary carbohydrates, and insoluble dietary fiber are considered as non-essential nutrients.

Malnutrition is a nutrition disorder that refers to deficits, excess, or imbalanced nutritional intake in a person's diet **[6]**. In both undernutrition and overnutrition, protein deficiency occurs along with other essential

micronutrients such as iron, vitamins B6, B12, C, D, and calcium **[7]**. Malnutrition is a global concern. In developed countries, the excess intake of diet deficit in protein leads to obesity, whereas, developing countries are struggling with widespread micronutrient deficiencies that cause problems of maternal undernutrition, child stunting, and wasting **[8]**. The main causes of malnutrition in developing countries are due to poverty, low income, large family size, poor hygiene conditions, and food insecurity **[9]**. These bases of malnutrition result in stunted adults and increased risks of NCDs with the ultimate result of increased morbidity and mortality **[10]**. Essential nutrients are inevitable in the diet to overcome malnutrition. Among six, two are the most important i.e., protein and carbohydrates.

Protein is an essential macronutrient for the prevention of malnutrition. The deficiency of protein leads to acute malnutrition, a condition that specifically occurs due to low protein consumption. Jelliffe replaced the term "acute malnutrition" with "protein-calorie malnutrition" in 1959 **[11]**. European adults are at risk of protein-calorie malnutrition among 23% of the total population **[12]** and the United States of America adults have 2-3% of poor indication according to the Healthy Eating Index (HEI) **[13]**. Protein calorie malnutrition is the cause of one-third of mortalities among children under 5 years and those who survive that disease are at risk of intellectual or cognitive impairment **[14]**. Infants are at high risk of malnutrition due to low consumption of essential nutrients. For newborn children, 5g of protein intake per day is required to overcome the nutrient deficiency **[15]**. For older adults, 35 g of a healthy protein meal is necessary per day to prevent the risk of protein-calorie malnutrition **[16]**.

Carbohydrates are the second most vital essential macronutrient for the proper body growth. Since the start of the 21st century, low intake of carbohydrates in the diet has been trending for weight loss in adults. This low intake results in poor growth and improper functioning of organs which makes it a high risk of different diseases including malnutrition. The amount of energy that carbohydrate has in foods is usually 4 kcal/g **[17]**. To overcome the carbohydrate deficiency <10% i.e., a 20-25 g/day diet is necessary for infants, 130 g/day for toddlers, and 250-300 g/day for adults **[18]**. Carbohydrates that have plant-based origin consist of fibers. The risk of certain diseases like cancer, obesity, and diabetes can be reduced by consuming a fiber-rich diet **[19]**. Highly rich fiber diets are good for health, such as grains, nuts, fruits, and vegetables because their effects are associated with a lower incidence of various diseases. Fiber can be used (women with 9.6 g/day and men with 10.3 g/day) in various functional foods, such as baked goods, beverages, and meat **[20]**.

The rush in economic development around the globe is a major concern for food security which relates to malnutrition, as for processed food, the waste is increasing due to the use of destructive technologies implemented during operating procedures. This waste directly relates to food security **[21]**. The growth of the world's population is an alarming concern. To increase the production of foods, researchers and scientists are working to introduce new varieties of different foods to overcome the issues of food insecurity. To ensure food security, reliable and inexpensive sources are needed, which will help to reduce malnutrition risks. The food commodities enriched with protein, carbohydrates, and other essential nutrients readily available for people can be processed to get proper nutritional value. The lack of complete nutritional values from a single food source prompted researchers around the globe to explore different processing techniques to produce nutrient-rich products. To fulfill that purpose, it is inevitable to process a combination of more than one nutritious food commodity to get a healthy, balanced, and complete nutrient-enriched diet. Numerous technologies have the potential for the processing of foods to improve nutritional density, nutrient bioavailability, food safety, and storage stability. Processed food can be prepared by using these technologies, like roasting, fermentation, germination, grinding, spraying, baking, drying, and extrusion **[22]**. Extrusion is a productive procedure in the sense of remedy for malnutrition. Many food-derived by-products contain potential nutrient content such as pomace, oil seed, grits, peels, etc. which can be processed for value-addition purposes. Extrusion technology has the high potential to utilize these by-products' nutrients and get nutrient-rich extruded snacks **[23]**. Extrusion is a standard food manufacturing method that involves mixing, shaping, texturing, and heating to create a unique food product. It is a modern food processing technology used to create various snacks and supplemental meals. It can help to efficiently utilize the by-products (i.e., corn grits and apple pomace, etc.) to convert them into highly nutritious snacks that have a high melting temperature, enhanced physical appearance, reduced bulk density, and crispiness **[24]**. Using maximum screw speed during extrusion operation for apple pomace results in high bulk density and starch degradation, lower expansion ratio, porosity, and moisture **[25]**. The (HTST) high-temperature short-time extrusion process made it novel from other technologies as it ensures product safety with maximum nutrient retention **[26]**. Extrusion technology's flexibility enables the creation of nutritionally dense extruded foods using diverse raw materials and serves as a mechanism for value addition. Extruded commodities have a low moisture content, a longer shelf life, and resistance to microbial activity. Additionally, many methods exist to create value-added and fortified extruded products by combining various raw ingredients **[27]**. It's a food-processing technology that employs a single or more screws to shove raw & mixed food ingredients through a narrow opening. Extrusion cooking in HTST, combined with pressure, temperature, and shear force to obtain the starch and protein from the raw products, is

used widely. This technology's adaptability allows the manufacturer of rich fortified foods and value-added products to use various low-cost raw ingredients. Nutritional food is unavailable for everyone by using expensive raw materials with irrelevant and old-fashioned processing operations and technologies. Extrusion is just a very flexible device procedure that can be placed on several delicacy steps. The product's versatility along with high quality with new food productivity and low processing time allows us to meet the demand for enriched products for remedy against malnutrition. Loss of nutritional quality occurs at unstable temperatures; therefore, technology like extrusion, which operates at high temperatures, is used for this research. Food processed from the extruders is rich in dietary fibers and antioxidants **[28]**.

Carbohydrate and protein-enriched snacks prepared with different commodities like chickpeas and sour gum lack nutritional value. Researchers have prepared these snacks and are being used just as a general diet **[29]**. Extruded snacks evaluated on a physical basis like adhesiveness and factorability are suggested by researchers for improvements **[30]**. In other previous literature, Zhang and Liu **[31]** consider only the temperature and moisture content as decisive factors for the physical properties of snacks. Cueto et al. **[32]** consider expansion rate and density for physical attributes of snacks, and Jozinović et al. **[33]** state expansion ratio, bulk density, and water solubility index solely as parameters. The use of nutrients according to body need which has the potential to overcome malnutrition based on engineering cum nutritional assessment, has not been addressed yet. The primary goal of this study is to develop snack products by combining food commodities in specific ratios using extrusion technology by keeping in mind the physical properties and making a comprehensive quality assessment that has proper nutritional value, which will help communities overcome malnutrition by consuming it with low processing cost and by using raw material that is readily available with high protein and other nutrients. These prepared snacks have been evaluated based on daily energy requirements in a diet compared to food data central as standard, provided by the United States Department of Agriculture. The essential nutrient availability and energy content with a specific ratio of commodities give a solution to malnutrition. Our research focused on the apparent ratio mix expertise of perfect diet ingredients to use them effectively to develop a healthy product. The main objective of our research was to develop corn-based extrudates, fortified with fibre cum carbohydrate and proteins, and to evaluate the quality characteristics and energy content of prepared extrudates that have a standard nutritional value which will help children to meet the demand of nutrition to get rid of malnutrition.

Scientific Hypothesis

Adding supplements, apple pomace, and mung bean significantly enhances the physical properties and increases the nutritional value of the engineered food product. Using different ratios of supplements, we expect to get the best of these two parameters, i.e., physical properties and nutritional value.

MATERIAL AND METHODOLOGY

Samples

The research was performed at the extrusion laboratory of the Department of Food Engineering, University of Agriculture, Faisalabad. Apple pomace, mung bean, and corn grits were used as raw commodities because of their high nutritional values and easy availability in commercial markets worldwide. Combining these three commodities with the best nutritional value can potentially be used as a remedy for malnutrition.

Apple pomace: Apple (*Malus Sylvestris*) belongs to the *Rosacea* family and is the fourth highest produced in the fruits market globally after red grapes and bananas **[34]**. It contains a balanced ratio of phytochemical soluble and insoluble solids **[35]**. Juicing operations are done to obtain pomaces from the apples. Apple pomace is a nutritious food ingredient **[36]**. It contains protein, fat, ash, and phenolic content **[37]**. Pomace from apples is the main by-product of juice extraction. It contains 6.8% cellulose, 5.3% proteins, 0.38% ash, 3.6% sugars, and 0.42% acid **[38]**. Total carbohydrate is 44.5-57.4%, Simple carbohydrates such as fructose and glucose make 44% and 18.1-18.3% mass of pomace respectively. Total fiber, insoluble fiber, and soluble fiber constitute 4.4-47%, 33.8-60%, and 13.5-14.6% respectively **[39]**.

Mung bean: Beans are a good source of protein. Worldwide, it is called green gram and golden gram. Its seeds contain 24.3% protein and 0.67% fats **[40]**. Mung bean (*Vigna radiate* (L.)) belongs to legumes, been processed for more than 2,000 years in the world **[41]**. Amino acid composition in mung bean protein isolate has the highest efficiency of (73.25%) i.e., 0.5 g/ml, pH 9.0 **[42]**. Mung beans comprise protein, fiber, minerals, vitamins, and biologically active compounds and are considered functional food **[43]**. The macronutrient composition of mung bean consists of moisture 9.80 (g/100g), crude protein 23.8 (g/100g dm), crude lipid 1.22 (g/100g dm), crude fiber 4.57 (g/100g dm), ash 3.51(g/100g dm), carbohydrate 61.0 (g/100g dm), energy 344 (Kcal/100g dm) **[44]**.

Corn grits: Corn grits are the fundamental snacks that help them gain specific size, shape, and texture after extrusion. Corn grits are endosperm particles **[45]** low in fiber content and have about 1% oil **[46]**. Corn grits are

inevitable for snack extrudates and fortified with other nutrients that can meet the demand of the nutritional status of the body **[33]**. The nutritional value of corn comprises 9% protein, 0.37% potassium, 0.29% phosphorus, 0.11% magnesium, 50% iron, and 21% zinc **[47]**.

The potential availability of nutrients in these readily available commodities made it possible to use them as inexpensive snack products to fulfill the nutritional needs of children.

Chemicals

All chemical reagents were of analytical grade and utilized as per described standards. Glass Beads: Used as a displacement moderator for volumetric displacement methodology (diameter of 1 mm) En1423 Origin China.

Animals, Plants and Biological Materials

Apple (*Malus Sylvestris*) has 6.8% cellulose, 5.3% proteins, 0.38% ash, 3.6% sugars, and 0.42% acid. Mung bean (*Vigna radiate* (L.)) with moisture 9.80 (g/100g), crude protein 23.8 (g/100g dm), crude lipid 1.22 (g/100g dm), crude fiber 4.57 (g/100g dm). Corn grits with 9% protein, 0.37% potassium, 0.29% phosphorus, and 0.11% magnesium.

Instruments

Lab scale twin screw extruder having a 3 mm diameter of die with 102 rpm was used to get extrudate by using different concentrations of apple pomace, mung bean, and corn grits. The steady pumping mechanism in the twin screw extruder comprises to generate high die pressure by using a motor having 2.5 kW power. A mixed sample with a specific mixing ratio (Table 1) was placed into an extruder hopper, which passed to the barrel that cooked the raw material under pressure and shear at high temperature, The mass was forced through a die and cut into individual pieces of particular shapes and collected into food grade stainless steel bin. After extrusion processing, extrudates were cooled at 20 to 22 ℃ (room temperature) and sealed into bags for further analysis. The chosen ratios (as outlined in Table 1) were designed to investigate the impact of varying proportions of apple pomace and mung bean on extruded snacks' physical and sensory attributes. The aim was to cover a spectrum that included formulations with no supplementation (T1), different levels of apple pomace enrichment (T2 and T3), various concentrations of mung bean (T4 and T5), and combinations of both supplements (T6, T7, and T8). This comprehensive approach allows for a nuanced understanding of how these specific ingredients and their concentrations contribute to the overall characteristics of the extrudates, providing valuable insights for potential applications in the development of nutritious and appealing snack products.

Laboratory Methods

Apples were purchased from the market in August and September 2020 when they were firm, mature, and fully grown and stored at 4 ℃ until processed. Mung bean and corn grits were stored at room temperature in a clean environment. Mung bean and Corn grits were cleaned to remove dust, straws, small stones, and any other contaminated materials. After cleaning Mung bean and corn grits were ground to obtain fine flour and packed in sealed polythene bags (Figure 1)for further analysis. Apples were placed in the stainless-steel plates after washing and rested for 30 minutes for surface drying. The simple knife was used to cut each apple into six equal pieces, then processed in a lab juicer to collect apple pomace as described below (Figure 2). A hot air oven was used for drying apple pomace at 70 ℃ for 940 minutes until complete drying. The powder was prepared by crushing and mixing the mung beans (Figure 3) and dry matter of apple pomace. To get uniform size, powder was passed through a sieve of 80 mesh sizes. The rationale behind the drying temperature and duration, as well as the grinding parameters, lies in preserving the nutritional content of the raw commodities. The selected parameters were optimized to achieve thorough drying without compromising the quality of the apple pomace and to attain a fine and uniform powder from mung beans and corn grits. Standardized procedures, including sieving, further contributed to the homogeneity of the final powder product.

Figure 1 Packing of corn grits, apple pomace, and mung bean powders respectively.

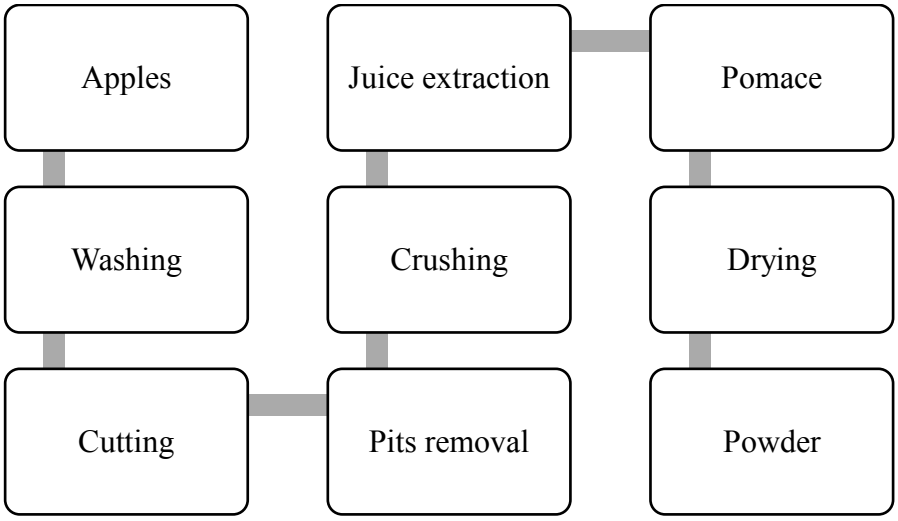


Figure 2 Process flow chart for extracting powder from apples.

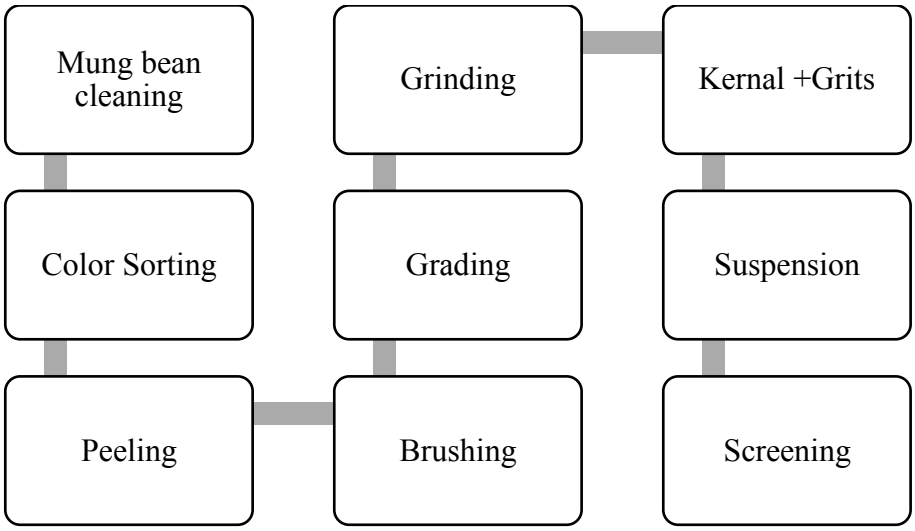


Figure 3 Process flow chart for extracting powder from mung beans.

Description of the Experiment

Sample preparation: A mixed sample with a specific mixing ratio (Table 1) was placed into an extruder hopper which passed to the barrel that cooked the raw material under pressure and shear at high temperature, then mass was forced through a die, and cut into individual pieces of particular shapes and collected into food grade stainless steel bin. After extrusion processing, extrudates were cooled at 20 to 22 °C (room temperature) and sealed into bags for further analysis.

Number of samples analyzed: 8.

Number of repeated analyses: 3.

Number of experiment replication: 3.

Design of the experiment:

Table 1 Mixing ratios of mung bean, apple pomace, and corn grits.

Treatment	Mung bean (%)	Apple pomace (%)	Corn grits (%)
T_1	0	0	100
T_2	0	10	90
T_3	0	20	80
T_4	10	0	90
T_5	20	0	80
T_6	15	5	80
T_7	5	15	80
T_8	10	10	80

Color: The color results were determined in accordance with the methodology mentioned by Lara. et al., (2011) with a colorimeter (Color Test-II Neuhaus Neotec). Tone principles as "L*" (lightness), "a*" (–a greenness; +a inflammation), and "b*" (–b blueness; +b yellowness) were recorded by puffed treats. The outcome results were used to determine hue directions and chroma **[48]**. The white tile control was used under constant light conditions to tape record the specifications i.e., (L* 97.46, a* 0.02, b* 1.72).

Bulk Density: The volumetric displacement methodology outlined by **[49]** was used to determine the bulk density (g/cm^3) of extrudates. Glass beans were utilized as displacement moderators through a diameter of 1 mm. Extrudate density was calculated as (1):

$$\text{Bulk density} = \frac{W_{ex}(\text{g})}{\text{Vc}\ (cm^3)\times(1-\frac{W_{gbd}(g)}{W_{gb}(g)})} \quad (1)$$

Rehydration Ratio: RR (rehydration ratio) was determined following a treatment explained by **[50]**. The extrudate was sliced into 30-mm lengths (M1) with an average weight of 18g and placed in 500 ml of liquid at 30 °C for 10 to 15 minutes. The water was evaporated, and the hydrated trials were weighed (M2). RR was defined as follows (2):

$$\text{Rehydration ratio} = \frac{M2-M1}{M1} \times 100 \quad (2)$$

Porosity: Bulk and apparent volume are used to calculate the porosity of extrudates using the methodology defined by **[51]** by the following equation (3):

$$\text{Porosity} = \frac{\text{Bulk volume} - \text{Apparent volume}}{\text{Bulk volume}} \quad (3)$$

Where:

B.V. = (1/ρb) and A.V. = (1/ρs).

Texture: Extrudate texture was determined by the method outlined by **[52]** the structure analyzer (Mod. TA-XT2 steady Microsystems, Surrey, UK). Extrudates were tested using the Texture Expert Program Version 1.21. A three-aim fold ridge is used to make texture assessments. Extrudates were twisted to determine textural characteristics. For curve examination, products were positioned in the center of a heavy-weight dish design. Before each test, the cellular strain and probe were both calibrated. Fold analysis was used to describe items'

hardness and fracture potential by displaying energy (grams) vs range (mm). The weight (grams) was used for flex examination to test firmness and fracture ability. A texture analyzer was used to measure hardness and crispiness (TA-XT Plus, Stable Microsystems, UK) through the method described by **[28]**.

Water Solubility Index: Extrudate powder was sieved to ensure a uniform size distribution of 1.5 g and suspended in 10 ml of water for 15 minutes at room temperature with constant stirring, then centrifuged for 15 minutes at 3000 rpm. For uniform size distribution, extrudates were crushed to powder and sieved using a 60-mesh size. Crushed powder (2.5 g) was suspended in 25 ml water for 30 minutes at room temperature, with intermediate stirring, and then centrifuged for 15 minutes at 3000 rpm. To obtain dry solids, the supernatant was poured into a vanishing dish and water was dissipated until a steady weight was achieved. The WSI measured the amount of dry solids in the supernatant and expressed it as a percentage.

Sensory Evaluation: The method of sensory evaluation of extrudates was used as described by **[53]**. In a sensory evaluation laboratory of food engineering, a group of ten judges (students and faculty) evaluated the extrudates for color, crispiness, taste, texture, and overall acceptability on a 9-point hedonic scale **[54]** ranging from 1 to 9. Panelists washed their mouths with water before testing each sample. The faculty members are well trained and have certifications regarding quality and sensory evaluation from the Government of Pakistan under the organization Punjab Food Authority. The students selected in the panel are particular alumnus of the very institute and working in FMCG industries. The use of a 9-point hedonic scale is a well-established method in sensory science, allowing for a nuanced and detailed assessment of consumer preferences. The 9-point scale offers a higher resolution compared to scales with fewer response points, enabling more precise discrimination between product attributes and facilitating a more comprehensive understanding of consumer preferences.

Statistical Analysis

The obtained triplicate data was subjected to the Tukey test **[55]** to get variance analysis of mean values for each parameter to check the level of significance among different treatments, statistical analysis was performed using Statistics 8.1 (USA). The choice of the Tukey test for statistical analysis in this study was driven by its suitability for multiple comparisons, specifically designed to identify significant differences between treatment means efficiently. Given that the study involves multiple treatments with various concentrations of apple pomace, mung bean, and corn grits, the Tukey test provides a robust approach to compare the means of all treatments while controlling the experiment-wise error rate. Its ability to handle multiple pairwise comparisons without inflating the overall Type I error rate makes it well-suited for exploring differences in physical and sensory attributes across the diverse set of treatments. The assurance of normality and homoscedasticity in the data, crucial assumptions for the application of parametric statistical tests like Tukey, typically involves preliminary analyses.

RESULTS AND DISCUSSION

The objective of the current research work was to prepare fiber- and protein-enriched extrudates using the physical properties of commodities, their quality, and nutritional assessment. To increase the protein contents, mung beans were used, while apple pomace was used as a dietary fiber-rich source. Prepared extrudates were analyzed for color, rehydration ratio, and porosity. These extrudates were also analyzed for bulk density, water solubility index, texture, and sensory assessment. The results are presented and discussed as under.

Physical properties analysis of extruded snacks

The physical properties of fortified snacks were evaluated, including color, bulk density, rehydration ratio, porosity, texture (hardness), and water solubility index. As shown in the tables, the mean results of the treatments indicated significant differences among treatments.

The eight treatments comprise the following ratios in grams. T1 extrudate was prepared with no supplementation, T2 has 10g/100g apple pomace, T3 has 20g/100g, T4 has 10g/100g mung beans, T5 has 20g/100g mung beans, T6 has 5g/100g apple pomace and 15g/100g mung beans, T7 has 15g/100g apple pomace and 5g/100g mung beans, T8 has 10g/100g apple pomace and 10g/100 mung beans.

Bulk density: Table 2 shows the mean values for bulk density of extruded snacks enriched with apple pomace and mung bean. The results for bulk density revealed that extruded snacks with treatment T1 recorded the lowest value of bulk density i.e., 0.033. At the same time, T3 showed the highest bulk density value at 0.143. The fortified snacks T2, T4, T5, T6, T7, and T8 were observed with bulk density values of 0.063, 0.053, 0.043, 0.073, 0.083, and 0.093 respectively. The statistical analysis showed a significant difference among treatments. The bulk density of extrudates increased significantly due to adding apple pomace. The observations are supported by the findings of **[56]** those who reported that the bulk density of extrudates increases with the addition of grape pomace **[32]** suggesting no change in bulk density with the addition of supplement whereas the addition of two

supplements shows significance in treatment 3. An increase in bulk density is an indicator of an increase in weight which directly enhances the texture and nutrition of snacks.

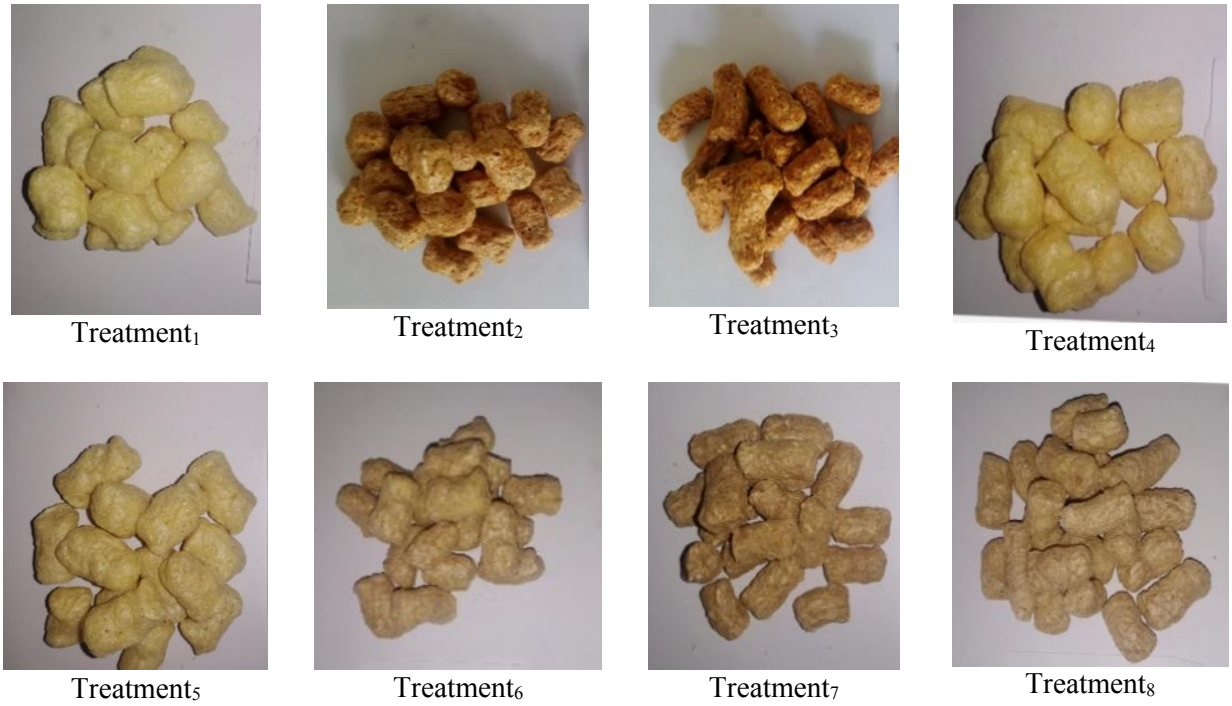


Figure 4 Extruded snacks with all eight treatments.

Water solubility index of extrudates: Table 2 shows the mean values for the water solubility index (WSI) of extruded snacks enriched with apple pomace and mung bean. The results for WSI revealed that extruded snacks with treatment T1 were recorded with a maximum value of 48. Meanwhile, the snacks with treatment T7 showed a minimum value of 37 for WSI. The fortified snacks T2, T3, T4, T5, T6, and T8 were observed with water solubility indexes of 46, 47, 46, 42, 47, and 46 respectively. The statistical analysis showed the T3, T6 and T4, T8 are non-significant. The water solubility index drops as apple pomace and mung bean concentrations rise. The Stickiness of extruded items is proportional to the water solubility index. Therefore, snacks without any supplements have a high stickiness. As a result, T1 has the highest water solubility index. These observations were also found by **[57]**.

Texture of snacks (Hardness): Table 2 shows the mean values for extruded snacks enriched with apple pomace and mung bean texture. The results revealed that extruded snacks with treatment T2 had a minimum hardness value of 2.4. Meanwhile, the snacks with treatment T5 recorded the maximum hardness value, which is 5.87. The fortified snacks having treatments T1, T3, T4, T6, T7, and T8 were recorded with hardness values of 2.87, 3.68, 3.83, 4.13, 2.84, and 3.26 respectively. The statistical analysis showed that T2, T5, and T6 are significant. These findings revealed that when the percentage of mung bean in a snack increased, the hardness of the snack increased. The addition of supplements is directly proportional to the texture of snacks. These results correspond to that of **[58]**, who reported that as the percentage of grape pomace in snacks increased, the hardness of the snacks increased.

Porosity: Table 2 shows the mean values for porosity of extruded snacks enriched with apple pomace and mung bean. The results revealed that extruded snacks with treatment T3 were observed to have the highest porosity value of 0.80. Meanwhile, the snacks that were treated with T1 showed the lowest porosity value, 0.56. The fortified snacks T2, T4, T5, T6, T7, and T8 were observed with porosity values of 0.76, 0.58, 0.6, 0.70, 0.66, and 0.72 respectively. The statistical analysis showed that T1 and T3 are significant. Porosity increased as the percentage of apple pomace increased. By the addition of supplements, porosity increased which is inversely proportional to the water solubility index, which was decreased by the addition of a supplement. Hence, the decrease of water solubility index and increase in porosity help children easily digest the snacks. These results follow the findings of **[59]**, who stated that porosity increases as grapes pomace increases, whereas **[32]** suggested no change in porosity by increasing supplement.

Rehydration Ratio: Table 2 shows the mean values for extruded snacks enriched with apple pomace and mung bean rehydration ratio. The results revealed that extruded snacks T1 was observed with the lowest value of rehydration ratio of 57.81. Meanwhile, the snacks T2 showed the highest rehydration ratio value, 77.82. The

fortified snacks T2, T4, T5, T6, T7, and T8 were observed with rehydration values of 68.04, 62.05, 70.76, 62.11, 65.23, and 63.39, respectively. The statistical analysis showed a significant difference among treatments, $p < 0.05$.

Color analysis of extruded snack: Color is a key factor in determining whether or not a product will be accepted by consumers. The table revealed the results of a variance of color analysis of snacks, which indicated that color attributes for extruded snacks had a significant impact on the treatments.

L* value: The mean for L* value of extruded snacks as in Table 2 showed the lowest value of 39.81 for the snacks T2. Corn snacks T1 showed the highest value of 62.26. Snacks with treatment T2, T4, T5, T6, T7 and T8 showed 47.79, 56.79, 55.93, 55.97, 46.21 and 48.61 values respectively. As the amount of apple pomace is added, the L* value of extruded snacks decreases.

a* value: The mean for a* value of all treatments is in Table 2. The results showed the lowest value for the snacks prepared without supplementation T1 i.e., 4.01. Corn snacks T3 showed the highest value of 13.69. Snacks T2, T4, T5, T6, T7, and T8 had 13.17, 5.38, 6.39, 10.6, 12.97, and 11.86 values respectively. The statistical analysis showed that T2, T3, and T7 are non-significant. The addition of apple pomace and mung bean increased the a* value of extruded snacks.

b* value: The mean for b* value of all treatments is in Table 2. The results showed the lowest value for the snacks prepared without supplementation T1 i.e., 19.26. Corn snacks T2, T4, T5, T6, T7, and T8 had 22.82, 19.66, 20.06, 21.93, 24.76 and 23.68 values respectively. Snacks with treatment T2 showed the highest value of 28.24. The statistical analysis showed the T3, T8, and T4, T5 are non-significant to each other. The b* value of extruded snacks gradually increased as apple pomace and mung bean were added. The findings of **[56]**, determined that as the amount of grape pomace increased, the L* value decreased and the a* and b* values increased.

Sensory evaluation

For extruded snacks, a nine-point hedonic scale **[54]** was used for evaluating the sensory characteristics i.e. color, texture, crispiness, taste, and overall acceptability.

Color: The color of a product is the first impression that a customer has while deciding whether to acquire it. Mean values for the color of extruded snacks fortified with apple pomace and mung bean are presented in Table 3 which describes how the supplementation of apple pomace and mung bean in extruded snacks has significantly changed the color score of snacks. Corn snacks T1 were liked more as compared to the other snacks with a score of 8 because of their good appearance and similarities with corn snacks commercially available. The color score of other treatments T2, T3, T5, T6, T7, and T8 were 5.5, 5, 7, 6.5, 5.25, and 6 respectively. As the amount of apple pomace increased, the color score decreased. The statistical analysis showed the significance among treatments. This was due to the color of corn snacks fading as the degree of supplementation was increased. A study showed that muffin color decreased with supplementation of apple pomace **[60]**.

Texture: The hardness and softness of extruded snacks are described by the texture parameter. The mean value for the texture of extruded snacks is presented in Table 3 which describes the supplementation of apple pomace and mung bean in extruded snacks has significantly changed the texture score of snacks. The results revealed that snacks T5 were most liked with a score of 8. However, snacks T3 were less liked by the panelists with the lowest value 5. Snack scores of other T1, T2, T4, T6, T7, and T8 were 6.75, 5.53, 7.5, 7, 6, and 6.5 respectively. A study showed that texture likeness decreases in snacks by increasing the apple pomace **[61]**.

Crispiness: Crispiness is described as the crunchiness sensation of snacks. The mean value for the texture of extruded snacks is presented in Table 3 which describes the supplementation of apple pomace and mung bean in extruded snacks has significantly changed the texture score of snacks. The results revealed that snacks T3 were most liked with a score of 8. The lowest value 5 was observed in snacks T1. The mean value of all other treatments T2, T4, T5, T6, T7, T8, are 7.5, 5.5, 6, 5.75, 7, and 6.5 respectively.

Taste: Snack quality and acceptance are strongly influenced by taste. Mean values for taste of extruded snacks are presented in Table 3 which described the supplementation of apple pomace and mung bean in extruded snacks has significantly changed the taste score of snacks. The results revealed that snacks T5 were most liked with a score of 8. Snacks T3 were observed with the lowest value 5. The mean values of all other treatments T1, T2, T4, T6, T7, and T8 were 7, 5.5, 7.5, 6.75, 6, and 6.5 respectively. Increased mung bean supplementation increased the taste of extruded snacks. This could be due to the taste of mung bean, which is popular in Asia.

Overall acceptability: Mean values for taste of extruded snacks are presented in Table 3 which describes the significant relationship between them. The results showed that with treatment T5 was most liked with a score of 8. The lowest value 5 was observed in snack T3. The mean values of all other treatments T1, T2, T4, T6, T7, and T8 are 6.5, 5.5, 7.5, 7, 7.25, and 7.75 respectively **[33]** suggested not to incorporate supplement i.e., spelled flour while extrusion whereas in current study two supplement added to enhance physical as well as nutritional attributes of snacks.

The graphical illustration of the physical properties of snacks (Table 2) and sensory evaluation (Table 3) is shown in Figure 5 and Figure 6 respectively.

Table 2 Effects of treatments on different parameters of physical properties of extruded snacks.

		Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8
Bulk Density		0.03^{h}	0.06^{e}	0.14^{a}	0.05^{f}	0.04^{g}	0.07^{d}	0.08^{c}	0.09^{b}
Water Solubility Index		48.0^{a}	46.0^{c}	47.0^{b}	46.0^{c}	42.0^{d}	47.0^{b}	37.0^{e}	46.0^{c}
Texture		2.87^{de}	2.40^{e}	3.68^{bcd}	3.83^{bc}	5.87^{a}	4.13^{b}	2.84^{de}	3.26^{cde}
Porosity		0.56^{e}	0.76^{ab}	0.80^{a}	0.58^{de}	0.60^{de}	0.70^{bc}	0.66^{cd}	0.72^{abc}
Rehydration Ratio		57.8^{e}	77.8^{a}	68.0^{c}	70.7^{b}	62.0^{d}	62.1^{d}	61.9^{d}	58.3^{e}
Color	**L* Value**	62.2^{a}	47.7^{d}	39.8^{f}	56.7^{b}	55.9^{c}	55.9^{bc}	46.2^{e}	48.6^{d}
	a* Value	4.01^{f}	13.1^{a}	13.6^{a}	5.38^{e}	6.39^{d}	10.6^{c}	12.9^{a}	11.8^{b}
	b* Value	19.26^{e}	28.2^{a}	22.8^{c}	19.6^{e}	20.0^{e}	21.9^{d}	24.7^{b}	23.6^{c}

Note: Mean values having the same alphabetic letters are statistically non-significant (p >0.05).

Table 3 Effects of treatments on different parameters of sensory evaluation.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8
Color	8.00^{a}	5.50^{f}	5.00^{h}	7.50^{b}	7.00^{c}	6.50^{d}	5.25^{g}	6.00^{e}
Texture	6.75^{d}	5.53^{g}	5.00^{h}	7.50^{b}	8.00^{a}	7.00^{c}	6.00^{f}	6.50^{e}
Crispiness	5.00^{h}	7.50^{b}	8.00^{a}	5.50^{g}	6.00^{e}	5.75^{f}	7.00^{c}	6.50^{d}
Taste	7.00^{c}	5.50^{g}	5.00^{h}	7.50^{b}	8.00^{a}	6.75^{d}	6.00^{f}	6.50^{e}
Overall acceptability	6.50^{f}	5.50^{g}	5.00^{h}	7.50^{c}	8.00^{a}	7.00^{e}	7.25^{d}	7.75^{b}

Note: Mean values having the same alphabetic letters are statistically non-significant (p >0.05). T_1 = 100% Corn grits; T_2= 90% corn grits + 10% apple pomace; T_3= 80% corn grits + 20% apple pomace; T_4= 90% corn grits + 10% mung bean; T_5= 80% corn grits + 20% mung bean; T_6= 80% corn grits + 5% apple pomace + 15% mung bean; T_7= 80% corn grits + 15% apple pomace + 5% mung bean; T_8= 80% corn grits + 10% apple pomace + 10% mung bean.

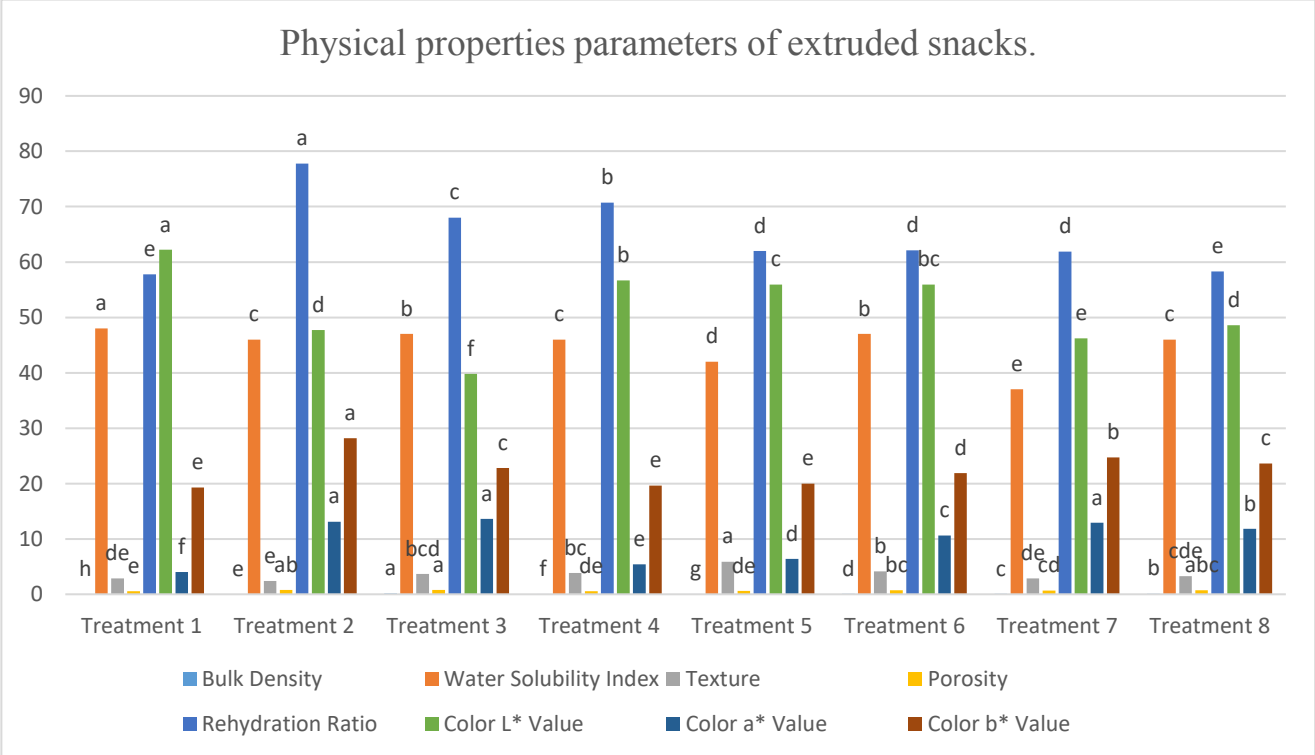


Figure 5 Effects of treatments on different parameters of physical properties of extruded snacks.

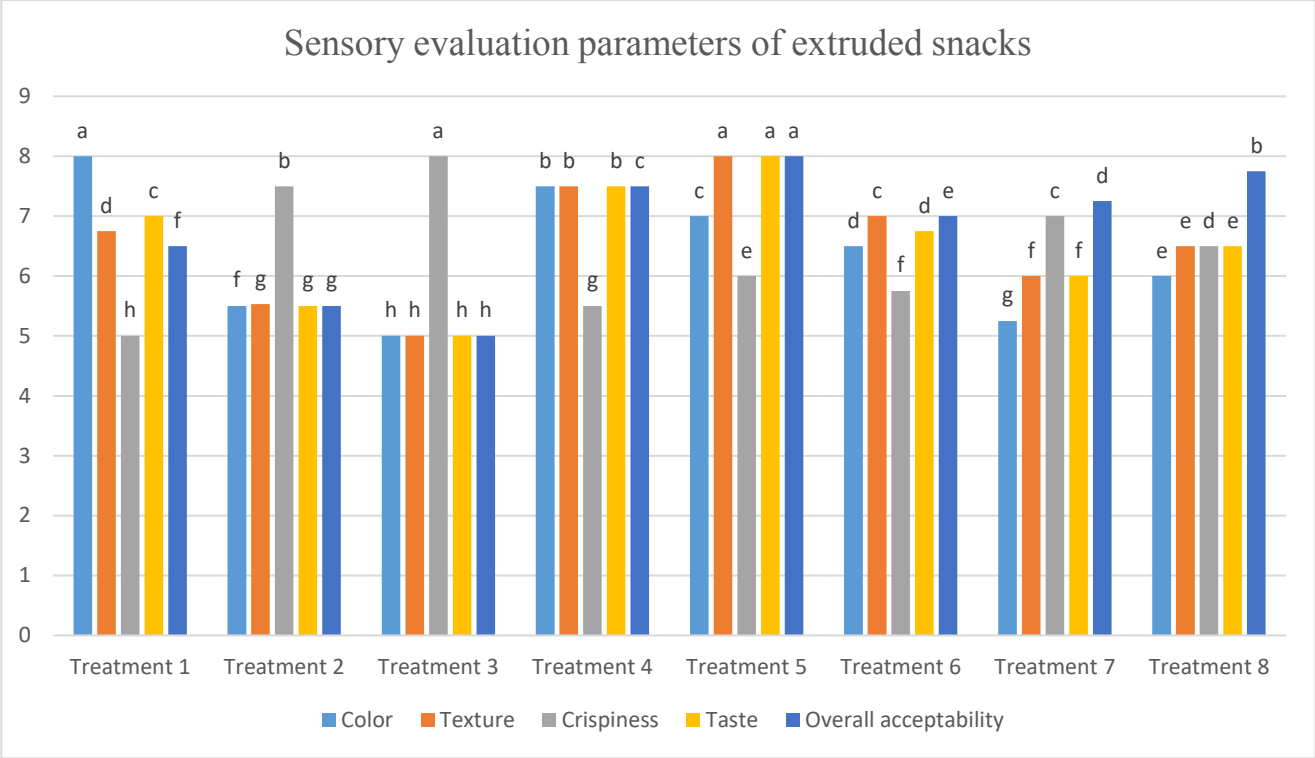


Figure 6 Effects of treatments on different parameters of sensory evaluation.

Energy Content

The energy content was obtained by making food data central as standard (Table 4), provided by United States Department of Agriculture.

Table 4 Energy content of apple pomace, mung bean, and corn grits per 100-gram of protein and carbohydrate as per food data central USA.

	Protein/100g	Carbohydrate/100g	Energy kcal/100g
Apple pomace	0.19	67.72	62.00
Mung Bean	23.90	62.60	347.00
Corn Grits	8.80	79.60	370.00

Note: The eight treatments comprise the following ratios in grams. T_1 extrudate was prepared with no supplementation, T_2 has 10g/100g apple pomace, T_3 has 20g/100g apple pomace, T_4 has 10g/100g mung beans, T_5 has 20g/100g mung beans, T_6 has 5g/100g apple pomace and 15g/100g mung beans, T_7 has 15g/100g apple pomace and 5g/100g mung beans, T_8 has 10g/100g apple pomace and 10g/100 mung beans.

Table 5 Protein and carbohydrate energy content for all treatments.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8
Protein	0	0.019	0.038	2.3	4.6	3.589	1.218	2.40
Carbohydrate	0	6.77	13.54	6.26	12.52	12.77	13.28	13.03
Energy (kcal) /100g	1548	1418.46	1238.27	1538.24	1528.35	1467.95	1349.35	1409.3

The calculated energy content for all treatments is presented in Table 5. The treatment T_1 comprises 1548 kcal/100g of extruded snacks. The energy content of all other treatments T_2, T_3, T_4, T_5, T_6, T_7, & T_8 are 1418.46, 1238.27, 1538.24, 1528.35, 1467.95, 1349.35, 1409.3 kcal/100g respectively.

The discussion section of the analysis on the physical properties of extruded snacks enriched with apple pomace and mung bean offers a nuanced exploration of the findings, their implications, and the broader context within the existing scientific literature. Bulk density is a critical parameter influencing extruded snacks' texture and nutritional aspects. The observed increase in bulk density with the addition of apple pomace aligns with

previous studies **[62]**, **[63]**, **[64]**, **[65]**, **[66]**, **[67]**, **[68]**. Some other researchers found an experiment conducted on the enrichment of apple pomace (AP) in the dough and cookies, as AP contains high fiber content which increases the attributes of cookies like color, texture, and rheological properties **[69]**. Kaur and colleagues suggested that to check the effect of apple pomace on the rheology, microstructure, and texture of yogurt, the gelation pH increased, and the fermentation time decreased of yogurt by adding apple pomace powder **[70]**. In another study, researchers found Sample must ferment till the drying process is completed in other applications of apple pomace **[71]**. Others found dried out and powdered fruit pomace is passed through 30, 50, and 60 interlock sieves to get pomace of differing particle proportions **[72]**. Hossain Brishti suggested that along with dietary fiber, apple pomace has a rich amount of phenolics **[73]**. Nguyen et al. found professional apple pomace as a result of a contemporary fruit juice creation herbal can be viewed as a natural product for immediate planning of nutritional fiber, as it has above 50% TDF **[74]**. Akharume and colleagues found pomace will be the biggest result from fruit juices and cider part. The enhancement in bulk density suggests an increase in weight, directly impacting the texture and nutritional content of the snacks **[75]**. This finding underscores the potential of utilizing fruit pomace, such as apple pomace, as a supplementary ingredient to modify the physical properties of extruded snacks, catering to both sensory preferences and nutritional demands. The water solubility index (WSI) serves as a crucial indicator of stickiness in extruded snacks. The negative correlation between WSI and the concentrations of apple pomace and mung beans is a notable observation. As the supplements increase, the stickiness decreases, highlighting the potential of these supplements to influence the texture and overall quality of extruded snacks. The relationship between stickiness and WSI has practical implications for snack manufacturing, as it allows for the customization of texture based on consumer preferences. Texture, specifically hardness, is a key attribute in determining the overall sensory experience of snacks. The positive correlation between hardness and the percentage of mung beans emphasizes the direct impact of supplements on the textural qualities of extruded snacks. This aligns with similar studies on mung bean pomace **[76]**, **[77]**, **[78]**, **[79]**, **[80]**, **[81]**. Hou and colleagues found mung bean (*Vigna radiata* L.) is a vital heartbeat drink all over the globe, particularly in parts of Asia, and contains an extended reputation of practices as an old-fashioned drug. Delić and researchers found enrichment with pomegranate strips increasing the width, angle, and WVP associated with the mung bean healthy protein movies but reduced her MC and WS **[82]**. Şahin et al. found mung bean seeds had been developed an assortment of times to dissect the effect of germination on the design and physicochemical properties of starch. Understanding the influence of specific supplements on texture is essential for optimizing formulations to meet consumer preferences and nutritional requirements. Porosity, a parameter influencing the digestibility of snacks, showed an increase in the percentage of apple pomace **[83]**.

This is a noteworthy finding, as increased porosity is inversely proportional to WSI. The combination of decreased stickiness (lower WSI) and increased porosity suggests potential benefits for the digestibility of these snacks, particularly for children. The correlation observed supports findings in the literature **[84]**, **[85]**, **[86]**, **[87]**, **[88]**, **[89]**, **[90]** and adds to the growing body of knowledge on the interplay between porosity, WSI, and digestibility in extruded snacks. The rehydration ratio, a critical quality parameter, exhibited significant variations among treatments. The highest rehydration ratio observed in snacks with 10% apple pomace suggests a positive impact of this supplement on the rehydration properties of extruded snacks. This finding has implications for the overall quality of the snacks, particularly in terms of moisture retention during processing and storage. Color analysis is crucial for consumer acceptance, and the L*, a*, and b* values provide insights into the color attributes of the snacks. The decrease in L* value with the addition of apple pomace indicates a fading color. The increase in a* and b* values, on the other hand, suggests changes in hue and intensity. These color changes, though influenced by the supplements, need to be balanced to ensure consumer appeal. The findings align with a study on muffins that reported a decrease in color with apple pomace supplementation **[91]**, **[92]**, **[93]**, **[94]**, **[95]**. Yadav et al. found that fiber-enriched chicken nuggets are manufactured by including grain bran (WB) and dried-out fruit pomace (DAP) each at 3, 6, and 9% **[96]**. Other researchers suggested the role of dietary fiber in providing suffered wellness is learned for a number of many years along with adults there, clearly close research that food diets abundant with high-fiber food items lessen the likelihood of long-term conditions, like CVD and cancers **[97]**. Ötles et al. suggested dietary fiber is a set of food elements and is resilient to digestive minerals and discovered generally in grains, vegetables, and fruits **[98]**. Dahl et al. found fiber is helpful to health insurance and, if eaten in enough amounts **[99]**. Lin et al. found protein is a vital constituent of the system and takes on a broad spectral range of functionality in a system **[100]**. Rodríguez-Miranda et al. suggested the extrusion of combinations of taro flour with nixtamalized flour or with non-nixtamalized flour on a single-screw extruder triggered the creation of food of varied traits **[101]**. In another study, a researcher found after a total examination of the many features for real variables like mass thickness, development proportion, drinking water intake list, and liquid solubility **[102]**. Korkerd et al. unearthed that wealthy sourced elements of healthy protein and fiber from dinners handled by way of merchandise, defatted soybean food, germinated rice that will be brown, and

mango strip dietary fiber, happened to be put into corn determination at 20% (w/w) to give fortified extruded food **[103]**. Sriwattana et al. propose a three-element aggregate test that was utilized to improve the strategy of damaged-rice-based treatment fortified with proteins and dietary fiber. Inside their patent discovered that system for manufacturing fiber-fortified nutrition that features creating prepared the foodstuff and then externally implementing dietary fiber **[104]**. Sharma et al. learned the impact of the mixing stage (zero, 5, 10, 15. and 20%) of corn wheat, defatted germ, and gluten with grain flour in the physical substance **[105]**. Researchers discovered that extruded food comprised cooked from flour combinations created using grain flour, corn flour, and egg albumin powder inside an amount of 35-50: 35-50: 5-30 correspondingly, and dampness changed into modified to 17-20 **[106]**. In another study, researchers recommend fortification of extrusion feed preparations with protein. The sensory evaluation conducted using a nine-point hedonic scale sheds light on the overall acceptability of the extruded snacks. The significant changes in color, texture, crispiness, taste, and overall acceptability across treatments underscore the multifaceted impact of supplementation on the sensory characteristics of snacks. These findings provide valuable information for product development, helping manufacturers tailor formulations to meet consumer expectations. The comprehensive analysis of physical properties and sensory evaluation in this study contributes to the evolving understanding of the impact of apple pomace and mung bean supplementation on extruded snacks. The observed relationships between supplements and various parameters offer practical insights for the food industry, guiding the development of snacks that not only meet nutritional demands but also align with consumer preferences. As the demand for nutritious and appealing snack options continues to rise, research in this domain remains critical for advancing both scientific knowledge and product innovation **[107]**.

Challenges and Future Directions

The comprehensive study on the physical properties, sensory evaluation, and nutritional aspects of extruded snacks enriched with apple pomace and mung bean has provided valuable insights. However, challenges and future directions in this research domain still exist. One challenge lies in further optimizing the formulations to achieve an ideal balance between sensory attributes and nutritional content. Understanding the molecular interactions during the extrusion process, especially with the addition of apple pomace and mung bean, could unveil opportunities for fine-tuning the snack formulations. Additionally, investigating the long-term effects of these fortified snacks on consumer health and understanding the bioavailability of nutrients in the human digestive system could enhance the practical application of the findings. Further studies could focus on the bioactive compounds present in apple pomace and mung bean and their potential health benefits, contributing to the broader understanding of functional foods. Collaborating with experts in food engineering and nutritional science, exploring novel ingredients for fortification, considering sustainability aspects, and developing innovative processing techniques are crucial for addressing malnutrition on a broader scale **[108]**, **[109]**, **[110]**, **[111]**, **[112]**, **[113]**, **[114]**. Moreover, studying the economic feasibility and scalability of the developed snacks for potential commercialization is essential. As technology advances, incorporating data-driven approaches and artificial intelligence in food research may open new avenues for optimizing formulations and predicting consumer preferences. Ultimately, the journey towards addressing malnutrition through fortified snacks requires a multidisciplinary approach, integrating insights from food science, nutrition, and technology to create impactful and sustainable solutions.

CONCLUSION

The research aimed to develop corn-extruded snacks enriched with protein and carbohydrates based on physical properties and quality cum nutritional assessment to overcome malnutrition. To combat malnutrition deficiency mung bean as a protein source was added in extrudates, and apple pomace was used as a dietary fiber source. The quality assessment of snacks was studied deeply, and eight treatments were done on each parameter. Different mixing ratios of ingredients were applied on extrudates, and quality cum sensory parameters were evaluated. The bulk density of the snacks increased with the addition of apple pomace, indicating a higher weight and enhanced texture. The water solubility index decreased as the concentrations of apple pomace and mung bean rose, leading to reduced stickiness in snacks. The hardness of the snacks increased with a higher percentage of mung bean, showing a direct relationship between supplements and texture. Porosity increased with the addition of apple pomace, inversely proportional to the water solubility index, making the snacks easily digestible. The rehydration ratio varied among treatments, with significant differences observed. Additionally, the color analysis highlighted the impact of supplementation on L*, a*, and b* values, influencing the overall acceptance by consumers. The results of the water solubility index showed that extruded snacks prepared without supplementation (T1) were observed with maximum value. The porosity of extruded snacks fortified with apple pomace and mung bean results showed that extruded (T3) was observed with highest value of porosity. Rehydration ratio of extruded snacks fortified with apple pomace and mung bean results showed (T1) was with

lowest value of rehydration ratio. In a nutshell, supplementation with apple pomace and mung bean significantly affected all the parameters measured through testing and sensory evaluation. Treatments showed that the mixing ratio comprises 80% corn grits + 15% apple pomace + 5% mung bean i.e T7 was best among all and has best nutrient content that is adequate, balanced, and effective for malnutrition remedy.

REFERENCES

1. Crittenden, A. N., & Schnorr, S. L. (2017). Current views on hunter-gatherer nutrition and the evolution of the human diet. In American Journal of Physical Anthropology (Vol. 162, Issue S63, pp. 84–109). Wiley. https://doi.org/10.1002/ajpa.23148
2. Wattick, R., Hagedorn, R., & Olfert, M. (2018). Relationship between Diet and Mental Health in a Young Adult Appalachian College Population. In Nutrients (Vol. 10, Issue 8, p. 957). MDPI AG. https://doi.org/10.3390/nu10080957
3. Ohlhorst, S. D., Russell, R., Bier, D., Klurfeld, D. M., Li, Z., Mein, J. R., Milner, J., Ross, A. C., Stover, P., & Konopka, E. (2013). Nutrition research to affect food and a healthy life span1,2. In The Journal of Nutrition (Vol. 143, Issue 8, pp. 1349–1354). Elsevier BV. https://doi.org/10.3945/jn.113.180638
4. Greene, J. M., & McAdam, P. A. (1983). Nutritional Requirements and the Appropriate Use of Supplements. In Nutrition in the Middle and Later Years (pp. 17–45). Elsevier. https://doi.org/10.1016/b978-0-7236-7046-9.50008-4
5. Organization W.H.O. (2003). In Diet, nutrition, and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation (Vol. 916). World Health Organization.
6. Mehta, N. M., Corkins, M. R., Lyman, B., Malone, A., Goday, P. S., Carney, L. (Nieman), Monczka, J. L., Plogsted, S. W., & Schwenk, W. F. (2013). Defining Pediatric Malnutrition. In Journal of Parenteral and Enteral Nutrition (Vol. 37, Issue 4, pp. 460–481). Wiley. https://doi.org/10.1177/0148607113479972
7. Hawkes, C., Ruel, M. T., Salm, L., Sinclair, B., & Branca, F. (2020). Double-duty actions: seizing programme and policy opportunities to address malnutrition in all its forms. In The Lancet (Vol. 395, Issue 10218, pp. 142–155). Elsevier BV. https://doi.org/10.1016/s0140-6736(19)32506-1
8. Majekodunmi, A., Ajiboye, E. A., Odaibo, A. (2011). Single cell proteins: as nutritional enhancer. In Adv. Appl. Sci. Res. (Vol. 2, Issue 5, pp. 396–409). Prime Scholars.
9. Vorster, H. H. (2010). The link between poverty and malnutrition: A South African perspective. In Health SA Gesondheid (Vol. 15, Issue 1). AOSIS. https://doi.org/10.4102/hsag.v15i1.435
10. Vorster, H. H. (2010). The link between poverty and malnutrition: A South African perspective. In Health SA Gesondheid (Vol. 15, Issue 1). AOSIS. https://doi.org/10.4102/hsag.v15i1.435
11. Olsen, E. M., Petersen, J., Skovgaard, A. M., Weile, B., Jorgensen, T., & Wright, C. M. (2006). Failure to thrive: the prevalence and concurrence of anthropometric criteria in a general infant population. In Archives of Disease in Childhood (Vol. 92, Issue 2, pp. 109–114). BMJ. https://doi.org/10.1136/adc.2005.080333
12. Vorster, H. H. (2010). The link between poverty and malnutrition: A South African perspective. In Health SA Gesondheid (Vol. 15, Issue 1). AOSIS. https://doi.org/10.4102/hsag.v15i1.435
13. Hengeveld, L. M., Wijnhoven, H. A., Olthof, M. R., Brouwer, I. A., Harris, T. B., Kritchevsky, S. B., Newman, A. B., & Visser, M. (2018). Prospective associations of poor diet quality with long-term incidence of protein-energy malnutrition in community-dwelling older adults: the Health, Aging, and Body Composition (Health ABC) Study. In The American Journal of Clinical Nutrition (Vol. 107, Issue 2, pp. 155–164). Elsevier BV. https://doi.org/10.1093/ajcn/nqx020
14. Koletzko, B., Bhatia, J., Bhutta, Z. A., Cooper, P., Makrides, M., Uauy, R., Wang, W. (2015). Pediatric nutrition in practice. Karger Medical and Scientific Publishers. https://doi.org/10.1159/isbn.978-3-318-02691-7
15. Hudson, J. A., Charron, E., Maple, B., Krom, M., Heavner-Sullivan, S. F., Mayo, R. M., Dickes, L., & Rennert, L. (2020). Baby-Friendly Hospital Initiative Is Associated with Lower Rates of Neonatal Hyperbilirubinemia. In Breastfeeding Medicine (Vol. 15, Issue 3, pp. 176–182). Mary Ann Liebert Inc. https://doi.org/10.1089/bfm.2019.0220
16. Seemer, J., Volkert, D., Fleckenstein-Sußmann, D., Bader-Mittermaier, S., Sieber, C. C., & Kiesswetter, E. (2021). Usual Protein Intake Amount and Sources of Nursing Home Residents with (Risk of) Malnutrition and Effects of an Individualized Nutritional Intervention: An enable Study. In Nutrients (Vol. 13, Issue 7, p. 2168). MDPI AG. https://doi.org/10.3390/nu13072168
17. Trumbo, P., Schlicker, S., Yates, A. A., & Poos, M. (2002). Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein and Amino Acids. In Journal of the American Dietetic Association (Vol. 102, Issue 11, pp. 1621–1630). Elsevier BV. https://doi.org/10.1016/s0002-8223(02)90346-9

18. Oh, R., Gilani, B., & Uppaluri, K. R. (2019). Low carbohydrate diet. In StatPearls. StatPearls Publishing.
19. Thilagavathi, T., Pandiyan, M., Suganyadevi, M., Sivaji, M., Yuvaraj, M., & Sasmitha, R. (2020). Dietary fibre-health benefits. In Biotica Research Today (Vol. 2, Issue 6, pp. 519–522. Society for Biotic and Environmental Research (SBER) & BIOTICA.
20. Mahmood, M. W., Abraham-Nordling, M., Håkansson, N., Wolk, A., & Hjern, F. (2018). High intake of dietary fibre from fruit and vegetables reduces the risk of hospitalisation for diverticular disease. In European Journal of Nutrition (Vol. 58, Issue 6, pp. 2393–2400). Springer Science and Business Media LLC. https://doi.org/10.1007/s00394-018-1792-0
21. Nations, U. (2015). Transforming our world: The 2030 agenda for sustainable development. Department of Economic and Social Affairs.
22. Bressani, R., Harper, J., & Whickstrom, B. (1984). Processed and packaged weading foods: development, manufacture and marketing. In Improving the nutritional status of children during weaning period (pp. 51–64). Moi University.
23. Leonard, W., Zhang, P., Ying, D., & Fang, Z. (2019). Application of extrusion technology in plant food processing byproducts: An overview. In Comprehensive Reviews in Food Science and Food Safety (Vol. 19, Issue 1, pp. 218–246). Wiley. https://doi.org/10.1111/1541-4337.12514
24. Wang, Y.-Y., & Ryu, G.-H. (2013). Physical properties of extruded corn grits with corn fibre by CO2 injection extrusion. In Journal of Food Engineering (Vol. 116, Issue 1, pp. 14–20). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2012.10.041
25. O'Shea, N., Arendt, E., & Gallagher, E. (2013). Enhancing an Extruded Puffed Snack by Optimising Die Head Temperature, Screw Speed and Apple Pomace Inclusion. In Food and Bioprocess Technology (Vol. 7, Issue 6, pp. 1767–1782). Springer Science and Business Media LLC. https://doi.org/10.1007/s11947-013-1181-x
26. Arêas, J. A. G., Rocha-Olivieri, C. M., & Marques, M. R. (2016). Extrusion Cooking: Chemical and Nutritional Changes. In Encyclopedia of Food and Health (pp. 569–575). Elsevier. https://doi.org/10.1016/b978-0-12-384947-2.00266-x
27. Pathak, N., & Kochhar, A. (2018). Extrusion Technology: Solution to Develop Quality Snacks for Malnourished Generation. In International Journal of Current Microbiology and Applied Sciences (Vol. 7, Issue 1, pp. 1293–1307). Excellent Publishers. https://doi.org/10.20546/ijcmas.2018.701.158
28. de Morais, E. C., Cruz, A. G., & Bolini, H. M. A. (2013). Gluten-free bread: multiple time–intensity analysis, physical characterisation and acceptance test. In International Journal of Food Science & Technology (Vol. 48, Issue 10, pp. 2176–2184). Wiley. https://doi.org/10.1111/ijfs.12202
29. Shah, F., Sharif, M. K., Butt, M. S., & Shahid, M. (2016). Development of protein, dietary fiber, and micronutrient enriched extruded corn snacks. In Journal of Texture Studies (Vol. 48, Issue 3, pp. 221–230). Wiley. https://doi.org/10.1111/jtxs.12231
30. Paula, A. M., & Conti-Silva, A. C. (2014). Texture profile and correlation between sensory and instrumental analyses on extruded snacks. In Journal of Food Engineering (Vol. 121, pp. 9–14). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2013.08.007
31. Zhang, J., Liu, L., Jiang, Y., Faisal, S., & Wang, Q. (2020). A new insight into the high-moisture extrusion process of peanut protein: From the aspect of the orders and amount of energy input. In Journal of Food Engineering (Vol. 264, p. 109668). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2019.07.015
32. Cueto, M., Porras-Saavedra, J., Farroni, A., Alamilla-Beltrán, L., Schöenlechner, R., Schleining, G., & Buera, P. (2015). Physical and mechanical properties of maize extrudates as affected by the addition of chia and quinoa seeds and antioxidants. In Journal of Food Engineering (Vol. 167, pp. 139–146). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2015.07.027
33. Jozinović, A., Šubarić, D., Ačkar, Đ., Babić, J., & Miličević, B. (2016). Influence of spelt flour addition on properties of extruded products based on corn grits. In Journal of Food Engineering (Vol. 172, pp. 31–37). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2015.04.012
34. Asghar, A., Ali, S. M., & Yasmin, A. (2012). Effect of climate change on apple (Malus domestica var. ambri) production: A case study in Kotli Satian, Rawalpindi, Pakistan. In Pakistan Journal of Botany (Vol. 44, Issue 6, pp. 1913–1918). Pakistan Botanical Soc.
35. Eberhardt, M. V., Lee, C. Y., & Liu, R. H. (2000). Antioxidant activity of fresh apples. In Nature (Vol. 405, Issue 6789, pp. 903–904). Springer Science and Business Media LLC. https://doi.org/10.1038/35016151
36. Li, W., Yang, R., Ying, D., Yu, J., Sanguansri, L., & Augustin, M. A. (2020). Analysis of polyphenols in apple pomace: A comparative study of different extraction and hydrolysis procedures. In Industrial Crops and Products (Vol. 147, p. 112250). Elsevier BV. https://doi.org/10.1016/j.indcrop.2020.112250

37. Diñeiro García, Y., Valles, B. S., & Picinelli Lobo, A. (2009). Phenolic and antioxidant composition of by-products from the cider industry: Apple pomace. In Food Chemistry (Vol. 117, Issue 4, pp. 731–738). Elsevier BV. https://doi.org/10.1016/j.foodchem.2009.04.049
38. Shalini, R., & Gupta, D. K. (2010). Utilization of pomace from apple processing industries: a review. In Journal of Food Science and Technology (Vol. 47, Issue 4, pp. 365–371). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-010-0061-x
39. Skinner, R. C., Gigliotti, J. C., Ku, K.-M., & Tou, J. C. (2018). A comprehensive analysis of the composition, health benefits, and safety of apple pomace. In Nutrition Reviews. Oxford University Press (OUP). https://doi.org/10.1093/nutrit/nuy033
40. Rasul, F., Cheema, M., Sattar, A., Saleem, M. F., & Wahid, M. A. (2012). Evaluating the performance of three mungbean varieties grown under varying inter-row spacing. In J. Anim. Plant Sci. (Vol. 22, Issue 4, pp. 1030–1035). Elewa Biosciences Journals.
41. Dahiya, P. K., Linnemann, A. R., Van Boekel, M. A. J. S., Khetarpaul, N., Grewal, R. B., & Nout, M. J. R. (2014). Mung Bean: Technological and Nutritional Potential. In Critical Reviews in Food Science and Nutrition (Vol. 55, Issue 5, pp. 670–688). Informa UK Limited. https://doi.org/10.1080/10408398.2012.671202
42. Du, M., Xie, J., Gong, B., Xu, X., Tang, W., Li, X., Li, C., & Xie, M. (2018). Extraction, physicochemical characteristics and functional properties of Mung bean protein. In Food Hydrocolloids (Vol. 76, pp. 131–140). Elsevier BV. https://doi.org/10.1016/j.foodhyd.2017.01.003
43. Hou, D., Yousaf, L., Xue, Y., Hu, J., Wu, J., Hu, X., Feng, N., & Shen, Q. (2019). Mung Bean (Vigna radiata L.): Bioactive Polyphenols, Polysaccharides, Peptides, and Health Benefits. In Nutrients (Vol. 11, Issue 6, p. 1238). MDPI AG. https://doi.org/10.3390/nu11061238
44. Shahrajabian, M. H., Sun, W., & Cheng, Q. (2019). A short review of health benefits and nutritional values of mung bean in sustainable agriculture. Instytut Uprawy Nawożenia i Gleboznawstwa - Państwowy Instytut Badawczy. https://doi.org/10.26114/PJA.IUNG.381.2019.37.05
45. Serna-Saldivar, S. O., & Perez Carrillo, E. (2019). Food Uses of Whole Corn and Dry-Milled Fractions. In Corn (pp. 435–467). Elsevier. https://doi.org/10.1016/b978-0-12-811971-6.00016-4
46. Serna-Saldivar, S. O. (2004). MAIZE | Foods from Maize. In Encyclopedia of Grain Science (pp. 242–253). Elsevier. https://doi.org/10.1016/b0-12-765490-9/00095-1
47. Loy, D. D., & Lundy, E. L. (2019). Nutritional Properties and Feeding Value of Corn and Its Coproducts. In Corn (pp. 633–659). Elsevier. https://doi.org/10.1016/b978-0-12-811971-6.00023-1
48. Guyot, S., Marnet, N., Sanoner, P., & Drilleau, J.-F. (2003). Variability of the Polyphenolic Composition of Cider Apple (Malus domestica) Fruits and Juices. In Journal of Agricultural and Food Chemistry (Vol. 51, Issue 21, pp. 6240–6247). American Chemical Society (ACS). https://doi.org/10.1021/jf0301798
49. Selani, M. M. (2015). Characterization and application of fruit byproducts in the development of beef burger and corn extruded product. [Doctoral dissertation, Universidade de São Paulo].
50. Yu, J., & Anchordoquy, T. J. (2009). Synergistic effects of surfactants and sugars on lipoplex stability during freeze-drying and rehydration. In Journal of Pharmaceutical Sciences (Vol. 98, Issue 9, pp. 3319–3328). Elsevier BV. https://doi.org/10.1002/jps.21564
51. Rathod, R. P., & Annapure, U. S. (2016). Effect of extrusion process on antinutritional factors and protein and starch digestibility of lentil splits. In LWT - Food Science and Technology (Vol. 66, pp. 114–123). Elsevier BV. https://doi.org/10.1016/j.lwt.2015.10.028
52. Piga, A., Catzeddu, P., Farris, S., Roggio, T., Sanguinetti, A., & Scano, E. (2005). Texture evolution of "Amaretti" cookies during storage. In European Food Research and Technology (Vol. 221, Issues 3–4, pp. 387–391). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-005-1185-5
53. Meilgaard, M. C., Carr, B. T., & Carr, B. T. (2006). Sensory Evaluation Techniques. CRC Press. https://doi.org/10.1201/b16452
54. Peryam, D. R., & Pilgrim, F. J. (1957). Hedonic scale method of measuring food preferences. In Food technology (Vol. 11, pp. 9–14). IFT.
55. Tukey, J. W. (1977). Exploratory data analysis. 2 ed. Addison-Wesley Publishing Company, 688 p.
56. Altan, A., McCarthy, K. L., & Maskan, M. (2008). Twin-screw extrusion of barley–grape pomace blends: Extrudate characteristics and determination of optimum processing conditions. In Journal of Food Engineering (Vol. 89, Issue 1, pp. 24–32). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2008.03.025
57. Hashimoto, J. M., & Grossmann, M. V. E. (2003). Effects of extrusion conditions on quality of cassava bran/cassava starch extrudates. In International Journal of Food Science & Technology (Vol. 38, Issue 5, pp. 511–517). Wiley. https://doi.org/10.1046/j.1365-2621.2003.00700.x

58. Altan, A., McCarthy, K. L., & Maskan, M. (2009). Effect of extrusion process on antioxidant activity, total phenolics and β-glucan content of extrudates developed from barley-fruit and vegetable by-products. In International Journal of Food Science & Technology (Vol. 44, Issue 6, pp. 1263–1271). Wiley. https://doi.org/10.1111/j.1365-2621.2009.01956.x

59. Yağcı, S., & Göğüş, F. (2008). Response surface methodology for evaluation of physical and functional properties of extruded snack foods developed from food-by-products. In Journal of Food Engineering (Vol. 86, Issue 1, pp. 122–132). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2007.09.018

60. Younas, M. B., Rakha, A., Sohail, M., Rashid, S., & Ishtiaq, H. (2015). Physicochemical and sensory assessment of apple pomace enriched muffins. In Pakistan Journal of Food Sciences (Vol. 25, Issue 4, pp. 224–234). Pakistan Society of Food Scientists and Technologists.

61. Sudha, M. L., Baskaran, V., & Leelavathi, K. (2007). Apple pomace as a source of dietary fiber and polyphenols and its effect on the rheological characteristics and cake making. In Food Chemistry (Vol. 104, Issue 2, pp. 686–692). Elsevier BV. https://doi.org/10.1016/j.foodchem.2006.12.016

62. Sahu, C., Patel, S., & Tripathi, A. K. (2022). Effect of extrusion parameters on physical and functional quality of soy protein enriched maize based extruded snack. In Applied Food Research (Vol. 2, Issue 1, p. 100072). Elsevier BV. https://doi.org/10.1016/j.afres.2022.100072

63. Sobowale, S. S., Kewuyemi, Y. O., & Olayanju, A. T. (2021). Process optimization of extrusion variables and effects on some quality and sensory characteristics of extruded snacks from whole pearl millet-based flour. In SN Applied Sciences (Vol. 3, Issue 10). Springer Science and Business Media LLC. https://doi.org/10.1007/s42452-021-04808-w

64. Rani, P., Kumar, A., Purohit, S. R., & Rao, P. S. (2020). Extrusion of fermented rice-black gram flour for development of functional snacks: Characterization, optimization and sensory analysis. In Journal of Food Science and Technology (Vol. 58, Issue 2, pp. 494–509). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-020-04558-4

65. Preethi, P., Mangalassery, S., Shradha, K., Pandiselvam, R., Manikantan, M. R., Reddy, S. V. R., Devi, S. R., & Nayak, M. G. (2021). Cashew apple pomace powder enriched the proximate, mineral, functional and structural properties of cereal based extrudates. In LWT (Vol. 139, p. 110539). Elsevier BV. https://doi.org/10.1016/j.lwt.2020.110539

66. Kloepfer, I. N. (2022). Sensory Characterization and Consumer Acceptance of Corn-Based Extruded Puffed Snacks with Apple or Cranberry Pomace. [Doctoral dissertation, Washington State University].

67. Asif, M., Javaid, T., Razzaq, Z. U., Khan, M. K. I., Maan, A. A., Yousaf, S., Usman, A., & Shahid, S. (2023). Sustainable utilization of apple pomace and its emerging potential for development of functional foods. In Environmental Science and Pollution Research. Springer Science and Business Media LLC. https://doi.org/10.1007/s11356-023-28479-9

68. O'Shea, N., Arendt, E., & Gallagher, E. (2013). Enhancing an Extruded Puffed Snack by Optimising Die Head Temperature, Screw Speed and Apple Pomace Inclusion. In Food and Bioprocess Technology (Vol. 7, Issue 6, pp. 1767–1782). Springer Science and Business Media LLC. https://doi.org/10.1007/s11947-013-1181-x

69. Sharma, C., Singh, B., Hussain, S. Z., & Sharma, S. (2017). Investigation of process and product parameters for physicochemical properties of rice and mung bean (Vigna radiata) flour based extruded snacks. In Journal of Food Science and Technology (Vol. 54, Issue 6, pp. 1711–1720). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-017-2606-8

70. Kaur, J., Singh, B., & Singh, A. (2023). Sorghum–mung bean combination snacks: Effect of extrusion temperature and moisture on chemical, functional, and nutritional characteristics. In Legume Science (Vol. 5, Issue 3). Wiley. https://doi.org/10.1002/leg3.186

71. Brishti, F. H., Chay, S. Y., Muhammad, K., Ismail-Fitry, M. R., Zarei, M., & Saari, N. (2021). Texturized mung bean protein as a sustainable food source: Effects of extrusion on its physical, textural and protein quality. In Innovative Food Science & Emerging Technologies (Vol. 67, p. 102591). Elsevier BV. https://doi.org/10.1016/j.ifset.2020.102591

72. Hossain Brishti, F., Chay, S. Y., Muhammad, K., Rashedi Ismail-Fitry, M., Zarei, M., Karthikeyan, S., Caballero-Briones, F., & Saari, N. (2021). Structural and rheological changes of texturized mung bean protein induced by feed moisture during extrusion. In Food Chemistry (Vol. 344, p. 128643). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.128643

73. Hossain Brishti, F., Chay, S. Y., Muhammad, K., Rashedi Ismail-Fitry, M., Zarei, M., Karthikeyan, S., Caballero-Briones, F., & Saari, N. (2021). Structural and rheological changes of texturized mung bean protein induced by feed moisture during extrusion. In Food Chemistry (Vol. 344, p. 128643). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.128643

74. Nguyen, T. P., Lin, J., & Songsermpong, S. (2023). Influence of feed moisture content on physical and sensory properties of extruded pure mung bean starch vermicelli. In International Journal of Food Science & Technology (Vol. 58, Issue 8, pp. 4205–4214). Wiley. https://doi.org/10.1111/ijfs.16513

75. Akharume, F., Xiong, Y. L., & Adedeji, A. (2019). Effects of Transglutaminase in Product Formulation on Physico-textural Properties of Protein Rich Extruded Snacks. In 2019 Boston, Massachusetts July 7- July 10, 2019. 2019 Boston, Massachusetts July 7- July 10, 2019. American Society of Agricultural and Biological Engineers. https://doi.org/10.13031/aim.201900306

76. Azzollini, D., Derossi, A., Fogliano, V., Lakemond, C. M. M., & Severini, C. (2018). Effects of formulation and process conditions on microstructure, texture and digestibility of extruded insect-riched snacks. In Innovative Food Science & Emerging Technologies (Vol. 45, pp. 344–353). Elsevier BV. https://doi.org/10.1016/j.ifset.2017.11.017

77. Lisiecka, K., Wójtowicz, A., Samborska, K., Mitrus, M., Oniszczuk, T., Combrzyński, M., Soja, J., Lewko, P., Kasprzak Drozd, K., & Oniszczuk, A. (2023). Structure and Texture Characteristics of Novel Snacks Expanded by Various Methods. In Materials (Vol. 16, Issue 4, p. 1541). MDPI AG. https://doi.org/10.3390/ma16041541

78. Gomes, K. S., Berwian, G. F., Tiepo, C. B. V., & Colla, L. M. (2023). Development and evaluation of extruded protein snacks added. In Food Science and Technology (Vol. 43). Zeppelini Editorial e Comunicacao. https://doi.org/10.5327/fst.1123

79. Kaur, J., Singh, B., Singh, A., Sharma, S., & Kidwai, M. K. (2023). Effect of extrusion processing on techno-functional properties, textural properties, antioxidant activities, in vitro nutrient digestibility and glycemic index of sorghum–chickpea-based extruded snacks. In Journal of Texture Studies (Vol. 54, Issue 5, pp. 706–719). Wiley. https://doi.org/10.1111/jtxs.12760

80. Eftekhariyazdi, M., Zenoozian, M. S., Milani, E., Rad, A. H. E., & Armin, M. (2023). Effect of extrusion parameters on physical, microstructural and functional quality of lentil-quinoa extruded snack enriched with pumpkin by stepwise response surface methodology. Research Square Platform LLC. https://doi.org/10.21203/rs.3.rs-2962834/v1

81. Kaur, J., Singh, B., Singh, A., Sharma, S., & Kidwai, M. K. (2023). Effect of extrusion processing on techno-functional properties, textural properties, antioxidant activities, in vitro nutrient digestibility and glycemic index of sorghum–chickpea-based extruded snacks. In Journal of Texture Studies (Vol. 54, Issue 5, pp. 706–719). Wiley. https://doi.org/10.1111/jtxs.12760

82. Delić, J., Ikonić, P., Jokanović, M., Peulić, T., Ikonić, B., Banjac, V., Vidosavljević, S., Stojkov, V., & Hadnađev, M. (2023). Sustainable snack products: Impact of protein- and fiber-rich ingredients addition on nutritive, textural, physical, pasting and color properties of extrudates. In Innovative Food Science & Emerging Technologies (Vol. 87, p. 103419). Elsevier BV. https://doi.org/10.1016/j.ifset.2023.103419

83. Şahin, N., Bilgiçli, N., & Sayaslan, A. (2023). Improvement of Functional and Nutritional Properties of Extruded Snacks with the Utilization of Red Dog Flour. In Brazilian Archives of Biology and Technology (Vol. 66). FapUNIFESP (SciELO). https://doi.org/10.1590/1678-4324-2023220180

84. Kantrong, H., Klongdee, S., Jantapirak, S., Limsangouan, N., & Pengpinit, W. (2021). Effects of extrusion temperature and puffing technique on physical and functional properties of purpled third-generation snack after heat treatment. In Journal of Food Science and Technology (Vol. 59, Issue 6, pp. 2209–2219). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-021-05234-x

85. Obradović, V., Babić, J., Dragović-Uzelac, V., Jozinović, A., Ačkar, Đ., & Šubarić, D. (2021). Properties of Extruded Snacks Prepared from Corn and Carrot Powder with Ascorbic Acid Addition. In Processes (Vol. 9, Issue 8, p. 1367). MDPI AG. https://doi.org/10.3390/pr9081367

86. Félix-Medina, J. V., Montes-Ávila, J., Reyes-Moreno, C., Perales-Sánchez, J. X. K., Gómez-Favela, M. A., Aguilar-Palazuelos, E., & Gutiérrez-Dorado, R. (2020). Second-generation snacks with high nutritional and antioxidant value produced by an optimized extrusion process from corn/common bean flours mixtures. In LWT (Vol. 124, p. 109172). Elsevier BV. https://doi.org/10.1016/j.lwt.2020.109172

87. Waseem, M., Majeed, Y., Nadeem, T., Naqvi, L. H., Khalid, M. A., Sajjad, M. M., Sultan, M., Khan, M. U., Khayrullin, M., Shariati, M. A., & Lorenzo, J. M. (2023). Conventional and advanced extraction methods of some bioactive compounds with health benefits of food and plant waste: A comprehensive review. In Food Frontiers (Vol. 4, Issue 4, pp. 1681–1701). Wiley. https://doi.org/10.1002/fft2.296

88. Waseem, M., & Majeed, Y. (2023). Cell-based food needs collaborative efforts for safe production and suitable consumption. In Food Safety and Health (Vol. 1, Issue 1, pp. 6–8). Wiley. https://doi.org/10.1002/fsh3.12009

89. Waseem, M., Majeed, Y., Nadeem, T., Naqvi, L. H., Khalid, M. A., Shafiq, M., & Sajjad, M. M. (2022). Climate Change, Flood Disaster, and Food Insecurity in Pakistan. Research Square Platform LLC. https://doi.org/10.21203/rs.3.rs-2284023/v1
90. Waseem, M., Tahir, A. U., & Majeed, Y. (2023). Printing the future of food: The physics perspective on 3D food printing. In Food Physics (Vol. 1, p. 100003). Elsevier BV. https://doi.org/10.1016/j.foodp.2023.100003
91. Waseem, M., Khan, M. U., Majeed, Y., Ntsefong, G. N., Kirichenko, I., Klopova, A., Trushov, P., & Lodygin, A. (2023). Seaweed-based films for sustainable food packaging: properties, incorporation of essential oils, applications, and future directions. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 899–917). HACCP Consulting. https://doi.org/10.5219/1908
92. Majeed, Y., & Waseem, M. (2022). Postharvest Handling Systems. In Encyclopedia of Smart Agriculture Technologies (pp. 1–9). Springer International Publishing. https://doi.org/10.1007/978-3-030-89123-7_125-1
93. Majeed, Y., Khan, M. U., Waseem, M., Zahid, U., Mahmood, F., Majeed, F., Sultan, M., & Raza, A. (2023). Renewable energy as an alternative source for energy management in agriculture. In Energy Reports (Vol. 10, pp. 344–359). Elsevier BV. https://doi.org/10.1016/j.egyr.2023.06.032
94. Kantrong, H., Klongdee, S., Jantapirak, S., Limsangouan, N., & Pengpinit, W. (2021). Effects of extrusion temperature and puffing technique on physical and functional properties of purpled third-generation snack after heat treatment. In Journal of Food Science and Technology (Vol. 59, Issue 6, pp. 2209–2219). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-021-05234-
95. Obradović, V., Babić, J., Dragović-Uzelac, V., Jozinović, A., Ačkar, Đ., & Šubarić, D. (2021). Properties of Extruded Snacks Prepared from Corn and Carrot Powder with Ascorbic Acid Addition. In Processes (Vol. 9, Issue 8, p. 1367). MDPI AG. https://doi.org/10.3390/pr9081367
96. Yadav, U., Singh, R. R. B., & Arora, S. (2018). Evaluation of quality changes in nutritionally enriched extruded snacks during storage. In Journal of Food Science and Technology (Vol. 55, Issue 10, pp. 3939–3948). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-018-3319-3
97. Edwards, E. J., Edwards, M. S., & Lyvers, M. (2015). Cognitive trait anxiety, situational stress, and mental effort predict shifting efficiency: Implications for attentional control theory. In Emotion (Vol. 15, Issue 3, pp. 350–359). American Psychological Association (APA). https://doi.org/10.1037/emo0000051
98. Ötles, S., & Ozgoz, S. (2014). Health effects of dietary fiber. In Acta Scientiarum Polonorum Technologia Alimentaria (Vol. 13, Issue 2, pp. 191–202). Uniwersytet Przyrodniczy w Poznaniu (Poznan University of Life Sciences). https://doi.org/10.17306/j.afs.2014.2.8
99. Dahl, W. J., & Stewart, M. L. (2015). Position of the Academy of Nutrition and Dietetics: Health Implications of Dietary Fiber. In Journal of the Academy of Nutrition and Dietetics (Vol. 115, Issue 11, pp. 1861–1870). Elsevier BV. https://doi.org/10.1016/j.jand.2015.09.003
100. Lin, D., Long, X., Huang, Y., Yang, Y., Wu, Z., Chen, H., Zhang, Q., Wu, D., Qin, W., & Tu, Z. (2020). Effects of microbial fermentation and microwave treatment on the composition, structural characteristics, and functional properties of modified okara dietary fiber. In LWT (Vol. 123, p. 109059). Elsevier BV. https://doi.org/10.1016/j.lwt.2020.109059
101. Rodríguez-Miranda, J., Ruiz-López, I. I., Herman-Lara, E., Martínez-Sánchez, C. E., Delgado-Licon, E., & Vivar-Vera, M. A. (2011). Development of extruded snacks using taro (Colocasia esculenta) and nixtamalized maize (Zea mays) flour blends. In LWT - Food Science and Technology (Vol. 44, Issue 3, pp. 673–680). Elsevier BV. https://doi.org/10.1016/j.lwt.2010.06.036
102. Seth, D., & Rajamanickam, G. (2012). Development of extruded snacks using soy, sorghum, millet and rice blend – A response surface methodology approach. In International Journal of Food Science & Technology (Vol. 47, Issue 7, pp. 1526–1531). Wiley. https://doi.org/10.1111/j.1365-2621.2012.03001.x
103. Korkerd, S., Wanlapa, S., Puttanlek, C., Uttapap, D., & Rungsardthong, V. (2015). Expansion and functional properties of extruded snacks enriched with nutrition sources from food processing by-products. In Journal of Food Science and Technology (Vol. 53, Issue 1, pp. 561–570). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-015-2039-1
104. Sriwattana, S., Laokuldilok, N., & Prinyawiwatkul, W. (2008). Sensory Optimization of Broken-Rice Based Snacks Fortified with Protein and Fiber. In Journal of Food Science (Vol. 73, Issue 6). Wiley. https://doi.org/10.1111/j.1750-3841.2008.00853.x
105. Sharma, S., Gupta, J. P., Nagi, H. P. S., & Kumar, R. (2011). Effect of incorporation of corn byproducts on quality of baked and extruded products from wheat flour and semolina. In Journal of Food Science and Technology (Vol. 49, Issue 5, pp. 580–586). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-011-0304-5

106. Kocherla, P., K. Aparna, K., & Lakshmi, D. N. (2012). Development and evaluation of RTE (Ready to Eat) extruded snack using egg albumin powder and cheese powder. In Agricultural Engineering International: CIGR Journal (Vol. 14, Issue 4, pp. 179–187). International Commission of Agricultural and Biosystems Engineering.

107. Day, L., & Swanson, B. G. (2013). Functionality of Protein-Fortified Extrudates. In Comprehensive Reviews in Food Science and Food Safety (Vol. 12, Issue 5, pp. 546–564). Wiley. https://doi.org/10.1111/1541-4337.12023

108. Waseem, M., Majeed, Y., Nadeem, T., Naqvi, L. H., Khalid, M. A., Sajjad, M. M., Sultan, M., Khan, M. U., Khayrullin, M., Shariati, M. A., & Lorenzo, J. M. (2023). Conventional and advanced extraction methods of some bioactive compounds with health benefits of food and plant waste: A comprehensive review. In Food Frontiers (Vol. 4, Issue 4, pp. 1681–1701). Wiley. https://doi.org/10.1002/fft2.296

109. Waseem, M., & Majeed, Y. (2023). Cell-based food needs collaborative efforts for safe production and suitable consumption. In Food Safety and Health (Vol. 1, Issue 1, pp. 6–8). Wiley. https://doi.org/10.1002/fsh3.12009

110. Waseem, M., Majeed, Y., Nadeem, T., Naqvi, L. H., Khalid, M. A., Shafiq, M., & Sajjad, M. M. (2022). Climate Change, Flood Disaster, and Food Insecurity in Pakistan. Research Square Platform LLC. https://doi.org/10.21203/rs.3.rs-2284023/v1

111. Waseem, M., Tahir, A. U., & Majeed, Y. (2023). Printing the future of food: The physics perspective on 3D food printing. In Food Physics (Vol. 1, p. 100003). Elsevier BV. https://doi.org/10.1016/j.foodp.2023.100003

112. Waseem, M., Khan, M. U., Majeed, Y., Ntsefong, G. N., Kirichenko, I., Klopova, A., Trushov, P., & Lodygin, A. (2023). Seaweed-based films for sustainable food packaging: properties, incorporation of essential oils, applications, and future directions. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 899–917). HACCP Consulting. https://doi.org/10.5219/1908

113. Majeed, Y., & Waseem, M. (2022). Postharvest Handling Systems. In Encyclopedia of Smart Agriculture Technologies (pp. 1–9). Springer International Publishing. https://doi.org/10.1007/978-3-030-89123-7_125-1

114. Majeed, Y., Khan, M. U., Waseem, M., Zahid, U., Mahmood, F., Majeed, F., Sultan, M., & Raza, A. (2023). Renewable energy as an alternative source for energy management in agriculture. In Energy Reports (Vol. 10, pp. 344–359). Elsevier BV. https://doi.org/10.1016/j.egyr.2023.06.032

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank Dr. Abid Aslam Maan for providing the equipment and other necessary sources to conduct our research work at the canning hall, University of Agriculture, Faisalabad.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Muzammal Shafiq, University of Agriculture, Faisalabad, Department of Food Engineering, Pakistan
Tel.: +923474943238
E-mail: muzammalshafiq833@gmail.com
ORCID: https://orcid.org/0000-0003-4052-8857

Muhammad Waseem, University of Agriculture, Faisalabad, Department of Food Engineering, Pakistan
Tel.: +923117002248
E-mail: waseemsofficial@gmail.com
ORCID: https://orcid.org/0000-0002-7387-6201

***Yaqoob Majeed**, University of Agriculture, Faisalabad, Department of Food Engineering, Pakistan
Tel.: +923006815542
E-mail: yaqoob.majeed@uaf.edu.pk
ORCID: https://orcid.org/0000-0003-1782-3436

Muhammad Arslan Khalid, University of Agriculture, Faisalabad, Department of Food Engineering, Pakistan
Tel.: +923136640046
E-mail: chadury.arslan@gmail.com
ORCID: https://orcid.org/0000-0002-4116-2669

Tayyaba Nadeem, Government College Women University Faisalabad, Faisalabad, Department of Food Science and Technology, Pakistan,
Tel.: +923356767248
E-mail: tayyabasofficial@gmail.com
ORCID: https://orcid.org/0000-0001-8089-9885

Maksim Rebezov, V. M. Gorbatov Federal Research Center for Food Systems, Department of Scientific Research, 26 Talalikhin Str., Moscow, 109316, Russia; Ural State Agrarian University, Department of Biotechnology and Food, 42 Karl Liebknecht Str., Yekaterinburg, 620075, Russia,
Tel.: +79999002365
E-mail: rebezov@ya.ru
ORCID: https://orcid.org/0000-0003-0857-5143

Mars Khayrullin, Moscow State University of technologies and management (The First Cossack University), Department of Scientific Research, K.G. Razumovsky, 73 Zemlyanoy Val, Moscow, 109004, Russia,
Tel.: +79049755219
E-mail: 89049755219@ya.ru
ORCID: https://orcid.org/0000-0003-1697-7281

Orynzhamal Sarsembenova, Almaty University of Energy and Communication, Department of Engineering Ecology and Occupational Safety; Gumarbek Daukeev, Almaty, 050000, Kazakhstan,
Email: oryn23@mail.ru
ORCID: https://orcid.org/0000-0001-8089-9885

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 22.2.2024
Revised: 24.6.2024
Accepted: 27.6.2024
Published: 16.7.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 654-665
https://doi.org/10.5219/1955
ISSN: 1337-0960 online
www.potravinarstvo.com

Organoleptic profile of high protein and dietary fibre biscuits based on soybean flour, tempeh flour, and Moringa leaf powder

Lily Arsanti Lestari, Susetyowati, Azizah Isna Rachmawati, Ajeng Saskia Sekar Rachmadanty, Amalia Sarah Sholikhati

ABSTRACT

Noncommunicable diseases (NCDs) are the major cause of mortality globally. Malnutrition and inadequate or excessive intake were associated with increased risk factors for NCD development. This study aimed to determine the organoleptic profile and nutritional value of biscuit food formula from local food based on soybean (*Glycine max*) flour, tempeh flour, and *Moringa* leaf powder. This study was conducted in Yogyakarta, a city located in Indonesia in Southeast Asia. The organoleptic test involved 36 semi-trained panellists who fulfilled the established standards. The organoleptic profile was evaluated for colour, taste, aroma, texture, aftertaste, and overall acceptability. Five biscuit formulas, including the control (F0) and four sample treatments with the addition of 0 g (F1), 2.5 g (F2), 5 g (F3), and 7.5 g of *Moringa* leaf powder, were tested. The ratio of soybean flour to tempeh flour was 5:2 without F0. Organoleptic properties were analysed using the Friedman test and the Wilcoxon test. The highest scores for organoleptic profile, including colour, taste, aroma, texture, aftertaste, and overall acceptability parameters, were observed in F2, which was added with 2.5 of g *Moringa* leaf powder, and scored 3.61 ±0.599, 3.47 ±0.654, 3.67 ±0.793, 4.06 ±0.475, 3.22 ±0.898, and 3.53 ±0.654 respectively. Selected biscuit formulation (F2) per 100 g contains 534.8 kcal of energy, 18.8 g of protein, 33.0 g of fat, 40.49 g of carbohydrate, and 18.08 g of dietary fibre.

Keywords: soybean, tempe, moringa, biscuit, nutrition for malnutrition

INTRODUCTION

Noncommunicable diseases (NCDs), including cancers, diabetes, cardiovascular diseases (CVDs), and chronic respiratory diseases, are the leading global burden and cause of death, accounting for up to 74% of deaths worldwide **[1]**. Malnutrition and nutritional imbalance can eventually lead to the development of several NCDs. Malnutrition may trigger metabolic syndromes such as insulin resistance, oxidative stress, and inflammation **[2]**. Malnutrition, also known as disease-related malnutrition, and sarcopenia frequently occur as complications in patients with chronic or severe diseases **[3]**. Functional foods, such as plant-based foods, offer a potential opportunity to meet nutritional needs that could prevent and treat malnutrition fully **[4]**. Indonesia has an abundance of locally grown plant foods, which have the potential to improve and optimise nutritional needs. Soybeans, tempeh, and *Moringa* leaves are potential sources of functional food.

Soybean (*Glycine max*) is a rich source of high-quality plant protein (approximately 40%) and has a well-balanced amino acid profile. Isoflavones are polyphenolic compounds found primarily in soybeans. Daidzein and genistein, exclusive isoflavones, have been found in soy. Beneficial biological activities such as anticancer, antiaging, anti-kidney disease, antiobesity, anticholesterolemic, and anti-inflammatory activities have been reported **[5]**, **[6]**, **[7]**. Unlike wheat biscuits, soybean biscuits exhibited improved nutritional value and functional properties **[8]**. Tempeh, a popular traditional Indonesian food, is produced by the fermentation of soybeans with

strains of *Rhizopus* species such as *R. oligosporus, R. arrhizus,* and *R. stolonifer* **[9]**, **[10]**. Tempeh's antioxidant capacity and digestibility are enhanced by the fungal fermentation of soybeans, which improves the ingredient's nutritional value and functional properties. The digestibility level of tempeh amounts to 83% compared with that of soy, which is only 75%. In addition, compared with other plant-based protein sources, tempeh has higher fibre concentrations, isoflavones, arginine, linolenic acid, linoleic acid, and branched-chain amino acids **[11]**, **[12]**.

Moringa oliifera, known as the "miracle tree" for its nutritional and health benefits, has been widely cultivated in Indonesia. *Moringa oleifera* is a rich source of macronutrients, vitamins, minerals, and antioxidants. It contains flavonoids, phenolic acids, and tannin. It exerts various biological effects, such as antioxidant, anti-inflammatory, antihyperlipidemic, antidiabetic, anticancer, antispasmodic, anti-asthma, antiulcer, hypocholesterolemic, hypoglycemic, cardioprotective, hepatoprotective, and antimicrobial properties **[13]**, **[14]**, **[15]**, **[16]**, **[17]**, **[18]**. In addition, *Moringa oleifera* leaves could be used for the prevention and treatment of malnutrition **[15]**, **[19]**, **[20]**, **[21]**. Prospective studies have shown that biscuits, a well-known and inexpensive ready-to-eat food, are an improved food that can meet dietary needs and prevent or mitigate diet-related diseases. Currently, biscuits can be a potential strategy for nutritional enrichment **[22]**, **[23]**.

Scientific hypothesis

We have evaluated the organoleptic profile (colour, aroma, texture, taste, and overall acceptability) of biscuits made from soybean flour, tempeh, and Moringa leaves. Substitution of wheat flour with soybean flour, tempeh powder, and Moringa leaves powder could affect the organoleptic profile, so we have evaluated four biscuit formulas compared to a control biscuit. The best formula with the best organoleptic profile was further analysed for nutritional content.

MATERIALS AND METHODOLOGY

Samples

Five biscuit formulations were created, including one control formula (F0) and four treatment formulas (F1-F4). The biscuit formulation was based on local food, including organic soybeans, tempeh with no genetically modified organisms, and m*oringa* leaves. Soybean and tempeh were floured first. *Moringa* leaf powder was purchased from a local certified distributor in Yogyakarta.

Table 1 Biscuit formula.

Biscuit Ingredients	Biscuits Formula				
	Control	Treatment			
	F0	F1	F2	F3	F4
Cake Flour	60 g	20 g	20 g	20 g	20 g
Soybean Flour	0 g	27.5 g	26.25 g	25 g	23.75 g
Tempeh Flour	0 g	12.5 g	11.25 g	10 g	8.75 g
***Moringa* Leaf Powder**	0 g	0 g	2.5 g	5 g	7.5 g

The control formula was made from cake flour and did not contain soybean flour, tempeh flour, or Moringa leaves. The ratio of soybean flour and tempeh flour for the four treatment formulas was 5:2, with 20 g of cake flour. Each of the five formulations was enriched with various ingredients: butter, soybean oil, palm sugar liquid, cornstarch, full-cream milk powder, sweetener, vanilla extract, and cinnamon powder.

The ingredients, such as butter, soybean oil, palm sugar liquid, vanilla extract, and sweetener, were combined using a mixing machine for around 3-5 minutes. Then, shift the dry ingredients like cake flour, soybean flour, tempeh flour, moringa flour, cornstarch, and full cream milk powder before adding them to the wet ingredients. Mixing dry and wet ingredients until homogenous. The biscuit dough was weighed and shaped using a stainless steel biscuit round cutter with a 3-cm diameter. The biscuits were baked in an electric oven at 140°C for 20 min.

As semitrained panellists, this study selected 36 Undergraduate Nutrition Study Program students at Gadjah Mada University (UGM). These individuals met the following inclusion criteria: aged >18 years, preferred biscuits, did not have any taste-related issues, were not allergic to soybean, milk, wheat, tempeh, and Moringa, and have completed the food technology course.

Chemicals

The chemicals used to analyse macronutrients, micronutrients, and amino acids were authority PT. Saraswanti Indo Genetech (SIG) Laboratory in Semarang, Indonesia.

Animals, Plants, and Biological Material

The study used Glycine max and *Moringa olifera* as plant materials. No animals or biological materials were involved in the research.

Instruments

Electric oven, digital kitchen scale, stand mixer, food dehydrator machine, disk mill machine, organoleptic profile form.

Laboratory Methods

Five biscuit formulations were analysed. The organoleptic profile, a variable examined in this study, thoroughly evaluated different aspects such as colour, aroma, texture, taste, aftertaste, and overall acceptability for each formula. The results of the organoleptic profile analysis were presented as a panellist-preferred analysis of the colour, aroma, texture, taste, aftertaste, and overall acceptability of the various biscuit formula products derived from soybean, tempeh, and *Moringa* leaves.

The organoleptic profile of the biscuit formula was evaluated using a 6-point Likert scale (1 = very strongly dislike, 2 = strongly dislike, 3 = dislike, 4 = like, 5 = strongly like, and 6 = very strongly like). The panellists also provided some recommendations on how to create a more acceptable biscuit in all aspects, such as colour, aroma, texture, taste, aftertaste, and overall acceptability.

PT Saraswanti Indo Genetech (SIG), located in Semarang, is an authorised laboratory for conducting tests and adheres to the ISO/IEC 17,025:2017 standard. Table 2 provides information on the analysis method employed to determine the nutrient content of the selected formula.

Table 2 Analysis method of nutrient content.

Nutrients	Analysis method
Energy total	Calculation
Energy from fat	Calculation
Protein	18-8-31/MU/SMM-SIG (Titrimetri)
Fat total	18-8-5/MU/SMM-SIG point 3.2.2 (Weibull)
Monounsaturated fat	18-6-1/MU/SMM-SIG (GC-FID)
Polyunsaturated fat	18-6-1/MU/SMM-SIG (GC-FID)
Saturated fat	18-6-1/MU/SMM-SIG (GC-FID)
Carbohydrate	18-8-9/MU/SMM-SIG (calculation)
Dietary fibre	18-8-6-2/MU/SMM-SIG
Sugar total	18-8-8/MU/SMM-SIG (Luff Schrool)
Cholesterol	18-6-5/MU/SMM-SIG (GC-FID)
Sodium (Na)	18-13-1/MU/SMM-SIG (ICP OES)
Flavonoid (RSA)	Spectrophotometry

Description of the Experiment

Sample preparation: Five grams of biscuits of each formulation were individually sealed in a plastic bag. The biscuit was given at one time.

Number of samples analysed: One sample was analysed.

Number of repeated analyses: 2

Amount of experiment replications: The experiment was conducted only once to determine a single value without any repetitions.

Design of the experiment: At the beginning of the experiment, local foods, including soybean flour, tempeh flour, and Moringa leaf powder, were selected to make biscuits. The amount of an ingredient was calculated, and another ingredient was added. Acceptability aspects of the biscuits, such as colour, aroma, texture, taste, aftertaste, and overall acceptability, were then determined. The recipe for each biscuit formulation product was determined based on the data obtained.

Statistical Analysis

The IBM SPSS Statistics version 25 was used to analyse all the organoleptic profile data for each aspect. The data are presented as the mean ± standard deviation of the mean to provide a comprehensive description.

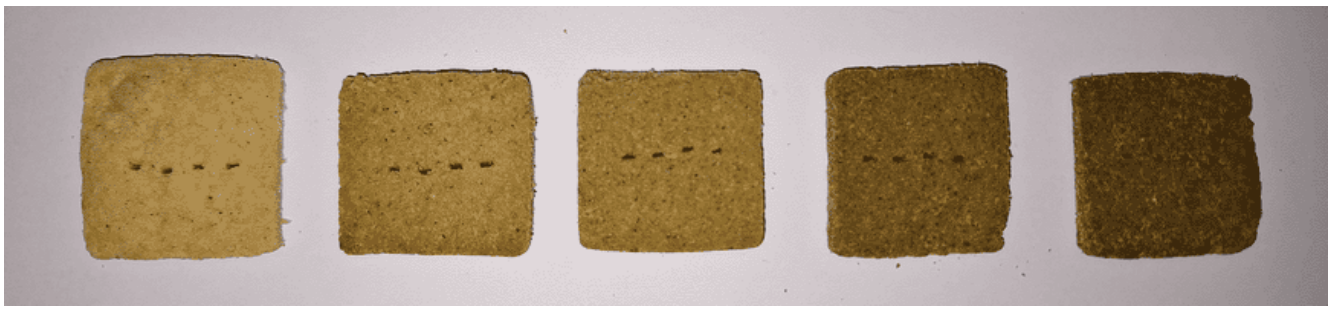
Statistical analysis assessed whether organoleptic profile differences exist among each biscuit formula. Data normality was evaluated using the Shapiro–Wilk test (n = 36). The results revealed that the data of each aspect of the organoleptic profile of the five biscuit formulas were nonnormally distributed (p <0.05). Furthermore, the

analysis data by Friedman's test and Wilcoxon's rank-sum test were used to evaluate the differences within the biscuit formula.

RESULTS AND DISCUSSION

The organoleptic test was performed at the Health Nutrition Building's Organoleptic Laboratory, UGM. Thirty-six semitrained panellists were involved in this organoleptic analysis. Each sample's organoleptic properties were assessed on a scale of 1-6 (1 = very strongly dislike, 2 = strongly dislike, 3 = dislike, 4 = like, 5 = strongly like 6 = very strongly like). Organoleptic panellists rated the Colour, aroma, texture, taste, aftertaste, and overall acceptability. Of the 36 panellists, 32 were female (88.9%) and 4 were male (11.1%).

The organoleptic test findings (Table 3) indicated that each biscuit formula exhibited distinct values across all test components, including colour, taste, aroma, texture, aftertaste, and overall evaluation. On a scale of 1-6, F0 received the highest rating from the panellists, with an average score of 4, indicating that it was the most preferred sample compared with the other samples. The panellist's personal preference solely determines the acceptance or rejection of food.



Figures 1 Biscuit organoleptic formulas, respectively F0, F1, F2, F3, F4.

Increasing the amount of *Moringa* leaf powder in the biscuits decreased the value perceived by the panellists. The panellists who expressed dissatisfaction with the biscuit identified various deficiencies in its organoleptic characteristics, including a bitter taste, beany flavour, and a less appealing scent. Furthermore, researchers analysed the data on organoleptic test results using Friedman's and Wilcoxons' rank-sum statistical test method (Table 3). This test observed highly significant variations (p <0.001) between each biscuit formula's colour, taste, aroma, texture, aftertaste, and overall acceptability quality.

Table 3 Biscuit organoleptic test results.

Biscuit Formula	Biscuit Organoleptic Component					
	Colour	**Taste**	**Aroma**	**Texture**	**Aftertaste**	**Overall**
F0	5.00 ±0.793^{a}	4.83 ±0.775^{a}	4.47 ±0.774^{a}	4.86 ±0.723^{a}	4.72 ±0.741^{a}	4.97 ±0.654^{a}
F1	4.56 ±0.695^{b}	4.19 ±0.624^{b}	4.42 ±0.806^{a}	4.42 ±0.692^{b}	3.69 ±0.710^{b}	4.19 ±0.624^{b}
F2	3.61 ±0.599^{c}	3.47 ±0.654^{c}	3.67 ±0.793^{b}	4.06 ±0.475^{c}	3.22 ±0.898^{c}	3.53 ±0.654^{c}
F3	3.33 ±0.862^{c}	3.19 ±749$^{c.d}$	3.33 ±0.676^{b}	4.11 ±0.575^{c}	3.08 ±0.649^{c}	3.28 ±0.741^{c}
F4	2.97 ±0.774^{d}	2.89 ±0.747^{d}	3.06 ±0.630^{c}	3.97 ±0.696^{c}	2.67 ±0.756^{d}	2.94 ±0.583^{d}
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: $^{a-d}$ means within the same row, and different uppercase letters represent significantly different biscuit formulas (p <0.05). All values are represented as the mean ± standard deviation.

According to the specifications outlined in BSN (National Standardization Agency of Indonesia) No. 2,973 of 2022 on biscuit products, a high-quality biscuit should exhibit colour consistent with its composition, possess a normal scent without any foreign odours, and have a normal taste without any foreign flavours. The sensory analysis results in the laboratory revealed that the biscuits possessed a typical aroma and appearance, a firm structure, a mildly sweet and slightly bitter flavour, a crunchy texture, and a slightly greenish-brown hue. These findings align with the specifications given in BSN No. 2,973 of 2022. As indicated by the laboratory findings, a difference was noted in the moisture level of the cookies, which reveals a moisture content of 5.39%, surpassing the maximum moisture content requirement of 5% stated in BSN No. 2,973 of 2022 **[24]**.

Colour

Colour is an important aspect of biscuits; it depends on the ingredients and can change during baking. The addition of soy and tempeh flour to biscuits affects the colour parameters of biscuits. During baking, the biscuits

undergo browning because of caramelisation and the Maillard reaction, which occurs when the protein in soybean flour reacts with sugar **[25]**, **[26]**, **[27]**, **[28]**. Furthermore, the high levels of protein and dietary fibre and presence of colour pigments in soy flour contribute to the darker hue of the biscuits **[29]**. Including *Moringa* leaves in the biscuit recipe results in a green hue, with the colour intensity increasing as more *Moringa* leaves are added. The green hue of *Moringa* leaves is attributed to their high chlorophyll content, which effectively covers the natural colour of the food **[16]**, **[30]**, **[31]**.

The findings of the organoleptic test revealed that F0 outperformed the other formulas in terms of colour. Increasing the amount of *Moringa* leaf powder in the biscuits decreased the score received, indicating that the panellists did not prefer it. The presence of chlorophyll in *Moringa* leaves gives the biscuits a green tint, which was considered uncommon by the panellists. The control biscuits received the highest score of 5. F1 scored lower than F0, specifically 4.56, whereas F2 and F3 scored 3.61 and 3.33, respectively; however, no significant difference was found between them. The panellists rated F4 the lowest, with a score of 2.97.

Taste

Taste influences consumers' decision to embrace or reject a food or product. Humans recognise four fundamental taste categories: sweet, salty, bitter, and sour **[11]**. The majority of panellists preferred biscuits without *Moringa* leaf powder. The panellists preferred F0, with a taste score of 4.83. F1 received a slightly lower score of 4.19. The higher the contents of *Moringa* leaf powder, the lower the taste preference of the panellists. The taste scores for F2, F3, and F4 were 3.47, 3.19, and 2.89, respectively.

The panellists remarked that the biscuits have a beany flavour and a bitter aftertaste. Increasing the amount of *Moringa* leaf powder resulted in bitter biscuits. The beany taste that discourages soy consumption may arise from the enzymatic breakdown of linoleic and linolenic acids by lipoxygenase or the autooxidation of these acids in soybeans, leading to the production of volatile compounds, including ketones, aldehydes, alcohols, furans, and furan derivatives **[32]**. An alternative method to diminish the strong taste of soybeans is to incorporate supplementary components into the product **[33]**. The biscuit was then enhanced with additional ingredients such as cinnamon powder, full cream milk, and vanilla to conceal the soybean's beany flavour and enhance the overall taste.

The tempeh flour and *Moringa* leaf powder are responsible for the bitter taste of the biscuits, as indicated by prior studies. Tempeh flour exhibits a disagreeable scent and imparts a bitter lingering taste in biscuit recipes. The bitterness detected in tempeh flour may result from the breakdown of amino acids during the Maillard reaction **[27]**. The bitterness of this substance is attributed to amino acids such as arginine, phenylalanine, proline, valine, and lysine. The bitter taste of soy flour is caused by the glycoside chemicals found in soybean seeds. The primary glycosides that contribute to bitterness are isoflavones, specifically genistin and daidzin, with their aglycone counterparts, genistein and daidzein **[11]**, **[34]**.

Moringa leaves are characterised by a slightly bitter taste. This affects the panellists' acceptance of biscuits supplemented with *Moringa* leaves **[30]**, **[31]**. A study mentioned that the decrease in biscuit flavour scores might be caused by adding large amounts of Moringa leaves, contributing to the bitter/grassy taste. The saponin content of *Moringa* leaves may contribute to the bitter taste in biscuits containing large amounts of *Moringa* leaves **[35]**. The effect of flavour on food acceptance is strongly linked to an individual's preferences. Individuals tend to choose and consume food items with many pleasant flavours. Panellists may have diverse sensations because of differences in sensory perception, which can be attributed to changes in the sensitivity of sensory organs or a lack of familiarity with specific flavours **[11]**.

Aroma

The aroma of food plays a crucial role in determining its deliciousness and flavour, thereby significantly influencing the assessment and quality of the food **[36]**. Volatile and aromatic substances generate aroma. Proteins in the ingredients degrade into amino acids when exposed to heat during baking. The interaction between these amino acids and sugars generates aroma, and simultaneously, the fats in the ingredients are oxidised and decomposed due to heat. As a result, some active components resulting from this decomposition react with amino acids and peptides, which contribute to aroma production **[11]**, **[37]**.

The organoleptic test results indicated that the panellists preferred the aroma of biscuits without *Moringa* leaf powder. A few panellists distended Moringa's earthy taste and unique scent. The average scores of F0 and F1, namely, 4.47 and 4.42, respectively, were not significantly different. F2, F3, and F4 scored 3.67, 3.33, and 3.06, respectively.

The combination of tempeh and *Moringa* leaf powder resulted in an undesirable scent in biscuits. The malodorous aroma of tempeh flour is attributed to the presence of lip oxidase enzymes in soybeans. The disagreeable scents are caused by the hydrolysis or breakdown of soybean fat into chemicals belonging to the

hexanal and hexanol groups, which is attributed to the lipoxidase enzyme. Even at low concentrations, these compounds can produce unpleasant odours **[11]**. The addition of *Moringa* leaf powder also affects the taste of the biscuits. Some panellists said the *Moringa*-added biscuits have a distinctive aroma like the leaves **[35]**.

Researchers have added other ingredients to the biscuits to mask the unpleasant aroma, such as powdered cinnamon and vanilla, so the biscuits would have a favourable aroma.

Texture

Texture is a pressure sensor that can be perceivable through the mouth (during biting, chewing, and swallowing) or touched with fingers. All biscuits had a texture favoured by panellists, namely, crispy and soft when bitten; however, some panellists said that the biscuits were too dry and fibrous, so they had to drink lots of water. The panellists favoured the control biscuit, which had an average score of 4.86. The score of F1 was nearly identical to that of F0 (4.42). The texture scores for F2, F3, and F4 were statistically indistinguishable, with values of 4.06, 4.11, and 3.97, respectively.

Soy flour has a very soft texture, making it suitable as a raw material for high-quality cookies because it can increase biscuit density **[29]**. Both flour composition and the interactions between the ingredients determine the firmness of biscuits. The high protein content in soy flour can enhance the firmness of biscuits because of the strong bond between protein and starch. Fibre can also influence texture by potentially densifying the dough structure when higher amounts of fibre are present **[17]**.

An inverse relationship was found between the fragility of biscuits and their crispness. The texture of the biscuits can be enhanced by reducing the particle size of soy flour and the moisture content of the biscuits **[29]**. Increasing the amount of soy flour in biscuits reduced the hydration and consistency of the dough, resulting in a more brittle biscuit **[32]**. The addition of *Moringa* leaf powder also affected the texture of the biscuits. Increasing the amount of *Moringa* leaf powder also increased the breaking strength of biscuits **[30]**, **[35]**. Tempe flour contains a significant amount of protein, ranging from 46% to 50.18%. This high protein content may lead to the hardening of biscuits because of denaturation that occurs during baking **[10]**, **[38]**.

Overall Acceptability

The findings indicated that increasing the proportion of soy flour and tempeh flour directly affected all sensory attributes, leading to a decline in the overall acceptability of the biscuits. In addition, the higher the amount of *Moringa* leaf powder added, the better the nutritional content; however, the acceptability of the biscuits decreased because of increased bitterness in flavour. Prior experiments in the sensory evaluation demonstrated that *Moringa*-enriched biscuits achieved approval ratings of 9% or 10% in distinct investigations. This result suggests interindividual differences between consumers regarding acceptance **[30]**.

Nutrient Content

Based on the test results, F2 was more acceptable regarding colour, taste, aroma, texture, aftertaste, and overall acceptability. Table 4 shows the results of the nutritional content analysis of F2, which meets the dietary needs of malnourished patients.

Table 4 Nutrient content of the selected formula.

Nutrients	Unit	Content per 100 g of Edible Weight
Energy total	Kcal	534.82
Energy from fat	kcal	297.68
Protein	g	18.80
Fat total	g	33.08
Monounsaturated fat	g	7.73
Polyunsaturated fat	g	9.68
Saturated fat	g	15.67
Carbohydrate	g	40.49
Dietary fibre	g	18.08
Sugar total	g	8.82
Cholesterol	mg	26.05
Sodium (Na)	mg	27.75
Flavonoid (RSA)	mg	78.6

According to regulation BPOM (Indonesian Food and Drug Authority) No. 9 of 2016, one serving (25 one serving or 25 g of cookies) provides 6% energy, 8% protein, 12% fat, 3% carbohydrates, and 13% dietary fibre based on percent daily values for a 2,150 calorie per day diet **[39]**. These biscuits can help meet nutritional needs and treat NCD-associated malnutrition. Eating balanced nutrition is essential for patients with NCD, especially foods high in protein, fibre, and antioxidants, such as those in biscuits. A balanced diet that meets individual needs is associated with a lower risk of disease-related malnutrition.

Dietary protein is one of the most commonly consumed macronutrients worldwide. Protein is a fundamental component of cell structure and plays a crucial role in forming essential substances such as antibodies. Inadequate protein consumption is associated with high susceptibility to infections, particularly in individuals with NCDs. Protein is also necessary to recover and repair body tissues after injury or disease. A study reported that the risk of NCDs can be reduced by consuming >40% plant-based proteins **[40]**. A systematic review and meta-analysis of studies showed that increased intake of plant-based proteins correlates with a lower risk of all-cause and CVD-related mortality **[41]**.

The biscuit's main ingredients were soybean flour and tempeh flour, which are good protein sources. Soybean is rich in proteins and amino acids, with up to 40% protein content, which is equivalent to animal sources such as meat, eggs, and milk **[6]**, **[7]**, **[42]**. Many randomised controlled trials have reported that soy protein intake improves metabolic status, which has implications for metabolic syndrome. This includes decreased total cholesterol levels, malondialdehyde **[43]**, low-density lipoprotein cholesterol (LDL-C), and nonhigh-density lipoprotein cholesterol (non-HDL-C) **[44]**. The total antioxidant capacity, a biomarker of lipid peroxidation and antioxidant activity, was also increased **[43]**.

Unsaturated fats exert anti-inflammatory effects that reduce inflammation in patients with cancer. It can also improve blood lipid profiles and support cardiovascular health **[45]**. The unsaturated fats in the biscuits can improve blood lipid profiles and support cardiovascular health. CVDs can be affected by several factors, such as high blood pressure, obesity, increased LDL-C, reduced HDL-C, high cholesterol, and hypertriglyceridemia. Soy protein consumption is considered low in saturated fat and reduces cholesterol, thereby minimising the incidence of CVDs. The potent antioxidant quality of isoflavones found in soy-derived products influences oxidative stress and reduces cholesterol levels **[46]**. *Moringa oleifera* leaves have shown beneficial therapeutic potential for preventing or treating dyslipidemia and CVDs **[47]**.

Sugars used in biscuits are zero-calorie sweeteners with lactose and sucralose. A study of the relationship between sugar absorption and carcinogenesis showed that consumption of foods or beverages with high sugar content positively correlated with cancer risk **[48]**. As a beneficial food, soy reduces fasting blood glucose, insulin **[43]**, and hyperglycemia **[49]**. Fermented soybean products can improve glucose metabolism disorders by acting as inhibitors of carbohydrate digestive enzymes, improving pancreatic function, preventing hepatic gluconeogenesis, enhancing muscle glucose utilisation, alleviating inflammation in adipose tissue, and addressing alterations in the gut microbiota **[49]**. Furthermore, *Moringa* showed encouraging in vitro properties for managing diabetes and reducing blood sugar levels. Overall, *Moringa* extracts can be easily used for postprandial blood glucose regulation, reducing the formation and buildup of advanced glycation end products and ultimately reducing complications associated with diabetes **[50]**.

Fibre has various benefits for patients with NCD, such as helping maintain gastrointestinal health by supporting the growth of good bacteria in the gut. In addition, fibre can help manage weight, control blood sugar, lower cholesterol levels, and prevent CVDs **[51]**. Consumption of soluble and insoluble dietary fibre has been associated with reduced risk of atherosclerosis and heart disease and reduced blood cholesterol levels **[52]**. *Moringa oleifera* leaves are a rich source of dietary fibre with a content of up to 19% **[53]**. The high fibre content of *Moringa* leaves was found to correlate with delayed gastric emptying, which may improve glycemic control and hypoglycemic effect in the postprandial state **[54]**.

Biscuits contain low sodium levels, which can prevent high blood pressure. Excessive salt intake can have adverse effects, including high blood pressure and increased CVD risk. A low-salt diet can decrease the volume load and blood pressure. Another possible explanation is that inflammation is a key factor in arterial stiffness. Adherence to a low-salt diet can prevent the infiltration of inflammatory cells, reduce the production of inflammatory factors, mitigate arterial stiffness, and lower blood pressure **[55]**.

Flavonoids are generally found in various plants. They modulate multiple signalling pathways and exert potent antioxidant and anti-inflammatory effects, which may reduce the risk of NCDs such as cancer and CVDs **[56]**. Because of flavonoids such as quercetin and kaempferol, Moringa leaves contain bioactive compounds and exert antioxidant activity. Moringa leaves benefit health and has been used as an antidiabetic, antibacterial, and anti-inflammatory alternative **[57]**. The radical scavenging activity of flavonoid content per 100 g of biscuits is 78.6 mg.

In addition to beneficial nutrient contents for patients, the organoleptic profile of biscuits must also be considered to ensure that they can be optimally consumed. According to regulation BPOM No. 1 of 2022, biscuits are labelled as high fibre and low sodium **[58]**. Furthermore, biscuits are a source of proteins, unsaturated fat, vitamins, minerals, and antioxidants, contributing to excellent health.

CONCLUSION

In conclusion, the study on the organoleptic profile and nutritional value of biscuits made from soybean flour, tempeh flour, and Moringa leaf powder has provided valuable insights into the potential of these ingredients in creating a nutritious food product. Among the various formulations tested, biscuit F2, containing 2.5 g of Moringa leaf powder, emerged as the preferred option regarding colour, taste, aroma, texture, aftertaste, and overall acceptability. The findings demonstrated that increasing the content of soybean and tempeh flour negatively impacted the biscuits' sensory attributes. At the same time, the inclusion of Moringa leaf powder, despite improving the nutritional profile, introduced a bitterness that reduced overall appeal. Nonetheless, the F2 formulation provided a balanced approach, offering optimal sensory acceptability and nutritional benefits. Nutritional analysis of the selected F2 biscuit revealed it to be a rich source of energy, protein, dietary fibre, and unsaturated fats while low in sodium. These attributes make the F2 biscuit particularly suitable for addressing malnutrition and associated non-communicable diseases (NCDs). The biscuit provides 6% energy, 8% protein, 12% fat, 3% carbohydrates, and 13% dietary fibre per serving, aligning well with dietary recommendations for a 2150-calorie per day diet. The high protein content derived from soybean and tempeh flours is crucial for cell structure and repair, supporting immune function and reducing susceptibility to infections, especially in individuals with NCDs. Including unsaturated fats contributes to anti-inflammatory effects and improved cardiovascular health, while the high dietary fibre content supports gastrointestinal health, weight management, blood sugar control, and cholesterol reduction. Moringa leaf powder, rich in flavonoids, antioxidants, and essential nutrients, further enhances the biscuits' health benefits. It offers anti-inflammatory, antihyperlipidemic, and antidiabetic properties. The biscuits' low sodium content aids in preventing high blood pressure and reducing cardiovascular risks. Overall, the study underscores the potential of functional food products, like the F2 biscuit, in improving nutritional intake and managing NCD-related malnutrition. The F2 biscuit's sensory acceptability and nutritional benefits position it as a promising dietary intervention for promoting health and preventing disease. Future research should continue to explore optimisation strategies for enhancing the sensory and nutritional attributes of such functional food products.

REFERENCES

1. World Health Organization. (2022). Noncommunicable Diseases Progress Monitor 2022. Retrieved from https://iris.who.int/bitstream/handle/10665/353048/9789240047761-eng.pdf.
2. Ahmad, R., Akter, F., & Haque, M. (2023). Editorial: Diet and Nutrition for Non-Communicable Diseases in Low and Middle-Income Countries. In Frontiers in Nutrition (Vol. 10, pp. 1–5). Frontiers Media SA. https://doi.org/10.3389/fnut.2023.1179640
3. Meyer, F., & Valentini, L. (2019). Disease-Related Malnutrition and Sarcopenia as Determinants of Clinical Outcome. In Visceral Medicine (Vol. 35, Issue 5, pp. 282–291). S. Karger AG. https://doi.org/10.1159/000502867
4. Ahmed, M. H., Vasas, D., Hassan, A., & Molnár, J. (2022). The Impact of Functional Food In Prevention of Malnutrition. In PharmaNutrition (Vol. 19, pp. 1–6). Elsevier BV. https://doi.org/10.1016/j.phanu.2022.100288
5. Kafeshani, M., Rafieian-Kopaei, M., Beigrezaei, S., & Nasri, H. (2017). Soy Protein and Chronic Kidney Disease: An Updated Review. In International Journal of Preventive Medicine (Vol. 8, Issue 1, pp. 1–6). Medknow. https://doi.org/10.4103/ijpvm.ijpvm_244_17
6. Yamagata, K., & Yamori, Y. (2021). Potential Effects of Soy Isoflavones on the Prevention of Metabolic Syndrome. In Molecules (Vol. 26, Issue 19, pp. 1–20). MDPI AG. https://doi.org/10.3390/molecules2619583
7. Kim, I.-S., Yang, W.-S., & Kim, C.-H. (2021). Beneficial Effects of Soybean-Derived Bioactive Peptides. In International Journal of Molecular Sciences (Vol. 22, Issue 16, pp. 1–23). MDPI AG. https://doi.org/10.3390/ijms22168570
8. Silva, F. de O., Miranda, T. G., Justo, T., Frasão, B. da S., Conte-Junior, C. A., Monteiro, M., & Perrone, D. (2018). Soybean Meal and Fermented Soybean Meal as Functional Ingredients for the Production of Low-Carb, High-Protein, High-Fibre and High Isoflavones Biscuits. In LWT (Vol. 90, pp. 224–231). Elsevier BV. https://doi.org/10.1016/j.lwt.2017.12.035

9. Widiany, F. L., Metty, M., Widaryanti, R., & Azizah, S. N. (2022). Comparison of IC50 Antioxidant Analysis of Local Soybean Tempeh and Imported Soybean Tempeh in Indonesia. In International Journal of Nutrition Sciences (Vol. 7, Issue 4, pp. 214–244. https://doi.org/10.30476/ijns.2022.97526.1213
10. Huang, L., Huang, Z., Zhang, Y., Zhou, S., Hu, W., & Dong, M. (2019). Impact of Tempeh Flour on the Rheology of Wheat Flour Dough and Bread Staling. In LWT (Vol. 111, pp. 694–702). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.04.004
11. Widiany, F. L., Sja'bani, M., Susetyowati, & Hurivati, E. (2021). The Organoleptic Quality of Liquid Food Formula Made From Snail (Pila Ampullacea), Tempeh, and Moringa Leaves. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 961–969). HACCP Consulting. https://doi.org/10.5219/1672
12. Erkan, S. B., Gürler, H. N., Bilgin, D. G., Germec, M., & Turhan, I. (2020). Production and Characterization of Tempehs from Different Sources of Legume by Rhizopus oligosporus. In LWT (Vol. 119, pp. 1–7). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.108880
13. Fapetu, A. P., Karigidi, K. O., Akintimehin, E. S., Olawuwo, T., & Adetuyi, F. O. (2022). Effect of Partial Substitution of Wheat Flour with Moringa oleifera Leaf Powder on Physical, Nutritional, Antioxidant and Antidiabetic Properties of Cookies. In Bulletin of the National Research Centre (Vol. 46, Issue 1, pp. 1–9). Springer Science and Business Media LLC. https://doi.org/10.1186/s42269-022-00746-8
14. Paikra, B. K., Dhongade, H. K. J., & Gidwani, B. (2017). Phytochemistry and Pharmacology of Moringa oleifera Lam. In Journal of Pharmacopuncture (Vol. 20, Issue 3, pp. 194–200). Korean Pharmacopuncture Institute. https://doi.org/10.3831/kpi.2017.20.022.2017.20.022
15. Giuberti, G., Rocchetti, G., Montesano, D., & Lucini, L. (2021). The Potential of Moringa Oleifera in Food Formulation: a Promising Source of Functional Compounds with Health-Promoting Properties. In Current Opinion in Food Science (Vol. 42, pp. 257–269). Elsevier BV. https://doi.org/10.1016/j.cofs.2021.09.001
16. Mahour, P. kumar, Lal, A. B., Khare, A., & Chauhan, D. S. (2022). Preparation and Optimization of Moringa Leaf Powder for Edible Purpose. In Journal of the Indian Chemical Society (Vol. 99, Issue 3, pp. 1–7). Elsevier BV.. https://doi.org/10.1016/j.jics.2022.100377
17. Giuberti, G., Bresciani, A., Cervini, M., Frustace, A., & Marti, A. (2021). Moringa oleifera L. Leaf Powder as Ingredient in Gluten-Free Biscuits: Nutritional and Physicochemical Characteristics. In European Food Research and Technology (Vol. 247, Issue 3, pp. 687–694). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-020-03656-z
18. Milla, P. G., Peñalver, R., & Nieto, G. (2021). Health Benefits of Uses and Applications of Moringa oleifera in Bakery Products. In Plants (Vol. 10, Issue 2, p. 318). MDPI AG. https://doi.org/10.3390/plants10020318
19. Saini, R. K., Harish Prashanth, K. V., Shetty, N. P., & Giridhar, P. (2014). Elicitors, SA and MJ Enhance Carotenoids and Tocopherol Biosynthesis and Expression of Antioxidant Related Genes in Moringa oleifera Lam. Leaves. In Acta Physiologiae Plantarum (Vol. 36, Issue 10, pp. 2695–2704). Springer Science and Business Media LLC. https://doi.org/10.1007/s11738-014-1640-7
20. Ma, Z. F., Ahmad, J., Zhang, H., Khan, I., & Muhammad, S. (2020). Evaluation of Phytochemical and Medicinal Properties of Moringa (Moringa oleifera) as a Potential Functional Food. In South African Journal of Botany (Vol. 129, pp. 40–46). Elsevier BV. https://doi.org/10.1016/j.sajb.2018.12.002
21. Kodi, G. M., Mustafa, H. A., & Idris, A. A. A. (2022). The Effects of Moringa oleifera Leaves on Complete Blood Count, Renal and Liver Functions as Potential Therapy for Malnutrition. In Molecular and Cellular Biomedical Sciences (Vol. 6, Issue 2, pp. 55–62). Molecular and Cellular Biomedical Sciences (MCBS) Office. https://doi.org/10.21705/mcbs.v6i2.234
22. Goubgou, M., Songré-Ouattara, L. T., Bationo, F., Lingani-Sawadogo, H., Traoré, Y., & Savadogo, A. (2021). Biscuits: a Systematic Review and Meta-Analysis of Improving the Nutritional Quality and Health Benefits. In Food Production, Processing and Nutrition (Vol. 3, Issue 1, pp. 1–18). Springer Science and Business Media LLC. https://doi.org/10.1186/s43014-021-00071-z
23. Nogueira, A. de C., & Steel, C. J. (2018). Protein Enrichment of Biscuits: a Review. In Food Reviews International (Vol. 34, Issue 8, pp. 796–809). Informa UK Limited. https://doi.org/10.1080/87559129.2018.1441299
24. Regulation of the National Standardization Agency of Indonesia (BSN) Number 2973 of 2022 on Biscuit Products. Retrieved from https://akses-sni.bsn.go.id/viewsni/baca/8816
25. Agustini, S., Priyanto, G., Hamzah, B., Santoso, B., & Pambayun, R. (2014). Changes on the Physical-Chemical Properties of Kue Delapan Jam on Various Steaming Time. In International Journal of Science and Engineering (Vol. 7, Issue 2, pp. 161–165). Faculty of Engineering Diponegoro University. https://doi.org/10.12777/ijse.7.2.161-165

26. Capuano, E., Ferrigno, A., Acampa, I., Ait-Ameur, L., & Fogliano, V. (2008). Characterization of the Maillard Reaction in Bread Crisps. In European Food Research and Technology (Vol. 228, Issue 2, pp. 311–319). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-008-0936-5

27. Ames, J. M. (1998). Applications of the Maillard Reaction in the Food Industry. In Food Chemistry (Vol. 62, Issue 4, pp. 431–439). Elsevier BV. https://doi.org/10.1016/S0308-8146(98)00078-8

28. Martins, S. I. F. S., Jongen, W. M. F., & van Boekel, M. A. J. S. (2000). A Review of Maillard Reaction in Food and Implications to Kinetic Modelling. In Trends in Food Science & Technology (Vol. 11, Issues 9–10, pp. 364–373). Elsevier BV. https://doi.org/10.1016/S0924-2244(01)00022-X

29. Yang, L., Wang, S., Zhang, W., Zhang, H., Guo, L., Zheng, S., & Du, C. (2022). Effect of Black Soybean Flour Particle Size on the Nutritional, Texture and Physicochemical Characteristics of Cookies. In LWT (Vol. 164, pp. 1–10). Elsevier BV. https://doi.org/10.1016/J.LWT.2022.113649

30. Hedhili, A., Lubbers, S., Bou-Maroun, E., Griffon, F., Akinyemi, B. E., Husson, F., & Valentin, D. (2021). Moringa oleifera Supplemented Biscuits: Nutritional Values and Consumer Segmentation. In South African Journal of Botany (Vol. 138, pp. 406–414). Elsevier BV. https://doi.org/10.1016/j.sajb.2021.01.017

31. Widodo, P., Budiningsari, D., & Susetyowati. (2019). Cookies Prepared using Peanuts, Pregerminated Soybean, and Moringa Leaf Flour Improve Food Intake and Nutritional Status in Malnourished Patients. In Journal of Food and Nutrition Research (Vol. 7, Issue 10, pp. 709–716). Science and Education Publishing Co., Ltd. https://doi.org/10.12691/JFNR-7-10-4

32. Pokharel, A., Dangal, A., Karki, S., Lamichhane, S., Timsina, P., & Bohara, A. (2023). Study on the Effect of Different Treatments on Soy Flour, and Quality and Sensory Evaluation of Prepared Biscuits Incorporated with Oats and Soy Flour. In Legume Science (pp. 1–10). Wiley. https://doi.org/10.1002/leg3.181

33. Yang, Lingyu, Ying, Z., Li, H., Li, J., Zhang, T., Song, Y., & Liu, X. (2023). Extrusion Production of Textured Soybean Protein: the Effect of Energy Input on Structure and Volatile Beany Flavour Substances. In Food Chemistry (Vol. 405, pp. 1–11). Elsevier BV. https://doi.org/10.1016/j.foodchem.2022.134728

34. Widiany, F. L., Sja'bani, M., Susetyowati, & Huriyati, E. (2022). Provision of Local Food-Based Formula Using Pila Ampullacea, Tempeh, and Moringa oleifera Leaves to the Acceptability and Nutrition Intake in Hemodialysis Patients. In Jurnal Gizi Indonesia (The Indonesian Journal of Nutrition) (Vol. 10, Issue 2, pp. 95–102). Institute of Research and Community Services Diponegoro University (LPPM UNDIP). https://doi.org/10.14710/jgi.10.2.95-102

35. Yadav, K., Bhattarai, S., Shiwakoti, L. D., Paudel, S., Subedi, M., Pant, B. R., Paudel, M., Dhugana, S., Bhattarai, S., Tiwari, T., Koirala, N., Aljarba, N. H., Alkahtani, S., El-Saber Batiha, G., Shiwakoti, R., & Upadhyaya, J. (2022). Sensorial and Chemical Analysis of Biscuits Prepared by Incorporating Moringa Flower Powder and Leaf Powder. In International Journal of Food Properties (Vol. 25, Issue 1, pp. 894–906). Informa UK Limited. https://doi.org/10.1080/10942912.2022.2069807

36. Maina, J. W. (2018). Analysis of the Factors that Determine Food Acceptability. In The Pharma Innovation Journal (Vol. 7, Issue 5, pp. 253–257). www.thepharmajournal.com.

37. Sinesio, F., Saba, A., Peparaio, M., Saggia Civitelli, E., Paoletti, F., & Moneta, E. (2018). Capturing Consumer Perception of Vegetable Freshness in a Simulated Real-Life Taste Situation. In Food Research International (Vol. 105, pp. 764–771). Elsevier BV. https://doi.org/10.1016/j.foodres.2017.11.073

38. Kristanti, D., Setiaboma, W., & Herminiati, A. (2020). Physicochemical and Organoleptic Characteristics of Mocaf Cookies with Tempeh Flour Additions. In Biopropal Industri (Vol. 11, Issue 1, pp. 1–8). Jurnal Biopropal Industri, Balai Riset dan Standardisasi Industri Pontianak. https://doi.org/10.36974/jbi.v11i1.5354

39. Regulation of the Indonesian Food and Drug Authority (BPOM) Number 9 of 2016 on Nutrition Label References. Retrieved from https://peraturan.go.id/files/bn792-2016.pdf. 1–9, 2016.

40. Azzini, E., Peluso, I., Intorre, F., Barnaba, L., Venneria, E., Foddai, M. S., Ciarapica, D., Maiani, F., Raguzzini, A., & Polito, A. (2022). Total and Plant Protein Consumption: The Role of Inflammation and Risk of Non-Communicable Disease. In International Journal of Molecular Sciences (Vol. 23, Issue 14, pp. 1–12). MDPI AG. https://doi.org/10.3390/ijms23148008

41. Qi, X.-X., & Shen, P. (2020). Associations of Dietary Protein Intake with All-Cause, Cardiovascular Disease, and Cancer Mortality: A Systematic Review and Meta-Analysis of Cohort Studies. In Nutrition, Metabolism and Cardiovascular Diseases (Vol. 30, Issue 7, pp. 1094–1105). Elsevier BV. https://doi.org/10.1016/j.numecd.2020.03.008

42. Movahedian, M., Tabibi, H., Atabak, S., Hedayati, M., Rahmani, L., & Yari, Z. (2021). Effects of Soy Isoflavones on Glycemic Parameters and Blood Pressure in Peritoneal Dialysis Patients: A Randomized, Double Blind, Placebo-Controlled Trial. In Iranian Journal of Kidney Diseases (Vol. 1, Issue 2, pp. 134–142). Iranian Society of Nephrology.

43. Bakhtiari, A., Hajian-Tilaki, K., Omidvar, S., & Nasiri-Amiri, F. (2019). Clinical and metabolic response to soy administration in older women with metabolic syndrome: a randomized controlled trial. In Diabetology; Metabolic Syndrome (Vol. 11, Issue 1, pp. 1–12). Springer Science and Business Media LLC. https://doi.org/10.1186/s13098-019-0441-y

44. Ruscica, M., Pavanello, C., Gandini, S., Gomaraschi, M., Vitali, C., Macchi, C., Morlotti, B., Aiello, G., Bosisio, R., Calabresi, L., Arnoldi, A., Sirtori, C. R., & Magni, P. (2016). Effect of Soy on Metabolic Syndrome and Cardiovascular Risk Factors: A Randomized Controlled Trial. In European Journal of Nutrition (Vol. 57, Issue 2, pp. 499–511). Springer Science and Business Media LLC. https://doi.org/10.1007/s00394-016-1333-7

45. Qian, L., Zhang, F., Yin, M., & Lei, Q. (2021). Cancer Metabolism and Dietary Interventions. In Cancer Biology & Medicine (Vol. 19, Issue 2, pp. 163–174). China Anti-Cancer Association. https://doi.org/10.20892/j.issn.2095-3941.2021.0461

46. Qin, P., Wang, T., & Luo, Y. (2022). A Review on Plant-Based Proteins From Soybean: Health Benefits and Soy Product Development. In Journal of Agriculture and Food Research (Vol. 7, pp. 1–8). Elsevier BV. https://doi.org/10.1016/j.jafr.2021.100265

47. Mbikay, M. (2012). Therapeutic Potential of Moringa oleifera Leaves in Chronic Hyperglycemia and Dyslipidemia: A Review. In Frontiers in Pharmacology (Vol. 3, pp. 1–12). Frontiers Media SA. https://doi.org/10.3389/fphar.2012.00024

48. Iizuka, K. (2022). Is the Use of Artificial Sweeteners Beneficial for Patients with Diabetes Mellitus? The Advantages and Disadvantages of Artificial Sweeteners. In Nutrients (Vol. 14, Issue 21, pp. 1–11). MDPI AG. https://doi.org/10.3390/nu14214446

49. Yu, S., Wang, W., Li, S., Li, J., Zhao, R., Liu, D., & Wu, J. (2023). Glucoregulatory Properties of Fermented Soybean Products. In Fermentation (Vol. 9, Issue 3, pp. 1–23). MDPI AG. https://doi.org/10.3390/fermentation9030254

50. Magaji, U. F., Sacan, O., & Yanardag, R. (2020). Alpha Amylase, Alpha Glucosidase and Glycation Inhibitory Activity of Moringa Oleifera Extracts. In South African Journal of Botany (Vol. 128, pp. 225–230). Elsevier BV. https://doi.org/10.1016/j.sajb.2019.11.024

51. Nguyen, H. D., Oh, H., & Kim, M.-S. (2022). Higher intakes of nutrients are linked with a lower risk of cardiovascular diseases, type 2 diabetes mellitus, arthritis, and depression among Korean adults. In Nutrition Research (Vol. 100, pp. 19–32). Elsevier BV. https://doi.org/10.1016/j.nutres.2021.11.003

52. Soliman, G. A. (2019). Dietary Fibre, Atherosclerosis, and Cardiovascular Disease. In Nutrients (Vol. 11, Issue 5, pp. 1–11). MDPI AG. https://doi.org/10.3390/nu11051155

53. Hodas, F., Zorzenon, M. R. T., & Milani, P. G. (2021). Moringa Oleifera Potential as a Functional Food and a Natural Food Additive: A Biochemical Approach. In Anais da Academia Brasileira de Ciências (Vol. 93, Issue suppl 4, pp. 1–8). FapUNIFESP (SciELO). https://doi.org/10.1590/0001-3765202120210571

54. Nova, E., Redondo-Useros, N., Martínez-García, R. M., Gómez-Martínez, S., Díaz-Prieto, L. E., & Marcos, A. (2020). Potential of Moringa oleifera to Improve Glucose Control for the Prevention of Diabetes and Related Metabolic Alterations: A Systematic Review of Animal and Human Studies. In Nutrients (Vol. 12, Issue 7, pp. 1–28). MDPI AG. https://doi.org/10.3390/nu12072050

55. Asri, M., Irwan, A. M., Sjattar, E. L., & Hardianto, Y. (2022). Effectiveness of a Low-Salt Diet in Rural Hypertensive Patients: A Systematic Review. In Clinical Epidemiology and Global Health (Vol. 15, pp. 1–7). Elsevier BV. https://doi.org/10.1016/j.cegh.2022.101024

56. Xia, J., Wan, Y., Wu, J., Yang, Y., Xu, J.-F., Zhang, L., Liu, D., Chen, L., Tang, F., Ao, H., & Peng, C. (2022). Therapeutic Potential of Dietary Flavonoid Hyperoside Against Non-Communicable Diseases: Targeting Underlying Properties of Diseases. In Critical Reviews in Food Science and Nutrition (pp. 1–31). Informa UK Limited. https://doi.org/10.1080/10408398.2022.2115457

57. Villarruel-López, A., López-de la Mora, D. A., Vázquez-Paulino, O. D., Puebla-Mora, A. G., Torres-Vitela, M. R., Guerrero-Quiroz, L. A., & Nuño, K. (2018). Effect of Moringa oleifera consumption on diabetic rats. In BMC Complementary and Alternative Medicine (Vol. 18, Issue 1, pp. 1–10). Springer Science and Business Media LLC. https://doi.org/10.1186/s12906-018-2180-2

58. Regulation of the Indonesian Food and Drug Authority (BPOM) Number 1 of 2022 on Control of Claim on Processed Food Labels and Advertisements. Retrieved from https://peraturan.go.id/files2/peraturan-bpom-no-1-tahun-2022_terjemah.pdf.

Funds:

This study was supported by a grant Directorate General of Higher Education, Research, and Technology, Number 3186/UN1/DITLIT/Dit-Lit/PT.01.03/2023.

Acknowledgments:

We thank the assistants and panellists for following this study from first to finish.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This study obtained ethical clearance from the Ethical Commission of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Indonesia, with Ref. No.: KE/FK/1514/EC/2023.

Contact Address:

Lily Arsanti Lestari, Universitas Gadjah Mada, Faculty of Medicine, Public Health and Nursing, Department of Nutrition and Health, Yogyakarta, 55281
Tel.: +62274547775
E-mail: lily_al@ugm.ac.id
ORCID: https://orcid.org/0000-0003-2930-4254

***Susetyowati**, Universitas Gadjah Mada, Faculty of Medicine, Public Health and Nursing, Department of Nutrition and Health, Yogyakarta, 55281
Tel.: +62274547775
E-mail: susetyowati@ugm.ac.id
ORCID: https://orcid.org/0000-0002-3990-6403

Azizah Isna Rachmawati, Universitas Gadjah Mada, Faculty of Medicine, Public Health and Nursing, Department of Nutrition and Health, Yogyakarta, 55281
Tel.: +62274547775
E-mail: azizah.isna.rachmawati@mail.ugm.ac.id
ORCID: https://orcid.org/0000-0003-0859-116X

Ajeng Saskia Sekar Rachmadanty, Universitas Gadjah Mada, Faculty of Medicine, Public Health and Nursing, Department of Nutrition and Health, Yogyakarta, 55281
Tel.: +62274547775
E-mail: ajeng.saskia.sekar@mail.ugm.ac.id
ORCID: https://orcid.org/0009-0006-2698-2841

Amalia Sarah Sholikhati, Universitas Gadjah Mada, Faculty of Medicine, Public Health and Nursing, Department of Nutrition and Health, Yogyakarta, 55281
Tel.: +62274547775
E-mail: amalia.sarah.sholikhati@mail.ugm.ac.id
ORCID: https://orcid.org/0000-0002-4375-6874

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 28.4.2024
Revised: 19.6.2024
Accepted: 3.7.2024
Published: 16.7.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 666-682
https://doi.org/10.5219/1981
ISSN: 1337-0960 online
www.potravinarstvo.com

The influence of different methods of decontamination of microbial biofilms formed on eggshells

Mykola Kukhtyn, Zhanna Sverhun, Yulia Horiuk, Volodymyr Salata, Svitlana Laiter-Moskaliuk, Mykhailo Mocherniuk, Larysa Kladnytska, Victor Horiuk

ABSTRACT

According to "food legislation" requirements, all eggs entering the production of egg products must be disinfected. Therefore, developing technologies for decontaminating chicken eggs before use for food purposes is a promising work direction in chicken egg production and storage. This research aimed to identify the microbiota of chicken eggs with varying degrees of shell contamination and determine the influence of different methods of decontaminating microbial biofilms formed on eggshells. It was set up that the quantitative content of microorganisms on the surface of chicken eggs ranged from 10^3 CFU to 10^6 CFU/ml of washing and depended on the contamination of the shell with droppings. *Lactobacillus* spp., *Bacillus* spp., *Corynebacterium*, *Staphylococcus* were among the genera of bacteria that prevailed on the clean chicken shell, which were isolated in 30-50% of cases, and gram-negative microbiota was practically absent. The constant release of gram-positive bacteria is noted on the contaminated eggshell, and the frequency of identification of gram-negative microbiota of the *Enterobacteriaceae* genus and non-fermenting genera *Pseudomonas* and *Psychrobacter* increases. That is, the microbial scape of the microbiota of the chicken shell depends on its cleanliness, and the presence of a dirty surface increases the frequency of allocation of the resident microflora of the gastrointestinal tract. It was found that the working solution of the disinfectant Vircon S destroyed planktonic bacteria applied to the eggshell in an average of 2 minutes of exposure, stabilised water ozone for 1 minute, gaseous ozone for 3 minutes, and the action of ultraviolet rays with a length of 253.7 nm for 25-30 min. At the same time, using these disinfection methods on bacteria formed in a biofilm on the eggshell did not cause a bactericidal action during this time. To significantly reduce bacteria in the biofilm using these methods, it is necessary to increase the exposure time of the biocide by 2-3 times. Therefore, the complex structure of the eggshell and the multi-layered matrix of biofilms provide better protection for bacteria against the influence of the investigated disinfection methods.

Keywords: chicken egg, disinfection, biofilm, eggshell microbiota, planktonic bacteria

INTRODUCTION

In the world, the issue of long-term storage of edible eggs and egg products with guaranteed quality and safety characteristics is relevant. After all, the safety of manufactured egg products directly depends on the safety of eggs **[1]**, **[2]**, **[3]**. If technological storage modes have long been included in world standards, then the issue of sanitary tilling of eggs before processing remains debatable **[4]**, **[5]**.

A significant source of bacterial insemination of edible eggs in the process of getting is considered to be the low sanitary condition of premises, equipment **[6]**, apparatus **[7]**, inventory workers' overalls **[8]**, **[9]**, **[10]** etc. In

particular, the researchers found that the microbial contamination of chicken eggs ranges from 10^2 CFU to 10^6 CFU, which depends on the contamination of the surface of the shell with droppings **[4]**.

Egg products (melange, egg powder) are widely used in the food industry and public food. Egg products are made from edible chicken eggs using a technology that involves washing and disinfecting them in various ways **[8]**. Using molecular genetic methods, researchers isolated a variety of microflora from the surface of clean and contaminated chicken eggs, including genera *Lactobacillus*, *Staphylococcus*, *Psychrobacter*, *Pseudomonas*, *Salinicoccus*, *Clostridium*, *Bacteroides*, *Micobacterium*, *Aerococcus*, and others **[4]**, **[11]**, **[12]**. In addition, it is reported that the composition of the surface microbiota of eggs and the frequency of their release depends on surface contamination and sanitary conditions of getting and storing eggs **[13]**, **[14]**, **[15]**, **[16]**. Therefore, to reduce the contamination of egg products with microflora, disinfection of eggs with various biocidal preparations (based on formalin, quaternary ammonium compounds, polyhexabioguanidine, calcium hypochlorite, glutaraldehyde, hydrogen peroxide, chlorine-containing, etc.) is used **[17]**, **[18]**, **[19]**, **[20]**, **[21]**. The disadvantages of the significant use of disinfectants include the formation and spread of resistant microorganisms and the possible accumulation of biocide residues in products **[4]**, **[17]**, **[22]**, **[23]**. Researchers indicate that one of the mechanisms of survival of microbiota on the surface of the shell of chicken eggs is the presence of pores and channels through which bacteria quickly penetrate the middle of the membranes, especially if they are contaminated with faeces **[24]**. This prevents contact of biocidal preparations with bacterial cells. In addition, a contributing factor to the resistance of bacteria in the middle of shell membranes is their ability to form biofilms **[25]**, which further protect cells from disinfectants.

In addition to chemical means for decontaminating chicken eggs, researchers report other methods of reducing egg microbial contamination, in particular, ultrasonic washing [26], gaseous ozone [28], ultraviolet radiation [27], and activated plasma **[29]**, **[30]**. However, these methods have not yet been found to be of sufficient industrial use.

Despite the considerable arsenal of methods of decontaminating chicken eggs, manufactured egg products always contain microflora **[4]**, **[5]**. Therefore, the rapid reproduction of residual microflora begins during the thawing of the melange. Therefore, the quality of the sanitary processing of eggs and compliance with the sanitary and hygienic regime significantly influence the level of seeding of egg products with microorganisms. Considering the above, a promising direction in the technology of production of egg products with minimal microbial contamination is the development and use of simple, cheap, ecological and effective methods of disinfection of eggs.

The work aimed to identify the microbiota of chicken eggs at different levels of shell contamination and investigate the influence of four methods of disinfection of microbial biofilms formed on the eggshell.

Scientific Hypothesis

The use of Vircon S disinfectant, gaseous ozone, stabilized water ozone, and ultraviolet radiation will significantly reduce the microbial load in biofilms on eggshells, as compared to untreated eggshells, thereby enhancing the safety and shelf-life of egg products.

MATERIAL AND METHODOLOGY

Selection of chicken eggs was carried out at the Ternopil Poultry Factory (Ternopil, Ukraine), and microbiological studies were carried out in the laboratories of the Podillia State University (Kamianets-Podilskyi, Ukraine).

Samples

The microflora of 33 chicken eggs, which were clean (shell without visible signs of mechanical contamination), was investigated; 33 eggs – conditionally clean (traces of mechanical contamination on the shell); 33 – contaminated (the surface of the shell contained visible traces of contamination up to 30% of the entire area).

Chemicals

Virkon S disinfectant (Lanxess, Cologne, Germany) was used in the experiment; nutrient media: meat peptone agar, meat peptone broth, Saburo, Endo, Enterococcus-agar, Kesler (Pharmaktiv, Ukraine), MRS-agar, Bile Esculin Azide agar, *Streptococcus Selective agar* (HiMedia, India); Baird-Parker agar, cetramide agar (Merck KGaA, Germany).

Animals and Biological Material

The bacterial strains *S. aureus* ATCC 25923, *P. aeruginosa* 27/99, *E. coli* 055K59 No. 3912/41, *and Enterococcus faecalis ATCC 19433 were obtained from the State Scientific and Control Institute of Biotechnology and Strains of Microorganisms* (Kyiv, Ukraine). The bacterial culture was obtained by reviving the lyophilisates in the liquid nutrient broth after 24-48 h of incubation at 37 °C.

Instruments

Gaseous ozone produced by an (ATWFS, China), ozone generator, stabilized water ozone (Baerain, China), and a 253.7 nm ultraviolet lamp (laminar cabinet model AC2-4E8, ESCO, Singapore), were used in the experiment. Multiskan FC microbiological spectrophotometer (Thermo Scientifis, Finland). Collection of washings with the help of a sterile disposable cotton pad, soaked in a peptone-saline solution, the egg's surface was wiped, to which a 2×2 cm stencil was applied.

Laboratory Methods

Determination of the number of aerobic and facultatively anaerobic microorganisms

To determine the number of microorganisms, the selected washes in the amount of 1ml and their tenfold dilutions were sown in the meat peptone agar medium, incubated at a temperature of +30 °C for 72 hours, the number of colonies was counted and the average amount in 1 ml of washings was determined.

To determine the generic and species composition of the microbiota of chicken eggs, the selected washings were sown on selective media for a certain type of bacteria, and the isolated colonies were identified using commercial API test systems (bioMerieux, France).

Determination of the effect of biocides on planktonic bacteria

To determine the influence of the investigated biocides on planktonic bacteria applied to the eggshell, a suspension of test strains of microorganisms was prepared in the amount of 10^7 CFU/ml, applied 0.1 ml per 1 cm^2 of the shell surface, distributed evenly over the entire surface, processed with biocides, kept for a certain time and washings were collected and inoculated into storage medium meat peptone broth with glucose (Pharmaktiv, Ukraine) (Figure 1).

Figure 1 Study of the effect of biocides on planktonic bacteria on eggshells.

Determining the number of bacteria in biofilms on eggshells after exposure to biocides

It was carried out on daily microbial biofilms grown on chicken eggshells in Petri dishes. To do this, 1 ml of a suspension of test bacteria in peptone water with glucose was applied to 1 cm^2 of the area of the chicken shell, kept in a thermostat at a temperature of + 37 °C for 24 hours, after which the unattached bacteria were washed off with a sterile phosphate buffer, the shell was dried for 15-20 min and processed with biocides, kept for a specific time, the biocide was washed off with phosphate buffer and the washings was collected, which was sown on elective media to count the surviving bacteria.

Determination of the density of the formed biofilms on the eggshell after the influence of biocides

1 ml of a suspension of test bacteria in peptone water with glucose was applied to 1 cm^2 of the area of the chicken shell and kept in a thermostat at a temperature of + 37 °C for 24 hours after that, the unattached bacteria were washed off with a sterile phosphate buffer, the shell was dried for 15-20 minutes and processed with biocides, kept for a certain time, washed off the biocide with phosphate buffer, dried the shell, processed with ethyl alcohol for 10 min, dried, stained with crystal violet, then the shell was placed in the well of the tablet, ethyl alcohol was added, crystal violet was washed off, and the density of the solution was measured on a Multiskan FC (Thermo Scientific, Finland) multichannel microbiological spectrophotometer at a wavelength of 584 nm **[31]**. The results were interpreted as the average arithmetic value of the optical density of 3 experimental wells (Figure 2).

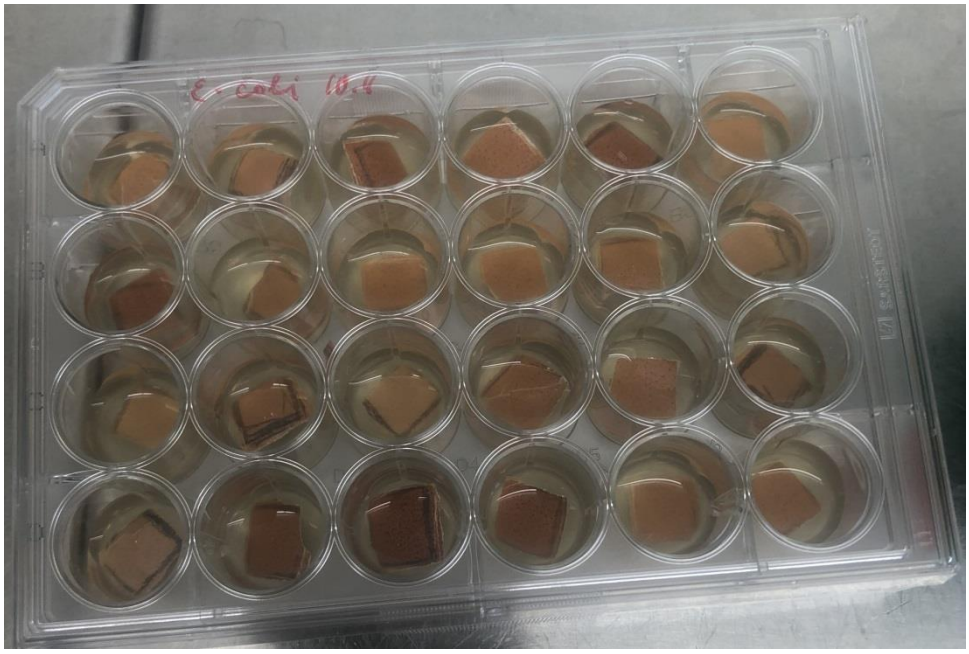


Figure 2 Study of the density of the formed biofilms on the eggshell.

Description of the Experiment

Sample preparation: Freshly laid chicken eggs were collected from poultry houses with caged chickens the eggs were visually evaluated for contamination, placed in a plastic container, and delivered to the microbiological laboratory for research within 2-4 hours at a temperature of +4 to +6 °C.

Number of samples analyzed: We analyzed 99 samples.

Number of repeated analyses: All measurements of instrument readings were performed three times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was three times.

Design of the experiment: To determine the quantitative content of mesophilic microorganisms on the surface of the shell of chicken eggs, depending on their contamination; to determine the generic composition of the microbiota of chicken eggs depending on their contamination; to investigate the time of the bactericidal action of the Vircon S disinfectant, gaseous and stabilized water ozone, and ultraviolet radiation on planktonic and bioplic strains of bacteria on eggshells.

Statistical Analysis

Statistical processing of the results was carried out using methods of variation statistics using Statistica 9.0 (StatSoft Inc., USA). Non-parametric methods of research were used (Wilcoxon-Mann-Whitney test). The arithmetic mean (x) and the mean (SE) standard error were determined. The difference between the comparable values was considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

During the investigation of the quantitative contamination of the surface of the shell of chicken eggs, we conditionally divided the eggs into three groups: the first – clean (shell without visible signs of mechanical contamination); the second - conditionally clean (traces of mechanical contamination on the shell); the third is contaminated (the surface of the shell contained clearly visible traces of contamination up to 30% of the entire area). Quantitative determination of the mesophilic microbiota of the shell of chicken eggs at different degrees of contamination revealed (Table 1) the naturally lowest content of microorganisms on the surface of the shell of the first group. In particular, the number of bacteria on the shell of these eggs was $7.3 \pm 0.3 \times 10^3$ CFU/cm^3 of washings. In the presence of minor contamination of the shell surface (the second group), an increase of one order of magnitude in the microbial number of the mesophilic microflora is noted compared to the first group.

Table 1 Microbial contamination of the surface of chicken eggs with different purity, x ±SE.

Conditional groups of egg purity	The number of examined eggs, n	The number of aerobic and facultative anaerobic microorganisms, CFU/cm^3 of washings
The first	7	7.3 ±0.3 ×10^3
The second	7	8.6 ±0.2 ×10^{4*}
The third	7	4.7 ±0.2 ×10^{6*}

Note: * – p <0.05 to the number in the first group.

In the third group, the eggshells were dirty, respectively, in the washings from the surface, the largest number of mesophilic bacteria was found – 4.7 ±0.2×10^6 CFU/cm^3, which is approximately two orders of magnitude higher than in the shells of the second group and three orders of magnitude higher, comparing with the first group.

Therefore, to reduce the microbiota in egg products, it is necessary to use eggs with the least contaminated shell and to use various safe methods to neutralize the microbiota on the surface.

The cleanliness of the surface of the egg shell, which is subjected to various methods of technological processing (washing, disinfection) before use, significantly influences the formation of the microbiota of egg products. The actual investigation of microbiota of the surface of chicken eggs is a necessary condition in developing a technology to disinfect them for the production of safe egg products. During the investigation of various methods of disinfection of chicken eggs, at the first stage, the microflora of the eggshell was investigated (Figure 3).

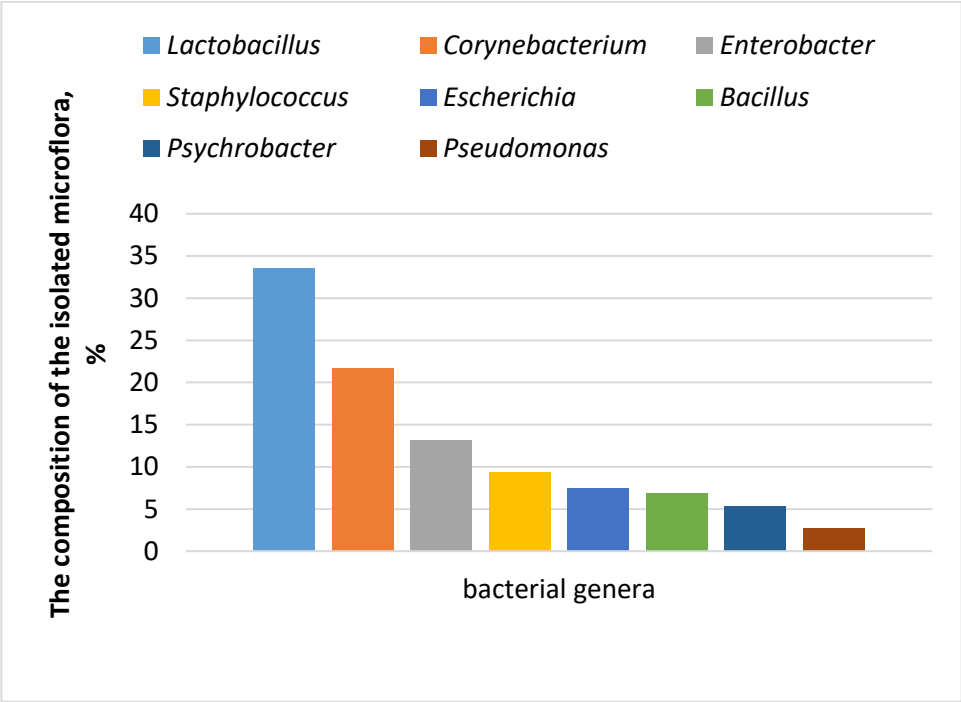


Figure 3 Genus composition of the microbiota identified on the shell of chicken eggs.

It was found that the microbiota of eggs is represented by genera of bacteria that usually belong to the resident microflora of the gastrointestinal tract of poultry. In particular, the largest share was made up of bacteria of the genus *Lactobacillus* spp. – 33.5 ±0.8% among the isolated microorganisms. In the second place were representatives of the genus *Corynebacterium* spp., which accounted for 21.7 ±0.5% of the studied bacteria. Ubiquitous bacteria of the genus *Staphylococcus* spp. in this biotope was 9.4 ±0.3% among the investigated aerobic and facultatively anaerobic bacteria, and aerobic spore-forming bacilli were 6.8 ±0.2%.

The representatives of the *Enterobacteriaceae* genus (*Enterobacter* spp. and *Escherichia* spp.) comprised 20.8 ±0.5% of the microbial population of the eggshell surface. At the same time, the share of *Escherichia* spp. was 1.8 times less than *Enterobacter* spp. Non-fermenting aerobic representatives of the genera *Psychrobacter* spp. and *Pseudomonas* spp. Occupied the smallest niche among the representatives of the eggshell surface microbiota – 5.3 ±0.2 and 2.7 ±0.1%, respectively.

The investigations on the frequency of isolation of different genera of bacteria from the surface of the eggshell were of interest since not all detected genera of bacteria are of equal importance, both from the point of view of

safety and compliance with sanitation and from the point of view of the presence of technically harmful microbiota that causes defects in egg products.

The frequency of bacterial release from the shell of chicken eggs at different degrees of contamination (Figure 4) revealed a significantly more diverse microbial population on the surface of a dirty shell than on a clean one. In addition, from the shells of clean eggs, much less bacteria were identified than those present on dirtier surfaces. In particular, bacteria of the genus *Lactobacillus* spp. and *Bacillus* spp. were revealed from clean surfaces in an average of 50% of cases. At the same time, the frequency of allocation of these genera of bacteria from the conditionally clean shell was increased by approximately 10 to 63.6%. These genera were identified from dirty shells in 100% of cases.

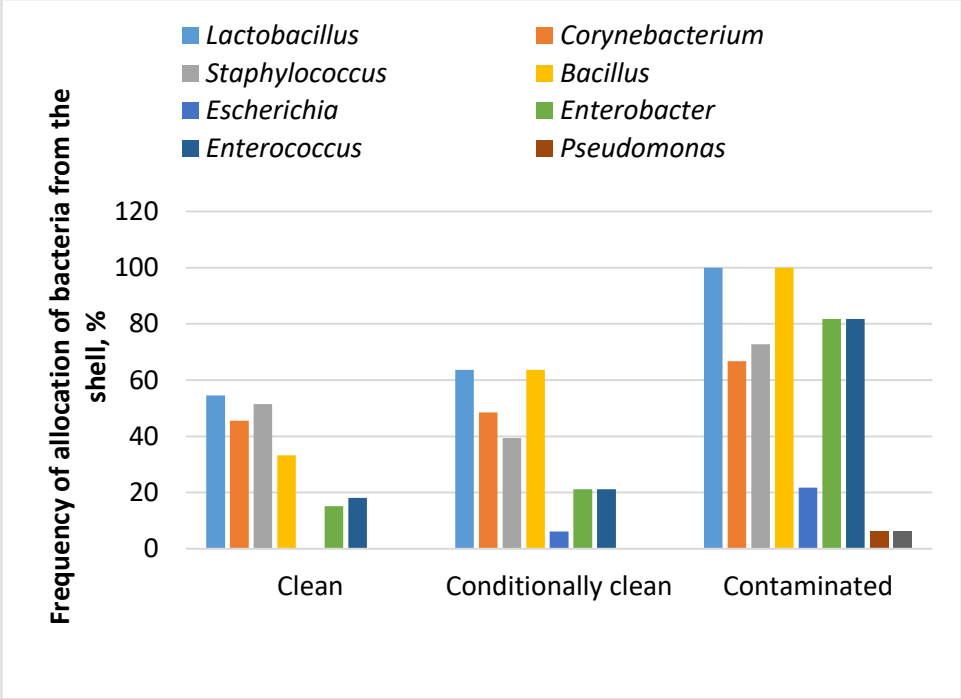


Figure 4 Identification of bacteria from the shell of chicken eggs at different degrees of contamination, n = 33.

The frequency of allocation of bacteria of the genus *Corynebacterium* from clean and conditionally clean shells was approximately the same and amounted to 45.5 and 48.5%, respectively. On the contaminated eggshell, the allocation frequency of corynebacteria was increased by 1.5 and 1.4 times, respectively. A similar tendency about the frequency of allocation was observed in bacteria of the genus *Staphylococcus* spp., which were released from the shell of eggs of the first and second groups in 33.3 and 39.4% of cases and from the surface of eggs of the third group in 2.2 and 1.8 times, respectively more often.

The frequency of identification of bacteria of the genus *Escherichia* depended on the cleanliness of the eggshell since they were not isolated from the surface of the first group of eggs, from the second group only in 6.1% of cases, and from the third (dirty) group the most – in 21.7% of cases. The frequency of identification of *Enterobacter* spp. and *Enterococcus* spp. from pure eggshells was insignificant and amounted to 15.1-18.1%, respectively, and from conditionally clean eggshells, a slight increase was noted to 21.2%. At the same time, the frequency of isolation of these types of bacteria from the dirty surface of the eggs was increased by an average of 4 times compared to the conditionally clean shell.

Gram-negative non-fermenting bacteria of the genera *Pseudomonas* and *Psychrobacter* were not identified on clean and conditionally clean shells, and the frequency of isolation from the dirty egg surface was 6.1% for the two genera.

Therefore, the microbial landscape of the chicken shell's microbiota depended on its cleanliness. In the presence of a dirty surface, the frequency of isolation of different genera of bacteria increases.

At the next research stage, the influence of disinfecting the shell of chicken eggs with Vircon S disinfectant, two forms of ozone: gaseous and stabilised water and ultraviolet rays, was determined. First, the time of the bactericidal action of selected biocides and ultraviolet rays at the concentration recommended by the instructions for use was investigated on planktonic forms of strains of bacteria and then on biofilms formed on the shell of chicken eggs.

It was set up (Table 2) that for the destruction of test strains of bacteria applied to the shell of chicken eggs in the amount of 10^6 CFU/cm^2 of the area, the Vircon S disinfectant in a working concentration of 1% must act for

an average of 2 minutes. The bactericidal action of stabilized aqueous ozone at a concentration of 0.0023% on these strains of bacteria on the chicken shell appeared after 1 minute of washing. Gaseous ozone at a concentration of 60 mg/h had a bactericidal action on plankton cultures of strains after three minutes of the influence. The longest time was required to achieve a bactericidal effect on planktonic bacteria using ultraviolet irradiation, an average of 30 min.

Table 2 The influence of the investigated biocides on planktonic bacteria applied to the eggshell, x ±SE.

Bacteria	The number of bacteria (CFU) in 1 cm^3 of washing from the surface before processing with a biocide	Disinfectant exposure time for which the bactericidal action was appeared, min			
		Vircon S (1% solution)	SAO (0.0023% conc. ozone)	gaseous ozone (60 mg/h)	ultraviolet radiation, 253.7 nm
S. aureus	$3.7 \pm 0.1 \times 10^6$	2 min	1 min	3 min	30 min
E. coli	$2.5 \pm 0.1 \times 10^6$	1 min	1 min	3 min	25 min
P. aeruginosa	$3.3 \pm 0.1 \times 10^6$	2 min	1 min	3 min	30 min
E. faecalis	$4.1 \pm 0.2 \times 10^6$	2 min	1 min	3 min	30 min

Therefore, the received results regarding the exposure time of disinfectants during which the bactericidal action on planktonic bacteria on the eggshell was used to investigate the influence of disinfection modes on biofilm forms of bacteria.

Since the relief of the eggshell is heterogeneous, it was essential to compare the time of bactericidal action of disinfectants on planktonic forms with biofilms formed on the surface of the shell.

Table 3 The influence of disinfectant Vircon S (1%) on bacteria in biofilms formed on eggshells, x ±SE.

Bacteria	The number of bacteria (CFU) in 1 cm^3 of washing from the surface before processing with a biocide	The number of bacteria (CFU) in the biofilm after the action of the biocide during, min			
		1	2	3	4
S. aureus	$4.7 \pm 0.2 \times 10^8$	$5.6 \pm 0.2 \times 10^4$	$8.4 \pm 0.3 \times 10^2$	$2.8 \pm 0.1 \times 10^1$	4.2 ± 0.2
E. coli	$5.8 \pm 0.2 \times 10^8$	$3.7 \pm 0.2 \times 10^3$	$2.6 \pm 0.1 \times 10^2$	$1.4 \pm 0.1 \times 10^1$	–
P. aeruginosa	$6.5 \pm 0.2 \times 10^8$	$4.1 \pm 0.2 \times 10^3$	$5.1 \pm 0.2 \times 10^2$	$1.7 \pm 0.1 \times 10^1$	1.8 ± 0.1
E. faecalis	$8.4 \pm 0.3 \times 10^8$	$5.1 \pm 0.2 \times 10^3$	$3.7 \pm 0.1 \times 10^2$	$2.0 \pm 0.1 \times 10^1$	2.1 ± 0.1

It was found (Table 3) that the disinfectant Vircon S in the concentration according to the instructions for use within one minute of the influence reduced the number of cells in the biofilm formed by *S. aureus* by four orders of magnitude. In the biofilms formed by *E. coli, P. aeruginosa* and *E. faecalis* the number of viable cells was reduced by five orders of magnitude, compared to the content before processing. During the next minute of action of the disinfectant, the bactericidal effect on the bacterial cells in the biofilm was less pronounced, as their number was decreased by only one order of magnitude. Three minutes later, after exposure to Vircon S on eggshells with microbial biofilms, a decrease in the number of bacteria by one order of magnitude was noted, compared to the two-minute action of the biocide. At the same time, during the five-minute exposure to the disinfectant, no live cells were detected from the biofilms formed by *E. coli,* and from the biofilms of *P. aeruginosa* and *E. faecalis,* the number of bacteria was, on average, 2.0 ±0.1 CFU/cm^3 of washing. The most cells were isolated after 5 minutes of Vircon S action from the staphylococcal biofilm – 4.2 ±0.2 CFU. Therefore, the bactericidal action of the Vircon S disinfectant against bacteria in biofilms on the eggshell is approximately 5 minutes, against 1 – 2 minutes for planktonic forms. In addition, single cells were released from the biofilms formed by *S. aureus, P. aeruginosa,* and *E. faecalis* even within 5 minutes of the biocide action.

The investigation of the anti-biofilm action of stabilised aqueous ozone is shown in Table 4.

Table 4 The influence of stabilized aqueous ozone (0.0023%) on bacteria in biofilms formed on eggshell, x ±SE.

Bacteria	The number of bacteria (CFU) in 1 cm^3 of washing from the surface before processing with a biocide	The number of bacteria (CFU) in the biofilm after the action of the biocide during, min			
		1	2	3	4
S. aureus	$5.1 \pm 0.2 \times 10^8$	$6.2 \pm 0.2 \times 10^3$	$8.4 \pm 0.3 \times 10^2$	$6.3 \pm 0.2 \times 10^1$	8.7 ± 0.3
E. coli	$5.9 \pm 0.2 \times 10^8$	$4.1 \pm 0.1 \times 10^3$	$5.4 \pm 0.2 \times 10^2$	$3.8 \pm 0.1 \times 10^1$	–
P. aeruginosa	$6.4 \pm 0.2 \times 10^8$	$5.4 \pm 0.2 \times 10^3$	$7.3 \pm 0.2 \times 10^2$	$5.4 \pm 0.2 \times 10^1$	–
E. faecalis	$8.2 \pm 0.3 \times 10^8$	$4.3 \pm 0.2 \times 10^3$	$7.7 \pm 0.3 \times 10^2$	$5.9 \pm 0.2 \times 10^1$	–

Stabilised water ozone in the concentration produced by the ozonator penetrated well into the pores of the eggshell and the matrix of the biofilm since within one minute of exposure, the number of viable cells was decreased by five orders of magnitude to 10^3 CFU/cm^3 of washing. During the next two minutes of action of stabilised water ozone, the number of bacteria was decreased by two orders of magnitude, on average to $5.0 \pm 0.2 \times 10^1$ CFU/cm^3 washed from the shell surface. At the same time, five minutes after the beginning of the processing of the eggshell with ozone, viable bacteria were isolated only from the biofilm formed by *S. aureus* in the amount of 8.7 ± 0.3 CFU/cm^3 of washing.

Thus, the bactericidal action of stabilised aqueous ozone at a concentration of 0.0023% on the biofilm forms of bacteria formed on the eggshell mainly appeared within 5 minutes of exposure. At the same time, bacterial cells in staphylococcal biofilms were more protected since even after 5 min of exposure, single viable bacteria were isolated.

Along with stabilised water ozone, the bactericidal action on biofilm forms of gaseous was determined (Table 5).

Table 5 The influence of gaseous ozone (60 mg/h) on bacteria in biofilms formed on eggshells, x ±SE.

Bacteria	The number of bacteria (CFU) in 1 cm^3 of washing from the surface before processing with a biocide	The number of bacteria (CFU) in the biofilm after the action of the biocide during, min			
		1	2	3	4
S. aureus	$5.0 \pm 0.2 \times 10^8$	$7.8 \pm 0.3 \times 10^5$	$8.4 \pm 0.3 \times 10^3$	$9.2 \pm 0.3 \times 10^1$	9.7 ± 0.3
E. coli	$6.0 \pm 0.2 \times 10^8$	$3.4 \pm 0.2 \times 10^5$	$3.6 \pm 0.2 \times 10^3$	$7.7 \pm 0.2 \times 10^1$	6.9 ± 0.2
P. aeruginosa	$6.4 \pm 0.2 \times 10^8$	$4.7 \pm 0.2 \times 10^5$	$4.1 \pm 0.2 \times 10^3$	$8.5 \pm 0.3 \times 10^1$	7.3 ± 0.3
E. faecalis	$7.9 \pm 0.3 \times 10^8$	$5.9 \pm 0.3 \times 10^5$	$6.5 \pm 0.3 \times 10^3$	$7.1 \pm 0.3 \times 10^1$	7.1 ± 0.3

It was set up that the bactericidal action of gaseous ozone was weaker on microbial biofilms formed on the eggshell than the influence of stabilized aqueous ozone. In particular, during one minute of exposure to gaseous ozone at a concentration of 60 mg/h, the number of bacteria in biofilms was decreased by approximately three orders of magnitude to 10^5 CFU/cm^3 of washing. Increasing the ozone exposure time to two minutes led to an increase in the bactericidal effect, since two orders of magnitude decreased the number of viable cells in the biofilm and was, on average, 10^3 CFU/cm^3 of washing from the shell. Within three minutes of biofilm processing with gaseous ozone, microbial cells were destroyed significantly, as the number of living bacteria was $8.1 \pm 0.3 \times 10^1$ CFU/cm^3 of washing.

It should be noted that when gaseous ozone acts on the planktonic forms of bacteria on the eggshell, they are destroyed after three minutes. After 5 min of exposure to gaseous ozone on the biofilm forms on the eggshell, up to 10 living cells were isolated from all biofilms. It was also noted that the largest number of viable cells after a three-minute exposure to gaseous ozone was in biofilms formed by *S. aureus* – 9.7 ± 0.3 CFU/cm^3.

So, gaseous ozone at a concentration of 60 mg/h effectively destroyed bacterial cells in the biofilm on the eggshell within 5 minutes of exposure, as 99.99% of living bacteria die during this time.

Ultraviolet rays are widely used to disinfect the surfaces of objects in various sectors of the national economy. The research on the influence of ultraviolet rays on microbial biofilms formed on eggshells (Table 6) found that the cells in the biofilm were much more resistant to the action of irradiation compared to their planktonic forms.

Table 6 The influence of gaseous ozone (60 mg/h) on bacteria in biofilms formed on eggshells, x ±SE.

Bacteria	The number of bacteria (CFU) in 1 cm^3 of washing from the surface before processing with a biocide	The number of bacteria (CFU) in the biofilm after the action of the biocide during, min			
		30	40	50	60
S. aureus	$4.9 \pm 0.2 \times 10^8$	$4.5 \pm 0.2 \times 10^5$	$7.0 \pm 0.2 \times 10^4$	$3.1 \pm 0.2 \times 10^4$	$9.3 \pm 0.3 \times 10^3$
E. coli	$5.9 \pm 0.2 \times 10^8$	$4.7 \pm 0.2 \times 10^5$	$5.7 \pm 0.2 \times 10^4$	$1.8 \pm 0.3 \times 10^4$	$8.1 \pm 0.3 \times 10^3$
P. aeruginosa	$6.3 \pm 0.2 \times 10^8$	$5.6 \pm 0.2 \times 10^5$	$6.8 \pm 0.2 \times 10^4$	$3.4 \pm 0.2 \times 10^4$	$9.1 \pm 0.3 \times 10^3$
E. faecalis	$8.3 \pm 0.3 \times 10^8$	$7.5 \pm 0.3 \times 10^5$	$7.2 \pm 0.3 \times 10^4$	$4.3 \pm 0.3 \times 10^4$	$9.5 \pm 0.3 \times 10^3$

In particular, the action of ultraviolet rays destroyed planktonic forms after approximately 30 minutes of exposure (Table 2), and when acting on biofilms during this time, the number of bacteria, although reduced by three orders of magnitude, was still quite significant (10^5 CFU/cm^3 of washing from the shell). Continuation of the surface processing with ultraviolet rays, even for 60 minutes, did not significantly destroy bacteria in biofilms since an average of $9.0 \pm 0.3 \times 10^3$ CFU/cm^3 of washing was released from the shell.

So, the research found that surface processing with ultraviolet rays effectively kills planktonic bacteria but weakly influences biofilm forms.

Along with investigating the action of selected biocides and ultraviolet rays on the survival of bacteria in the formed biofilms on the eggshell, we determined the influence on the density (degradation) of the biofilm matrix. After all, the resistance of microbial cells to environmental factors is related to the matrix. The results of the degradation of the matrix of microbial biofilms under the influence of Vircon S disinfectant (Table 7) found that already within 1 minute after surface processing with a biocide, the density of microbial biofilms was decreased by an average of 1.5 times, compared to that before processing. However, the biofilms were still of high density and amounted to about 2.0 units. Continuation of the action of the disinfectant Vircon S for 5 minutes increased the destruction of biofilms, which became, on average, 1.8 times less dense compared to the action for 1 minute. At the same time, the density of such biofilms was, on average, 8 times higher than that of destroyed biofilms on eggshells. This indicates that Vircon S penetrates the biofilm matrix, but does not cause its destruction within 5 min of exposure, during which planktonic bacteria and almost all cells in the biofilm die. In addition, it was found that biofilms formed by *E. coli* were the least dense on the eggshell.

Table 7 The influence of disinfectant Vircon S (1%) on the density of microbial biofilms formed on eggshells, x ±SE.

Bacteria	Density of biofilms (units) before the action of the biocide	Density of biofilms (units) after the action of the biocide during, min			
		1	2	3	5
S. aureus	2.79 ±0.07	1.90 ±0.06*	1.57 ±0.05*	1.30 ±0.04*	1.05 ±0.04*
E. coli	2.71 ±0.06	1.84 ±0.06*	1.51 ±0.06*	1.22 ±0.05*	0.98 ±0.04*
P. aeruginosa	2.98 ±0.09	1.94 ±0.07*	1.65 ±0.07*	1.41 ±0.06*	1.11 ±0.05*
E. faecalis	3.21 ±0.09	2.13 ±0.08*	1.90 ±0.08*	1.63 ±0.07*	1.17 ±0.05*
Control (shell without bacteria)	–	–	–	–	0,14 ±0,02

Note: * – $p > 0.05$, before the biocide action.

The research on the process of degradation of biofilms after processing with stabilised aqueous ozone (Table 8) found a tendency that during the first minute after processing with aqueous ozone, the biofilm density was decreased by an average of 1.6 times, and during the next 5 minutes by 1.7-2.0 times. Stabilised water ozone had an influence on the degradation of microbial biofilms from eggshells, similarly to the disinfectant Vircon S. That is, even within 5 min of action, the biofilm matrix was not destroyed and it was still of significant density, especially when compared to the control.

Table 8 The influence of stabilized aqueous ozone (0.0023%) on the density of microbial biofilms formed on eggshells, x ±SE.

Bacteria	The density of biofilms (units) before the action of the biocide	Density of biofilms (units) after the action of the biocide during, min			
		1	2	3	5
S. aureus	2.74 ±0.08	1.66 ±0.06*	1.35 ±0.05*	1.15 ±0.05*	0.96 ±0.04*
E. coli	2.65 ±0.07	1.61 ±0.6*	1.30 ±0.05*	1.08 ±0.05*	0.87 ±0.04*
P. aeruginosa	2.93 ±0.09	1.77 ±0.07*	1.43 ±0.05*	1.24 ±0.05*	1.03 ±0.04*
E. faecalis	3.25 ±0.11	1.98 ±0.08*	1.67 ±0.08*	1.39 ±0.05*	1.10 ±0.05*
Control (shell without bacteria*)*	–	–	–	–	0.15 ±0.02

Note: * – p >0.05, before the biocide action.

The research on the influence of gaseous ozone on the degradation of the matrix of biofilms (Table 9) found a weaker destructive action compared to a stabilised aqueous solution.

Table 9 The influence of gaseous ozone (60 mg/h) on the density of microbial biofilms formed on eggshells, x ±SE.

Bacteria	The density of biofilms (units) before the action of the biocide	Density of biofilms (units) after the action of the biocide during, min			
		1	2	3	5
S. aureus	2.70 ±0.07	2.41 ±0.06	2.00 ±0.06*	1.89 ±0.06*	1.60 ±0.05*
E. coli	2.67 ±0.05	2.38 ±0.06	2.01 ±0.06*	1.82 ±0.06*	1.55 ±0.05*
P. aeruginosa	2.95 ±0.06	2.46 ±0.07	2.09 ±0.06*	1.86 ±0.06*	1.63 ±0.05*
E. faecalis	3.18 ±0.07	2.57 ±0.07	2.21 ±0.07*	1.98 ±0.07	1.72 ±0.06*
Control (shell without bacteria)	–	–	–	–	0.12 ±0.02

Note: * – p >0.05, before the biocide action.

In particular, probable values regarding the decrease in the density of microbial biofilms during processing with gaseous ozone were noted only after two minutes of action. During this time, the density of biofilms was decreased by an average of 1.3 times to 2.00 units. In addition, even after a five-minute influence of gaseous ozone on biofilms, their density did not decrease significantly (1.7-1.8 times), compared to biofilms before processing. This indicates that gaseous ozone, penetrating the matrix, influences the biofilm forms of bacteria but does not cause a significant destructive action.

Ultraviolet rays had an even less destructive influence on the degradation of the microbial biofilm (Table 10).

Table 10 The influence of ultraviolet radiation (253.7 nm) on the density of microbial biofilms formed on eggshells, x ±SE.

Bacteria	The density of biofilms (units) before the action of the biocide	Density of biofilms (units) after the action of the biocide during, min			
		1	2	3	5
S. aureus	2.67 ±0.07	2.60 ±0.07	2.53 ±0.06	2.3 ±0.05	1.96 ±0.05*
E. coli	2.74 ±0.07	2.68 ±0.07	2.51 ±0.06	2.27 ±0.05	1.89 ±0.05*
P. aeruginosa	2.88 ±0.08	2.77 ±0.07	2.65 ±0.06	2.37 ±0.06	2.02 ±0.04*
E. faecalis	3.20 ±0.09	3.12 ±0.08	3.03 ±0.08	2.71 ±0.07	2.35 ±0.06*
Control (shell without bacteria)	–	–	–	–	0.12 ±0.02

Note: * – p >0.05, before the biocide action.

It was found that during the time (30 min), which had a detrimental influence on the planktonic forms of the investigated bacteria, a probable decrease in the density of microbial biofilms was not observed. Probable changes in the biofilm matrix degradation were noted after 60 min of exposure to ultraviolet rays. In particular, only after 1 h of irradiation the density of microbial biofilms was decreased by an average of 1.4 times. However, all of them were still quite dense, especially when compared with the control.

Therefore, ultraviolet rays during 30 minutes of irradiation do not influence biofilms' degradation, and their action during 60 minutes does not significantly reduce their density.

The use of safe methods of reducing microbial contamination of chicken eggs is an issue that has been relevant for a long time **[32]**, **[33]**, **[34]**. It is a guarantee of getting safe egg products is the use of eggs with minimal microbial contamination **[5]**, **[18]**. Therefore, developing technologies for decontaminating chicken eggs before use for food purposes is a promising area of work in the field of chicken egg production and storage. This research aimed to identify the microbiota of chicken eggs and determine the influence of different disinfection methods of microbial biofilms formed on the eggshell. Our research found that the quantitative content of microorganisms and the biodiversity of their generic and species composition on the surface of the eggshell depends on the area of litter contamination. In particular, three orders of magnitude more microorganisms (10^6 CFU) were isolated on the shell contaminated with droppings than on the relatively clean surface of the eggs. Scientists **[16]**, **[17]**, **[35]**, **[36]**, **[37]** also found a significantly higher number of microorganisms on the shell of chicken eggs provided they were in poor sanitary conditions. Therefore, we support the opinion of researchers **[4]**, **[5]**, **[38]** about the need to introduce various methods to get an egg with the cleanest possible surface. Such methods include washing eggs with chlorinated water **[39]**, quaternary ammonium salts **[40]**, hot water, or steaming at a temperature 5-10 °C higher than the surface temperature of the egg **[41]**. Processing with ultrasound, lysozyme **[26]**, pro- and prebiotics **[42]**, bacteriocins **[43]**, hot air **[44]**, steam, and infrared radiation **[45]**, **[46]**.

Therefore, various methods of reducing microorganisms on the surface of the eggshell are actually used because, in the conditions of industrial production, the presence of a certain number of dirty eggs is almost inevitable. Therefore, special attention must be paid to the disinfection of dirty eggs.

The evaluation of the generic composition of the microbiota of the shell of chicken eggs under different contamination found an increase in the frequency of the release of bacteria of faecal origin belonging to the *Enterobacteriaceae* genus. In particular, bacteria of the genus *Escherichia* were isolated 21.7% more often from the contaminated shell, *Enterobacter* and *Enterococcus* 5.0-5.2 times, and bacteria of the genera *Pseudomonas* and *Psychrobacter* were detected in 6.1% of the samples, which were absent on a clean eggshell. Therefore, it can be stated that the microbial contamination of the eggshell in most cases is the result of contact with dirty surfaces contaminated with chicken droppings, which is consistent with other researchers' data **[4]**, **[18]**.

The most common procedures used to reduce microbial contamination of eggs in the technological process of production of egg products are washing in tap water followed by soaking in chlorinated water **[4]**, **[5]**. However, this technological operation does not wholly decontaminate the shell from microorganisms **[17]**. We investigated the influence of four methods of decontaminating eggshells from applied strains of bacteria while determining the influence on planktonic bacteria and biofilms that were formed on the shell. It was found that the working solution of the disinfectant Vircon S destroyed planktonic bacteria applied to the eggshell in an average of 2 minutes of exposure, stabilised water ozone for 1 minute, gaseous ozone for 3 minutes, and the action of ultraviolet rays with a length of 253.7 nm – for 25-30 min. At the same time, using these disinfection methods on bacteria formed in a biofilm on the eggshell did not cause a bactericidal action during this time. For a significant reduction in bacteria in the biofilm under the influence of these methods, it is necessary to increase the exposure time of the biocide by 2-3 times. However, even raising the exposure time did not destroy the bacteria in the biofilm. This indicates that the complex structure of the matrix of biofilms **[47]**, **[48]** and not simple topography of the eggshell surface **[49]** provide better protection for bacteria against the influence of these disinfection methods. Literature data indicate that food pathogens such as salmonella **[50]** and pseudomonads **[51]** can produce biofilms on eggshells in a wide range of temperatures (20-37 °C) and thereby pose a danger to consumers. In addition, it is reported **[52]** that *Pseudomonas aeruginosa* formed a dense biofilm on the shell of quail eggs, which was difficult to remove with calcium oxide. At the same time, its degradation was much more accessible from rubber and plastic. However, in addition to the ability to form a biofilm, there are other mechanisms of resistance formation by bacteria to the used biocides on the eggshell **[53]**. In particular, resistance can be acquired in microorganisms, in which certain strains of bacteria survive at biocide concentrations that suppress the bulk of existing microorganisms **[54]**, **[55]**. Scientists note **[56]** that eggs can be a factor in the spread of antibiotic-resistant strains among consumers who consume them raw or unprocessed. In particular, it was found that 73.3% of microbial isolates were isolated from chicken eggs and had multiple medicinal resistance to antibiotics used to treat intestinal infections in consumers. Therefore, we consider that the practical use of biocides for egg disinfection should consider the formation of resistance and conduct monitoring investigations on the effectiveness of such means and regimes.

Researchers from Mexico **[57]** indicated that enterotoxigenic strains of *Bacillus cereus* survived on the shells of chicken eggs sold in the market and supermarkets due to their ability to form biofilms. Therefore, we believe it is necessary to use such eggshell disinfection regimes that affect the bacteria in biofilms, especially for eggs contaminated with droppings. Reliable control of such a regime will guarantee the sale of a safe egg and the production of high-quality, shelf-stable egg products. In addition, our research found that although Vircon S biocides, stabilised water, and gaseous ozone penetrated the microbial biofilms on the shell, they did not wholly destroy the matrix. This is probably due to the multi-layered nature of biofilms, which are intertwined with the pores and channels of the eggshell membranes. Thus, according to the data **[58]**, the shell of a chicken egg contains, on average, 7,000 to 17,000 pores, and the largest of them are visible to the naked eye as small indentations on the surface of the shell. On average, per 1 cm^2 of the surface of chicken eggs, there are about 154 pores, and their total area is 2.3 mm^2. It is precisely in these pores and the formed biofilm matrix that bacteria are more protected from the action of biocides due to the inability of the latter to penetrate deeply. Also, our researchers found that ultraviolet radiation had a bactericidal influence on planktonic bacteria on the eggshell during 30 exposures. At the same time, 10^3 CFU/washing were selected from biofilms even under the influence of ultraviolet rays for 60 min. Such data are consistent with reports **[59]** that ultraviolet radiation does not penetrate the matrix of biofilms well, and only the first few upper layers of microbial cells are exposed to its harmful influence. Therefore, we believe that the action of ultraviolet rays will be effective against planktonic bacteria, and in the case of the formation of biofilms, their survival is possible. This indicates the practicality of combining different methods of disinfection of the microbiota on the eggshell.

In general, the data obtained on the determination of the influence of various methods of disinfection of microbiota on the surface of chicken eggs indicate that bacterial pathogens, which are usually present in chicken droppings, can form dense biofilms on the shell and be the cause of contamination of egg products in the case of the use of ineffective methods of decontamination. In our opinion, it is necessary to combine various methods of reducing the microbial seeding of the egg, as well as chemical disinfectants, ozone, and ultraviolet radiation. At the same time, each disinfection method needs approval in production conditions. In addition, in our opinion, treatment with stabilised water ozone, a biocide, is effective and promising in terms of practical use in production conditions, which is safe both for the edible egg and the service personnel and consumers.

CONCLUSION

The quantitative content of microorganisms on the surface of chicken eggs ranged from 10^3 CFU to 10^6 CFU/ml washing, depending on the shell's contamination with droppings. *Lactobacillus* spp., *Bacillus* spp., *Corynebacterium, Staphylococcus* were found among the genera of bacteria that prevailed on the clean chicken shell, which were isolated in 30-50% of cases, and gram-negative microbiota was practically absent. On the contaminated eggshell, there is an almost constant excretion of gram-positive bacteria, and the frequency of identification of gram-negative microbiota of the *Enterobacteriaceae* genus and non-fermenting genera *Pseudomonas* and *Psychrobacter* increases. That is, the microbial landscape of the microbiota of the chicken shell depends on its cleanliness, and the presence of a dirty surface increases the frequency of allocation of the resident microflora of the gastrointestinal tract. It was found that the working solution of the disinfectant Vircon S destroyed planktonic bacteria applied to the eggshell in an average of 2 minutes of exposure, stabilised water ozone for 1 minute, gaseous ozone for 3 minutes, and the action of ultraviolet rays with a length of 253.7 nm – for 25-30 min. At the same time, using these disinfection methods on bacteria formed in a biofilm on the eggshell did not cause a bactericidal action during this time. To significantly reduce bacteria in the biofilm under the influence of these methods, it is necessary to increase the exposure time of the biocide by 2 – 3 times. Therefore, the complex structure of the eggshell and the multi-layered matrix of biofilms provide better protection for bacteria against the influence of the investigated disinfection methods.

REFERENCES

1. Park, J. H., Kang, M. S., Park, K. M., Lee, H. Y., Ok, G. S., Koo, M. S., Hong, S. I., & Kim, H. J. (2020). A dynamic predictive model for the growth of Salmonella spp. and Staphylococcus aureus in fresh egg yolk and scenario-based risk estimation. In Food Control (Vol. 118, p. 107421). Elsevier BV. https://doi.org/10.1016/j.foodcont.2020.107421
2. Wilson, P. B. (2017). Recent advances in avian egg science: A review. In Poultry Science (Vol. 96, Issue 10, pp. 3747–3754). Elsevier BV. https://doi.org/10.3382/ps/pex187
3. Tian, L., Hu, S., Jia, J., Tan, W., Yang, L., Zhang, Q., Liu, X., & Duan, X. (2021). Effects of short-term fermentation with lactic acid bacteria on the characterization, rheological and emulsifying properties of egg yolk. In Food Chemistry (Vol. 341, p. 128163). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.128163

4. Olsen, R., Kudirkiene, E., Thøfner, I., Pors, S., Karlskov-Mortensen, P., Li, L., Papasolomontos, S., Angastiniotou, C., & Christensen, J. (2017). Impact of egg disinfection of hatching eggs on the eggshell microbiome and bacterial load. In Poultry Science (Vol. 96, Issue 11, pp. 3901–3911). Elsevier BV. https://doi.org/10.3382/ps/pex182
5. Lin, C.-M., Chen, S.-Y., Lin, Y.-T., Hsiao, C.-P., Liu, C.-T., Hazeena, S. H., Wu, J.-S., & Hou, C.-Y. (2023). Inactivating Salmonella Enteritidis on shell eggs by using ozone microbubble water. In International Journal of Food Microbiology (Vol. 398, p. 110213). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2023.110213
6. Sharaf Eddin, A., Ibrahim, S. A., & Tahergorabi, R. (2019). Egg quality and safety with an overview of edible coating application for egg preservation. In Food Chemistry (Vol. 296, pp. 29–39). Elsevier BV. https://doi.org/10.1016/j.foodchem.2019.05.182
7. De Reu, K., Grijspeerdt, K., Herman, L., Heyndrickx, M., Uyttendaele, M., Debevere, J., Putirulan, F. F., & Bolder, N. M. (2006). The effect of a commercial UV disinfection system on the bacterial load of shell eggs. In Letters in Applied Microbiology (Vol. 42, Issue 2, pp. 144–148). Oxford University Press (OUP). https://doi.org/10.1111/j.1472-765x.2005.01825.x
8. Sharif, M. K., Saleem, M., & Javed, K. (2018). Food Materials Science in Egg Powder Industry. In Role of Materials Science in Food Bioengineering (pp. 505–537). Elsevier. https://doi.org/10.1016/b978-0-12-811448-3.00015-2
9. Kim, H.-J., Yong, H. I., Jayasena, D. D., Lee, H. J., Lee, H., & Jo, C. (2016). Microbial safety and physicochemical characteristics of electron beam irradiated whole egg powder. In Food Science and Biotechnology (Vol. 25, Issue 2, pp. 637–642). Springer Science and Business Media LLC. https://doi.org/10.1007/s10068-016-0089-4
10. Baron, F., Jan, S., & Techer, C. (2023). Microbiology of Egg and Egg Products. In Handbook of Egg Science and Technology (pp. 355–396). CRC Press. https://doi.org/10.1201/9781003254430-26
11. Kouam, M. K., Biekop, M. H. F., Katte, B., & Teguia, A. (2018). Salmonella status of table eggs in commercial layer farms in Menoua Division, West region of Cameroon. In Food Control (Vol. 85, pp. 345–349). Elsevier BV. https://doi.org/10.1016/j.foodcont.2017.09.037
12. Wan, Z., Chen, Y., Pankaj, S. K., & Keener, K. M. (2017). High voltage atmospheric cold plasma treatment of refrigerated chicken eggs for control of Salmonella Enteritidis contamination on egg shell. In LWT - Food Science and Technology (Vol. 76, pp. 124–130). Elsevier BV. https://doi.org/10.1016/j.lwt.2016.10.051
13. Holt, P. S., Davies, R. H., Dewulf, J., Gast, R. K., Huwe, J. K., Jones, D. R., Waltman, D., & Willian, K. R. (2011). The impact of different housing systems on egg safety and quality. In Poultry Science (Vol. 90, Issue 1, pp. 251–262). Elsevier BV. https://doi.org/10.3382/ps.2010-00794
14. De Reu, K., Grijspeerdt, K., Heyndrickx, M., Zoons, J., De Baere, K., Uyttendaele, M., Debevere, J., & Herman, L. (2005). Bacterial eggshell contamination in conventional cages, furnished cages and aviary housing systems for laying hens. In British Poultry Science (Vol. 46, Issue 2, pp. 149–155). Informa UK Limited. https://doi.org/10.1080/00071660500065359
15. Galvão, J. A., Biondo, A. W., Possebon, F. S., Spina, T. L. B., Correia, L. B. N., Zuim, C. V., Guerra Filho, J. B. P., Pantoja, J. C. F., & Pinto, J. P. D. A. N. (2018). Microbiological vulnerability of eggs and environmental conditions in conventional and free-range housing systems. In Semina: Ciências Agrárias (Vol. 39, Issue 1, p. 133). Universidade Estadual de Londrina. https://doi.org/10.5433/1679-0359.2018v39n1p133
16. Jones, D. R., Cox, N. A., Guard, J., Fedorka-Cray, P. J., Buhr, R. J., Gast, R. K., Abdo, Z., Rigsby, L. L., Plumblee, J. R., Karcher, D. M., Robison, C. I., Blatchford, R. A., & Makagon, M. M. (2015). Microbiological impact of three commercial laying hen housing systems. In Poultry Science (Vol. 94, Issue 3, pp. 544–551). Elsevier BV. https://doi.org/10.3382/ps/peu010
17. Zeweil, H. S., Rizk, R. E., Bekhet, G. M., & Ahmed, M. R. (2015). Comparing the effectiveness of egg disinfectants against bacteria and mitotic indices of developing chick embryos. In The Journal of Basic & Applied Zoology (Vol. 70, pp. 1–15). Elsevier BV. https://doi.org/10.1016/j.jobaz.2014.12.005
18. Mohammadi-Aragh, M. K., Linhoss, J. E., & Evans, J. D. (2022). Effects of various disinfectants on the bacterial load and microbiome of broiler hatching eggs using electrostatic spray. In Journal of Applied Poultry Research (Vol. 31, Issue 3, p. 100278). Elsevier BV. https://doi.org/10.1016/j.japr.2022.100278
19. Al-Ajeeli, M. N., Taylor, T. M., Alvarado, C. Z., & Coufal, C. D. (2016). Comparison of eggshell surface sanitization technologies and impacts on consumer acceptability. In Poultry Science (Vol. 95, Issue 5, pp. 1191–1197). Elsevier BV. https://doi.org/10.3382/ps/pew014
20. Carson, R. T., Larson, E., Levy, S. B., Marshall, B. M., & Aiello, A. E. (2008). Use of antibacterial consumer products containing quaternary ammonium compounds and drug resistance in the community. In Journal of

Antimicrobial Chemotherapy (Vol. 62, Issue 5, pp. 1160–1162). Oxford University Press (OUP). https://doi.org/10.1093/jac/dkn332

21. Cook, M. I., Beissinger, S. R., Toranzos, G. A., & Arendt, W. J. (2005). Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection. In Ecology Letters (Vol. 8, Issue 5, pp. 532–537). Wiley. https://doi.org/10.1111/j.1461-0248.2005.00748.x
22. Baron, F., & Jan, S. (2011). Egg and egg product microbiology. In Improving the Safety and Quality of Eggs and Egg Products (pp. 330–350). Elsevier. https://doi.org/10.1533/9780857093912.3.330
23. Garkavenko, T. O., Gorbatyuk, O. I., Dybkova, S. M., Kozytska, T. G., Andriiashchuk, V. O., Kukhtyn, M. D., & Horiuk, Y. V. (2021). Screening of Epidemiologically Significant Mechanisms of Antibiotics to β-Lactams in Enterobacteriaceae - Pathogens of Zoonoses. In Journal of Pure and Applied Microbiology (Vol. 15, Issue 3, pp. 1245–1256). Journal of Pure and Applied Microbiology. https://doi.org/10.22207/jpam.15.3.14
24. Jiang, W., Etienne, X., Li, K., & Shen, C. (2018). Comparison of the Efficacy of Electrostatic versus Conventional Sprayer with Commercial Antimicrobials To Inactivate Salmonella, Listeria monocytogenes, and Campylobacter jejuni for Eggs and Economic Feasibility Analysis. In Journal of Food Protection (Vol. 81, Issue 11, pp. 1864–1870). Elsevier BV. https://doi.org/10.4315/0362-028x.jfp-18-249
25. Kukhtyn, M., Kozhyn, V., Horiuk, V., Malimon, Z., Horiuk, Y., Yashchuk, T., & Kernychnyi, S. (2021). Dezenfektan Biyositler ve Proteaz ve Amilaz Enzimlerinin Biyofilmlerdeki Bakteriler Üzerine Aktivitesi. In Kafkas Universitesi Veteriner Fakultesi Dergisi. Kafkas University. https://doi.org/10.9775/kvfd.2021.25770
26. Bi, X., Wang, X., Chen, Y., Chen, L., Xing, Y., & Che, Z. (2020). Effects of combination treatments of lysozyme and high power ultrasound on the Salmonella typhimurium inactivation and quality of liquid whole egg. In Ultrasonics Sonochemistry (Vol. 60, p. 104763). Elsevier BV. https://doi.org/10.1016/j.ultsonch.2019.104763
27. Rodriguez-Romo, L. A., & Yousef, A. E. (2005). Inactivation of Salmonella enterica Serovar Enteritidis on Shell Eggs by Ozone and UV Radiation. In Journal of Food Protection (Vol. 68, Issue 4, pp. 711–717). Elsevier BV. https://doi.org/10.4315/0362-028x-68.4.711
28. Braun, S., Achermann, B., De Marco, A., Pleijel, H., Karlsson, P. E., Rihm, B., Schindler, C., & Paoletti, E. (2017). Epidemiological analysis of ozone and nitrogen impacts on vegetation – Critical evaluation and recommendations. In Science of The Total Environment (Vols. 603–604, pp. 785–792). Elsevier BV. https://doi.org/10.1016/j.scitotenv.2017.02.225
29. Hou, C.-Y., Lai, Y.-C., Hsiao, C.-P., Chen, S.-Y., Liu, C.-T., Wu, J.-S., & Lin, C.-M. (2021). Antibacterial activity and the physicochemical characteristics of plasma activated water on tomato surfaces. In LWT (Vol. 149, p. 111879). Elsevier BV. https://doi.org/10.1016/j.lwt.2021.111879
30. Lin, C.-M., Hsiao, C.-P., Lin, H.-S., Liou, J. S., Hsieh, C.-W., Wu, J.-S., & Hou, C.-Y. (2020). The Antibacterial Efficacy and Mechanism of Plasma-Activated Water Against Salmonella Enteritidis (ATCC 13076) on Shell Eggs. In Foods (Vol. 9, Issue 10, p. 1491). MDPI AG. https://doi.org/10.3390/foods9101491
31. Mocherniuk, M. M., Kukhtyn, M. D., Horiuk, Y. V., Horiuk, V. V., Tsvigun, O. A., & Tokarchuk, T. S. (2022). Microflora of boxes for holding veterinary patients in clinics. In Regulatory Mechanisms in Biosystems (Vol. 13, Issue 3, pp. 257–264). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/022233
32. Korowiecka, K., Trela, M., Tombarkiewicz, B., Pawlak, K., Niedziółka, J. W., Swadźba, M., & Lis, M. W. (2017). Assessment of the effect of selected substances used for disinfection of hatching eggs on hatching results in chickens. In Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego (Vol. 13, Issue 2, pp. 25–35). Index Copernicus. https://doi.org/10.5604/01.3001.0013.5221
33. Motola, G., Hafez, H. M., & Brüggemann-Schwarze, S. (2023). Assessment of three alternative methods for bacterial disinfection of hatching eggs in comparison with conventional approach in commercial broiler hatcheries. In S. El-Ashram (Ed.), PLOS ONE (Vol. 18, Issue 3, p. e0283699). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0283699
34. Liu, C., Zheng, W., Li, Z., Zhou, L., Sun, Y., & Han, S. (2022). Slightly acidic electrolyzed water as an alternative disinfection technique for hatching eggs. In Poultry Science (Vol. 101, Issue 3, p. 101643). Elsevier BV. https://doi.org/10.1016/j.psj.2021.101643
35. De Reu, K., Rodenburg, T. B., Grijspeerdt, K., Messens, W., Heyndrickx, M., Tuyttens, F. A. M., Sonck, B., Zoons, J., & Herman, L. (2009). Bacteriological contamination, dirt, and cracks of eggshells in furnished cages and noncage systems for laying hens: An international on-farm comparison. In Poultry Science (Vol. 88, Issue 11, pp. 2442–2448). Elsevier BV. https://doi.org/10.3382/ps.2009-00097

36. Rodríguez-Navarro, A. B., Marie, P., Nys, Y., Hincke, M. T., & Gautron, J. (2015). Amorphous calcium carbonate controls avian eggshell mineralization: A new paradigm for understanding rapid eggshell calcification. In Journal of Structural Biology (Vol. 190, Issue 3, pp. 291–303). Elsevier BV. https://doi.org/10.1016/j.jsb.2015.04.014

37. Samiullah, Roberts, J. R., & Chousalkar, K. K. (2014). Effect of production system and flock age on egg quality and total bacterial load in commercial laying hens. In Journal of Applied Poultry Research (Vol. 23, Issue 1, pp. 59–70). Elsevier BV. https://doi.org/10.3382/japr.2013-00805

38. Petrovič, J., Mellen, M., Čmiková, N., Schwarzová, M., & Kačániová, M. (2024). Effects of laying hens housing system on eggs microbial contamination. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 18, pp. 50–65). HACCP Consulting. https://doi.org/10.5219/1938

39. Hudson, L. K., Harrison, M. A., Berrang, M. E., & Jones, D. R. (2016). Alternative Antimicrobial Commercial Egg Washing Procedures. In Journal of Food Protection (Vol. 79, Issue 7, pp. 1216–1220). Elsevier BV. https://doi.org/10.4315/0362-028x.jfp-15-423

40. Chan, H. Y., Meor Hussin, A. S., Ahmad, N. H., Rukayadi, Y., & Farouk, A.-E. (2021). Effectiveness of Quaternary Ammonium in Reducing Microbial Load on Eggs. In Molecules (Vol. 26, Issue 17, p. 5259). MDPI AG. https://doi.org/10.3390/molecules26175259

41. James, C., Lechevalier, V., & Ketteringham, L. (2002). Surface pasteurisation of shell eggs. In Journal of Food Engineering (Vol. 53, Issue 2, pp. 193–197). Elsevier BV. https://doi.org/10.1016/s0260-8774(01)00156-x

42. Patterson, J., & Burkholder, K. (2003). Application of prebiotics and probiotics in poultry production. In Poultry Science (Vol. 82, Issue 4, pp. 627–631). Elsevier BV. https://doi.org/10.1093/ps/82.4.627

43. Riley, M. A., & Chavan, M. A. (Eds.). (2007). Bacteriocins. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-36604-1

44. Pasquali, F., Fabbri, A., Cevoli, C., Manfreda, G., & Franchini, A. (2010). Hot air treatment for surface decontamination of table eggs. In Food Control (Vol. 21, Issue 4, pp. 431–435). Elsevier BV. https://doi.org/10.1016/j.foodcont.2009.07.003

45. Holck, A. L., Liland, K. H., Drømtorp, S. M., Carlehög, M., & McLeod, A. (2018). Comparison of UV-C and Pulsed UV Light Treatments for Reduction of Salmonella, Listeria monocytogenes, and Enterohemorrhagic Escherichia coli on Eggs. In Journal of Food Protection (Vol. 81, Issue 1, pp. 6–16). Elsevier BV. https://doi.org/10.4315/0362-028x.jfp-17-128

46. Turtoi, M., & Borda, D. (2014). Decontamination of egg shells using ultraviolet light treatment. In World's Poultry Science Journal (Vol. 70, Issue 2, pp. 265–278). Informa UK Limited. https://doi.org/10.1017/s0043933914000282

47. Karygianni, L., Ren, Z., Koo, H., & Thurnheer, T. (2020). Biofilm Matrixome: Extracellular Components in Structured Microbial Communities. In Trends in Microbiology (Vol. 28, Issue 8, pp. 668–681). Elsevier BV. https://doi.org/10.1016/j.tim.2020.03.016

48. Flemming, H.-C., van Hullebusch, E. D., Neu, T. R., Nielsen, P. H., Seviour, T., Stoodley, P., Wingender, J., & Wuertz, S. (2022). The biofilm matrix: multitasking in a shared space. In Nature Reviews Microbiology (Vol. 21, Issue 2, pp. 70–86). Springer Science and Business Media LLC. https://doi.org/10.1038/s41579-022-00791-0

49. Solomon, S. E. (2010). The eggshell: strength, structure and function. In British Poultry Science (Vol. 51, Issue sup1, pp. 52–59). Informa UK Limited. https://doi.org/10.1080/00071668.2010.497296

50. Ashrafudoulla, Md., Kim, H. J., Her, E., Shaila, S., Park, S. H., & Ha, S.-D. (2023). Characterization of Salmonella Thompson-specific bacteriophages and their preventive application against Salmonella Thompson biofilm on eggshell as a promising antimicrobial agent in the food industry. In Food Control (Vol. 154, p. 110008). Elsevier BV. https://doi.org/10.1016/j.foodcont.2023.110008

51. Jung, S., Park, S. Y., Kim, S. E., Kang, I., Park, J., Lee, J., Kim, C., Chung, M., & Ha, S. (2017). Bactericidal Effect of Calcium Oxide (Scallop-Shell Powder) Against Pseudomonas aeruginosa Biofilm on Quail Egg Shell, Stainless Steel, Plastic, and Rubber. In Journal of Food Science (Vol. 82, Issue 7, pp. 1682–1687). Wiley. https://doi.org/10.1111/1750-3841.13753

52. Pande, V. V., McWhorter, A. R., & Chousalkar, K. K. (2016). Salmonella enterica isolates from layer farm environments are able to form biofilm on eggshell surfaces. In Biofouling (Vol. 32, Issue 7, pp. 699–710). Informa UK Limited. https://doi.org/10.1080/08927014.2016.1191068

53. Tessema, K., Bedu, H., Ejo, M., & Hiko, A. (2017). Prevalence and Antibiotic Resistance of Salmonella Species Isolated from Chicken Eggs by Standard Bacteriological Method. In Journal of Veterinary Science & Technology (Vol. 08, Issue 01). OMICS Publishing Group. https://doi.org/10.4172/2157-7579.1000421

54. Kapena, M. S., Muma, J. B., Mubita, C. M., & Munyeme, M. (2020). Antimicrobial resistance of Escherichia coli and Salmonella in raw retail table eggs in Lusaka, Zambia. In November-2020 (Vol. 13, Issue 11, pp. 2528–2533). Veterinary World. https://doi.org/10.14202/vetworld.2020.2528-2533

55. Fernández Márquez, M. L., Burgos, M. J. G., Pulido, R. P., Gálvez, A., & López, R. L. (2017). Biocide Tolerance and Antibiotic Resistance in Salmonella Isolates from Hen Eggshells. In Foodborne Pathogens and Disease (Vol. 14, Issue 2, pp. 89–95). Mary Ann Liebert Inc. https://doi.org/10.1089/fpd.2016.2182

56. Jain, A. KR., & Yadav, R. (2017). Study of antibiotic resistance in bacteria Isolated from table egg. In International Journal of pharma and Bio Science (Vol. 8, Issue 1). International Journal of Pharma and Bio Sciences. https://doi.org/10.22376/ijpbs.2017.8.1.b668-674

57. Cruz-Facundo, I., Adame-Gómez, R., Vences-Velázquez, A., Rodríguez-Bataz, E., Muñoz-Barrios, S., Pérez-Oláis, J., & Ramírez-Peralta, A. (2022). Bacillus Cereus in Eggshell: Enterotoxigenic Profiles and Biofilm Production. In Brazilian Journal of Poultry Science (Vol. 24, Issue 2). FapUNIFESP (SciELO). https://doi.org/10.1590/1806-9061-2021-1535

58. Hincke, M., T. (2012). The eggshell: structure, composition and mineralization. In Frontiers in Bioscience (Vol. 17, Issue 1, p. 1266). IMR Press. https://doi.org/10.2741/3985

59. de Carvalho, C. C. C. R. (2017). Biofilms: Microbial Strategies for Surviving UV Exposure. In Advances in Experimental Medicine and Biology (pp. 233–239). Springer International Publishing. https://doi.org/10.1007/978-3-319-56017-5_19

Funds:

This research received no external funding.

Acknowledgements:

The researchers also thanked the Ternopil Poultry Factory, which contributed to this research.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Mykola Kukhtyn, Ternopil Ivan Pului National Technical University, Faculty of Engineering of Machines, Structures and Technologies, Department of Food Biotechnology and Chemistry, Ruska, 56, 46001, Ternopil, Ukraine,
Tel.: +380972392057
E-mail: kuchtynnic@gmail.com
ORCID: http://orcid.org/0000-0002-0195-0767

Zhanna Sverhun, Podillia State University, Faculty of Veterinary Medicine and Technologies in Livestock, Department of Veterinary Obstetrics, Internal Pathology and Surgery, Schevchenko, 12, 32301, Kamianets-Podilskyi, Ukraine,
Tel.: +380677343101
E-mail: daniezana7@gmail.com
ORCID: https://orcid.org/0009-0004-6378-2836

***Yulia Horiuk**, Podillia State University, Faculty of Veterinary Medicine and Technologies in Livestock, Department of Veterinary Obstetrics, Internal Pathology and Surgery, Schevchenko, 12, 32301, Kamianets-Podilskyi, Ukraine,
Tel.: +380976617964
E-mail: goruky@ukr.net
ORCID: https://orcid.org/0000-0002-7162-8992

Volodymyr Salata, Lviv National University of Veterinary Medicine and Biotechnologies named after S. Z. Gzhytskyj, Faculty of Veterinary Hygiene, Ecology and Law, Department of Veterinary-Sanitary Inspection, Pekarska, 50, 79010, Lviv, Ukraine,
Tel.: +380677288933
E-mail: salatavolod@ukr.net
ORCID: http://orcid.org/0000-0002-7175-493X

Svitlana Laiter-Moskaliuk, Podillia State University, Faculty of Veterinary Medicine and Technologies in Livestock, Department of Animal Hygiene and Veterinary Support of the Cynological Service of the National Police of Ukraine, Schevchenko, 12, 32301, Kamianets-Podilskyi, Ukraine,
Tel.: +380974756864
E-mail: laytermoskalyuk@gmail.com
ORCID: http://orcid.org/0000-0001-5662-7636

Mykhailo Mocherniuk, Podillia State University, Faculty of Veterinary Medicine and Technologies in Livestock, Department of Veterinary Obstetrics, Internal Pathology and Surgery, Schevchenko, 12, 32301, Kamianets-Podilskyi, Ukraine,
Tel.: +380677954711
E-mail: vetpankockij@gmail.com
ORCID: https://orcid.org/0000-0001-6289-9080

Larysa Kladnytska, National University of Life and Environmental Sciences of Ukraine, FacultyVeterinary medicine, Department of Biochemistry and Physiology of Animals, Potechina Str., 16, 03127, Kyiv, Ukraine,
Tel.: + 380631866233
E-mail: kladlarisa@ukr.net
ORCID: https://orcid.org/0000-0002-9360-0587

Victor Horiuk, Podillia State University, Faculty of Veterinary Medicine and Technologies in Livestock, Department of Veterinary Obstetrics, Internal Pathology and Surgery, Schevchenko, 12, 32301, Kamianets-Podilskyi, Ukraine,
Tel.: +380679300964
E-mail: horiukv@ukr.net
ORCID: http://orcid.org/0000-0002-1633-7287

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 1.4.2024
Revised: 20.5.2024
Accepted: 17.7.2024
Published: 20.8.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 683-696
https://doi.org/10.5219/1976
ISSN: 1337-0960 online
www.potravinarstvo.com

Expanding the range of fortified meat products through the targeted combination of raw materials of animal and vegetable origin

Aleksander Borisenko, Yassin Uzakov, Ekaterina Greseva, Victoria Razinkova, Aleksey Borisenko

ABSTRACT

The problem of providing the population with a full-fledged balanced diet is currently quite acute worldwide. Therefore, one of the main tasks is to expand the range of fortified and functional food products, including those with prebiotic effects. The article presents the results of developing fortified boiled sausages, liver pates, and chopped semi-finished products, which have high consumer properties, nutritional and biological value, organoleptic characteristics, and a balanced nutritional composition. The minimum amino acid score value for the proposed boiled sausages is 95.6%, and, for liver pate – 99.6%, for chopped semi-finished products – 88.1%. The biological value of the protein of the developed products reaches 92.8%, 87.7%, and 99.7%, respectively. This is achieved through specially selected components of animal and vegetable origin. Meat and meat-plant products were developed based on an analysis of the nutritional status of North Caucasus Federal University students. The optimal formulation was determined, and the nutrient balance of the finished products was ensured using computer modelling. Using dry milk molasses with lactulose "LactuVet-1" in the formulations of fortified meat products made it possible to increase their organoleptic characteristics and enrich them with calcium (about 150 mg per 100 g of finished product) and other minerals. The developed meat products contain lactulose, g/100 g of product: boiled sausages – 0.46, chopped semi-finished products – 0.61, liver pate – 0.76. This helped to ensure the prebiotic effect of the finished product. The proposed meat products are a source of vitamin A and calcium, contain most B vitamins, macroelements potassium and magnesium, and microelements iron and zinc. The complex of tasks to reduce the deficit of the main types of nutrients, revealed during the study, can be solved by including the developed meat products in the diet of the target group of consumers.

Keywords: functional foods, meat product, lactulose, milk molasses, nutrient balance

INTRODUCTION

An unbalanced diet is one of the global problems of modern urbanised society. This leads to alimentary-dependent diseases. **[1]**, **[2]**, **[3]**. Introducing a variety of high-quality food into the diet and using products with a balanced composition of nutrients are two main ways to solve this problem **[4]**. The first way can be implemented in conditions of daily consumption of various high-quality food products of animal and vegetable origin without financial difficulties. The second way can be successfully implemented if there is a publicly available range of food products balanced in amino acids, lipids, vitamins, and minerals. Food products like these can be enriched with bioactive and functional ingredients to neutralise negative factors that affect human health through their diet **[5]**. The balance of nutrients in food products must necessarily be related to the

physiological needs of a certain group of consumers or a specific person. **[6]**. The second way is the most realistic and accessible when considering the world's economic situation and the lifestyle of a modern person.

Therefore, the most important task is to expand the range of fortified food products **[7]**, including meat products. This can be achieved by including high-quality functional ingredients in formulations, using low-cost secondary raw materials, and improving production technologies that ensure a maximum balance of finished products according to nutrients.

One effective direction in the selection and provision of the preventive orientation of new types of fortified and functional food products is the assessment of the nutritive (nutritional) status of the target consumer **[8]**, **[9]**, as well as the use of food ingredients with lactulose **[10]**, **[11]**, which can have a favourable effect on the human body through selective stimulation of the growth and activity of intestinal microflora **[12]**.

According to the results of analytical studies of the nutritive status of young people aged from 18 to 27 years (students of the North Caucasus Federal University), using computer modelling we developed recipes and technologies for such products as fortified cooked sausage products, liver pate and chopped meat and vegetable semi-finished products.

Scientific Hypothesis

The targeted combination of selected types of meat, vegetable raw materials, and functional ingredients using computer modelling will significantly increase the availability of finished meat products with high-grade protein, vitamins, and micro- and macroelements. This will achieve a prebiotic effect and high organoleptic characteristics.

MATERIAL AND METHODOLOGY

Samples

Raw materials of animal origin: veneered beef of the first grade, pork veneered semi-fat, side shpik, pork skin emulsion, duck meat, chicken meat, mechanically deboned chicken meat, beef liver, Bovine protein VT-Pro (collagen fibrilar), chicken eggs.

Raw materials of vegetable origin: carrots, onions, semolina, white cabbage, sweet pepper, rapeseed and soy oil, wheat bran, breadcrumbs, ground black pepper and allspice. Functional ingredients: mushroom powder from champignons, kelp, dry milk molasses with lactulose "LactuVet-1".

Chemicals

Hydrogen peroxide H_2O_2 according to GOST 177-88 (Medical class A, analytical grade, manufacturer: Russia), nitric acid HNO_3 according to GOST 4461-77 (analytical grade, manufacturer: Russia), sulfuric acid H_2SO_4 according to GOST 2184-2013 (analytical grade, manufacturer: Russia), phenol C_6H_6O according to the Technical conditions 6-06-5303-86 (analytical grade, manufacturer: Russia), ethanol C_2H_6O according to GOST 5962-2013 (excise duty, analytical grade, manufacturer: Russia), sodium nitroprusside $C_5FeN_6Na_2O$ according to GOST 4 218-48 (analytical grade, manufacturer: Russia), sodium hydroxide NaOH according to GOST 4328-77 (analytical grade, manufacturer: Russia), sodium hypochlorite NaClO according to GOST 11086-76 (grade A, manufacturer: Russia), distilled water H_2O according to GOSTP 58144 (analytical grade, manufacturer: Russia), standard solutions of carbohydrates arabinose, glucose, ribose, mannose, galactose, fructose, xylose, sucrose, lactose (analytical grade, Supelco USA), state standard samples of the composition of aqueous solutions of sodium, potassium, magnesium and manganese ions (analytical grade, manufacturer: Russia), caesium chloride (analytical grade, manufacturer: Russia), lanthanum chloride 7-aqueous (analytical grade, manufacturer: Russia), hydrochloric acid according to GOST 3118 (analytical grade, manufacturer: Russia), citric acid (analytical grade, manufacturer: Russia), phenolphthalein according to the Technical conditions 6-09-5360 (analytical grade, manufacturer: Russia), sodium N, N-diethyldithiocarbamate (analytical grade, manufacturer: Russia), copper sulfuric acid copper sulfuric acid according to GOST 4165 (analytical grade, manufacturer: Russia), lead nitric acid according to GOST 4236 (analytical grade, manufacturer: Russia), granular zinc according to the Technical conditions 6-09-5294 (analytical grade, manufacturer: Russia), cadmium metal (analytical grade, manufacturer: Russia), acetic acid ester according to GOST 22300 (analytical grade, manufacturer: Russia), calcone (eriochrome blue-black) (analytical grade, manufacturer: Russia), trilon B according to GOST 10652 (analytical grade, manufacturer: Russia), potassium hydroxide according to GOST 24363 (analytical grade, manufacturer: Russia), sodium citric acid, three-substituted, 5.5 aqueous according to GOST 22280 (analytical grade, manufacturer: Russia), hydroxylamine hydrochloride according to GOST 5456 (analytical grade, manufacturer: Russia), calcium carbonate according to GOST 4530 (analytical grade, manufacturer: Russia), technical chloroform according to GOST 20015 (analytical grade, manufacturer: Russia), acetone according to GOST 2603 (analytical grade, manufacturer: Russia), state standard samples of the composition of the selenium ion solution (analytical grade, manufacturer: Russia), hexane (analytical grade,

manufacturer: Russia), hydrochloric acid $HClO_4$ (analytical grade, manufacturer: Russia), ammonia is aqueous (analytical grade, manufacturer: Russia), potassium carbonate according to GOST 4221 (analytical grade, manufacturer: Russia), potassium iodide according to GOST 4232 (analytical grade, manufacturer: Russia), sodium sulphate 5-aqueous according to the Technical conditions 6-06-2540-87 (analytical grade, manufacturer: Russia), bromine according to GOST 4109 (analytical grade, manufacturer: Russia), soluble starch according to GOST 10163 (analytical grade, manufacturer: Russia), sodium carbonate according to GOST 83 (analytical grade, manufacturer: Russia), isobutane according to the Technical conditions 6-09-1708-77 (analytical grade, manufacturer: Russia).

Animals, Plants and Biological Materials

Raw animal and vegetable origin materials for research were purchased at a grocery store in Stavropol of Russian Federation. Bovine protein (collagen fibrilar 99%) produced on JSC "Volga Tannery" (Ostashkov, Tver region, Russian Federation, 172735, Declaration of conformity TS N RU D-RU.AY08.V.00745). Dry milk molasses with lactulose "LactuVet-1" produced on JSC "Dairy Plant" Stavropol (Stavropol, Stavropol region, Russian Federation, 355036, Declaration of conformity of the EAEU N RU D-RU.A21.V.03469/20).

Instruments

Total Protein Analyzer Kjeldahl UDK-149 (VELP Scientifica, Usmate, Italy), Memmert UFB 400 drybox (Memmert, Germany), Vilitek ASV-6M semi-automatic Soxhlet apparatus (Vilitek LLC, Moscow, Russia), Muffle furnace L 9/11/SKM (Nabertherm, Germany), liquid analyzer "Fluorat-02" (Lumex, Russia), liquid chromatography (HPLC) SHIMADZU LC-20AD Prominence (Shimadzu, Japan), MGA-915 atomic absorption spectrometer (Lumex, Russia), SF-102 spectrophotometer (Aquilon, Russia), analytical balance Sartorius A 120 S (SARTORIUS, Germany).

Laboratory Methods

Students' nutritional status was assessed using the computer program "Monitoring of Physical Development and Nutritional Status" **[13]**. Formulation modelling, computer analysis of the nutrient composition, calculation of the amount of saturated (EFAs), monounsaturated (MNFA), and polyunsaturated (PUFA) fatty acids, indicators of amino acid balance, biological value of protein, nutritional and energy value of finished products were carried out using the Etalon software package **[14]**, **[15]** following the methodology of multilevel modelling of food systems **[16]**.

The mass fraction of moisture in meat products was determined by drying in a drying cabinet at a temperature of 103 ±2 °C according to GOST 9793-2016 **[17]**. The mass fraction of the protein was determined by the Kjeldahl method **[18]**, the mass fraction of fat by the Soxlet method **[19]**, **[20]**, the mass fraction of ash by the method of salting in a muffle furnace **[21]**. The composition and mass fraction of carbohydrates were determined using high-performance liquid chromatography according to GOST 34134-2017 **[22]**. The mass fraction of vitamins was determined using high-performance liquid chromatography according to GOST R 55482-2013 **[23]** and GOST 32307-2013 **[24]**. The flame atomic absorption method determined the mass fraction of potassium, magnesium, zinc, and iron according to GOST 55484-2013 **[25]** and GOST 30178-96 **[26]**. The titrimetric method determined the mass fraction of calcium according to GOST R 55573-2013 **[27]**. The mass fraction of phosphorus was determined by the spectrophotometric method according to GOST 9794-2015 **[28]**. The mass fraction of selenium was determined by the fluorimetric method of M 04-33-2004 **[29]**. The titrimetric method determined the mass fraction of iodine according to Guidelines 4.1.1106-02 **[30]**.

Description of the Experiment

Sample preparation: During physico-chemical tests, point samples weighing 200 g were taken from sausage products, cutting off from the product in the transverse direction at a distance of at least 5 cm from the edge. Of the two-point samples from different production units, a combined sample weighing 400 g was made. From several point samples, two combined samples with a mass of 400 g were made. For liver pates, two combined samples weighing 600 g were made. To prepare samples from chopped semi-finished products, four culinary products weighing 75 g were ground twice in a meat grinder and mixed to obtain a homogeneous mass. The prepared samples were placed in dry glass jars and tightly closed with lids. Before each sample was taken, the contents of the jar were thoroughly mixed. The samples were stored at a temperature of 4 ±2 °C until the end of the tests.

Number of samples analyzed: In the computer modelling process, 610 formulations of the studied meat products were calculated and analysed. Five recipes of each type of meat product (boiled sausages, liver pate, and chopped semi-finished products) with the best values of nutrient balance indicators were obtained from modelling. Based on the development and testing results, one recipe for each type of meat product was selected, the properties of which are presented in this article. Eighteen samples were analysed during physicochemical tests.

Number of repeated analyses: All measurements of the indicators on the devices were carried out at least three times.

Number of experiment replications: Each experiment was repeated at least three times to determine one value of each indicator**.**

Design of the experiment: In the first stage, based on an assessment of the nutritional status, a lack of basic nutrients in the diet of North Caucasus Federal University students was revealed. Nutritional status was assessed by completing questionnaires on anthropometric data, physical activity, and diet. 265 students participated in the study. Balanced formulations of boiled sausages, liver paste, and chopped semi-finished products with high nutritional and biological value were developed using computer modelling to solve the problem of reducing nutrient deficiency in the diet of the target group of consumers. Experimental developments and physicochemical tests of meat product samples were carried out at the last stage of the research stage.

Statistical Analysis

During the research, a set of standard methods for determining the physicochemical properties of finished products was used. The reliability of the results is confirmed by repeated repetitions and reproducibility of experimental data, their statistical processing, and approbation of the technology of new meat products in laboratory conditions of the Faculty of Food Technology and Engineering named after Academician A.G. Khramtsov of the North Caucasus Federal University. Statistical analysis was performed in Microsoft Excel 2019 using XLSTAT statistical software. The authenticity of the obtained experimental data for all indicators was determined using the Student's test with a confidence probability of ≤ 0.05 for the number of parallel determinations of at least three.

RESULTS AND DISCUSSION

The need to develop fortified food products and their inclusion in the diet of the category of consumers under study has been confirmed by our research, the results of which have shown that the vast majority of young people have a nutritional deficiency of vitamins, as well as a deficiency of the main types of mineral substances, including calcium, potassium, magnesium, iron, zinc, selenium and iodine. A deficiency of B vitamins in the diet was found in 45% of young people, while vitamin A deficiency was found in 61% of young men and 77% of young women. Most consumers studied have a nutritional deficiency of vitamins C, E, and D. More than 64% of young people have insufficient calcium in their diet, and iodine deficiency was detected in more than 82% of the study participants (Figure 1).

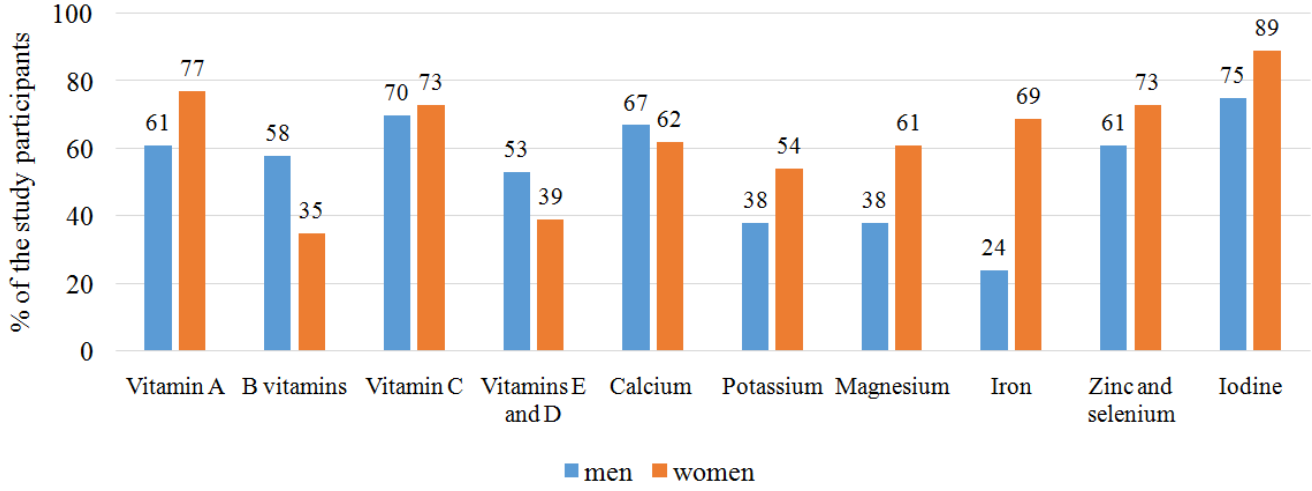


Figure 1 The lack of vitamins and minerals in the diet of the studied group of young people.

Most male and female respondents consume an excess of saturated fatty acids (SFAs) per day – 54% and 68%, respectively. At the same time, 61% of the studied men and 74% of female respondents are deficient in polyunsaturated fatty acids (PUFA).

To overcome the identified deficiency of nutrients, formulations of many meat and meat-vegetable products have been developed and proposed within the research framework. The composition of the developed cooked sausage includes veneered beef of the first grade, pork veneered semi-fat, pork skin emulsion, nitrite salt, and ground and allspice pepper. The recipe also includes chicken fillet, chicken eggs, and mushroom powder from

champignons as an additional source of protein **[31]** and to give the product a nutrient balance of amino acid composition, increase the stability of consumer properties, nutritional, biological value, and organoleptic characteristics.

Dry milk molasses with lactulose "LactuVet-1" is proposed as a source of prebiotics, sweetening agents, and micro- and macroelements in the formulation **[11]**. Kelp was used to enrich cooked sausage with iodine in an easily digestible form **[32]**.

Dry milk molasses with lactulose "LactuVet-1" is a product of deep processing of whey, has a low cost, and contains at least 14.3% lactulose, 25.2% lactose, 15.0% mineral substances, including 3.4% calcium, 1.4% phosphorus, 1.2% potassium, 0.5% magnesium **[33]**. It is obtained by manufacturing high-quality crystalline milk sugar (lactose). Dry milk molasses with lactulose "LactuVet-1" is a dry powder of light yellow or cream color, with a neutral odor characteristic of the raw material from which it is made.

The introduction of dry milk molasses with lactulose "LactuVet-1" and kelp in the recipe of cooked sausage enriches the product with mineral substances, including calcium, magnesium, phosphorus, potassium **[33]**, and iodine **[34]**, **[35]** due to their mutual combination and specially selected quantitative ratio.

The results of studies of boiled sausage are shown in Table 1.

Table 1 Physico-chemical parameters of the developed boiled sausage.

Name of indicators	Values
Mass fraction of moisture, %	66.7 ±0.9
Mass fraction of protein, %	10.3 ±0.4
Mass fraction of fat, %	15.7 ±0.9
Mass fraction of carbohydrates, %	2.9 ±0.5
Mass fraction of ash, %	3.56 ±0.15
Mass fraction of lactose, %	0.78 ±0.06
Mass fraction of lactulose, %	0.46 ±0.04
Energy value, Kcal/KJ	194/943

Developed cooked sausages contain not less than 0.42 grams of lactulose in their composition (in 100 grams) **[36]**, which corresponds to 20% of the minimum recommended daily intake of this prebiotic **[12]**. It is known **[37]** that lactulose retains its structure and bifidogenic activity during thermal processing in the production of food products **[38]**.

The high degree of balance of boiled sausage in terms of amino acid composition is confirmed by a sufficiently high value of the generalized desirability criterion (0.73 units) **[14]**, this corresponds to a rating of "good" on the Harrington desirability scale **[39]**, **[40]**. The minimum amino acid score value is 95.6%, and the biological value of the proposed product reaches 92.8% (Table 2).

Table 2 Indicators of amino acid, fatty acid balance, and biological value of the protein of the developed boiled sausage.

Name of indicators	Values
Generalized desirability criterion for essential amino acids **[14]**, percentage of units	0.73
Minimum amino acid score, %	95.6
Comparable excess ratio **[14]**, g/100g protein	3.8
Coefficient of difference of amino acid score **[14]**, %	7.2
Biological value of protein **[14]**, %	92.8
EFAs, g/100 g lipids	38.3
MUFAs, g/100 g lipids	34.5
PUFAs, g/100 g lipids	10.9

Note: The values of the indicators were calculated using the Etalon computer program.

Table 3 shows data on micro-, macronutrients, and vitamins in the developed product.

Table 3 Content of micro-, macronutrients and vitamins in boiled sausage.

Nutrient	Contents per 100 g of sausage	% of the RDA*
Vitamins		
Vitamin A, retinol equivalent, mg	0.141 ±0.012	15.7/17.6**
Vitamin B1, thiamine, mg	0.161 ±0.023	10.7
Vitamin B2, riboflavin, mg	0.214 ±0.041	11.9
Vitamin B3, niacin, mg	2.605 ±0.154	13.0
Vitamin B5, pantothenic acid, mg	0.285 ±0.039	5.7
Vitamin B6, pyridoxine, mg	0.167 ±0.029	8.4
Vitamin B12, cobalamin, mcg	0.337 ±0.083	11.2
Macronutrients		
Potassium, mg	346.19 ±30.81	9.9
Calcium, mg	153.04 ±16.98	15.3
Magnesium, mg	37.85 ±5.30	9.0
Phosphorus, mg	152.33 ±7.76	21.8
Micronutrients		
Iodine, μg	45.28 ±6.70	30.2
Iron, mg	0.979 ±0.123	9.8/5.4**
Zinc, mg	0.878 ±0.142	7.3
Selenium, mcg	15.56 ±2.53	22.2/28.3**

Note: *RDA – recommended daily requirement for over 18 of age by MR 2.3.1.0253-21 «Norms of physiological requirements in energy and food substances for different population groups of the Russian Federation»; ** values RDA for men/women.

The presented results of studies of the nutrient composition of the developed boiled sausage indicate that the product meets the needs of the target consumer group and fills the identified deficiency of the main types of vitamins and mineral substances.

The main raw materials of the developed pate recipe are beef liver, carrots, bacon, chicken skin, rapeseed and soya oil, onion, mushroom powder from mushrooms, dry milk molasses with lactulose (LaktuVet-1) **[11]**, **[33]**, semolina, and bone broth. Computer modelling and optimisation of the ratio of these components in the developed pate allowed the product to have consistently high consumer properties, balanced nutrient composition, and prebiotic action.

The useful properties of mushroom powder from dried champignon are determined primarily by its nutritional value. Mushrooms have a special chemical composition **[41]**; they are characterised by the content of a large number of vitamins, macro-, and microelements, and contain a significant amount of organic salts and sugars **[42]**, **[43]**. The useful properties of mushroom powder also manifest themselves in its saturation of vegetable protein **[44]**. It is digested by the body better than whole mushrooms and contains B vitamins, ascorbic acid, calcium, magnesium, and other vitamins and micro- and macronutrients **[45]**.

Soya oil contains 70% PUFAs **[46]**. It is also a source of vitamin E (17.1 mg per 100 g of product) **[47]**. Introducing soya oil into a new type of pate formulation increases the mass fraction of vegetable fat and useful unsaturated fatty acids **[48]**.

Rapeseed oil, which contains high polyunsaturated and monounsaturated fatty acids (MUFAs), is also used in the pate recipe **[49]**. MUFAs have a positive effect on reducing bad cholesterol and normalising heart function **[50]**. In addition, rapeseed oil contains a significant amount of a powerful antioxidant – vitamin E (18.9 mg per 100 g of product) **[48]**.

Finely ground chicken skin has a low production cost and is well suited for improving the visco-plastic properties of liver pate. The fat-soluble vitamins in chicken skin are vitamins A and D. The water-soluble ones are vitamins B1, B2, B3 (PP), B5, B6, B9, and B12. Its mineral composition is represented by macronutrients: potassium, magnesium, sodium, and phosphorus; and trace elements: iron, zinc, and selenium **[51]**.

Fortified liver pate contains at least 35.5% of the recommended daily intake of the prebiotic lactulose. Its energy value is 286 kcal, of which 15.1% is protein. This indicates that this product can be classified as a source of protein **[52]**.

Physicochemical quality parameters of the developed liver pate are presented in Table 4.

Table 4 Physico-chemical quality indicators of the developed pate.

Name of indicators	Values
Mass fraction of moisture, %	57.4 ±0.8
Mass fraction of protein, %	10.8 ±0.4
Mass fraction of fat, %	24.9 ±0.6
Mass fraction of carbohydrates, %	4.7 ±0.5
Mass fraction of ash, %	2.20 ±0.04
Mass fraction of lactose, %	1.29 ±0.06
Mass fraction of lactulose, %	0.76 ±0.05
Energy value, Kcal/KJ	286/1198

The developed pate has a high degree of balance in amino acid composition, which is confirmed by the value of the generalised desirability criterion (0.67 units), corresponding to a score of (good) on Harrington`s desirability scale **[39]**, **[40]**. At the same time, the value of the minimum amino acid score is 99.6%, and the biological value of the proposed product reaches 87.7% (Table 5).

Table 5 Indicators of amino acid, fatty acid balance and biological value of the protein of the developed pate.

Name of indicators	Values
Generalized desirability criterion for essential amino acids **[14]**, percentage of units.	0.67
Minimum amino acid score, %	99.6
Comparable excess ratio **[14]**, g/100g protein	4.5
Coefficient of difference of amino acid score **[14]**, %	12.4
Biological value of protein **[14]**, %	87.7
EFAs, g/100 g lipids	31.9
MUFAs, g/100 g lipids	33.4
PUFAs, g/100 g lipids	32.6

Note: The values of the indicators were calculated using the Etalon computer program.

Analysis of the data in Table 5 shows that by TR CU 022/2011 **[52]**, the developed liver pate has a high content of vitamins A, B2, and B12. It is a source of iron, calcium, phosphorus, and vitamins E, B3, and B5, therefore fulfilling the stated purpose of covering their deficiency in young people.

The recipes of the developed functional meat and vegetable semi-finished products with prebiotic action contain duck meat and mechanically deboned chicken meat in an optimally selected ratio, beef protein, carrots, white cabbage, sweet pepper, dry milk molasses with lactulose, semolina, egg powder, soybean oil, wheat bran, breadcrumbs, and spices.

The energy value of the developed semi-finished products is 177.8 kcal, of which 22.8% is provided by protein. It allows them to be classified as food products with a high protein content. The product contains the prebiotic lactulose (at least 28.5% of the recommended daily intake, Table 6).

Table 7 shows the pate's micro-, macronutrients, and vitamin content.

Table 6 Physicochemical quality indicators of chopped semi-finished products.

Name of indicators	Values
Mass fraction of moisture, %	65.1 ±0.9
Mass fraction of protein, %	10.2 ±0.5
Mass fraction of fat, %	11.2 ±0.6
Mass fraction of carbohydrates, %	1.68 ±0.05
Mass fraction of ash, %	1.05 ±0.05
Mass fraction of lactose, %	0.61 ±0.04
Mass fraction of lactulose, %	744.4/177.8

Table 7 Content of micro-, macronutrients and vitamins in liver pate.

Nutrient	Content in 100 g in the pate	% of the RDA*
Vitamins		
Vitamin A, retinol equivalent, mg	3.474 ±0.112	386/434**
Vitamin B1, thiamine, mg	0.111 ±0.016	7.4
Vitamin B2, riboflavin, mg	0.781 ±0.148	43.4
Vitamin B3, niacin, mg	4.434 ±0.212	22.2
Vitamin B5, pantothenic acid, mg	1.704 ±0.106	34.1
Vitamin B6, pyridoxine, mg	0.270 ±0.044	13.5
Vitamin B12, cobalamin, mcg	19.94 ±4.18	664.7
Vitamin C, mg	5.860 ±0.375	5.9
Vitamin D, calciferol, mcg	0.29 ±0.04	1.9
Vitamin E, alpha-tocopherol, mg	2.301 ±0.068	15.3
Macronutrients		
Potassium, mg	309.23 ±28.45	8.8
Calcium, mg	150.32 ±18.18	15.0
Magnesium, mg	35.68 ±4.71	8.5
Phosphorus, mg	202.44 ±10.02	28.9
Micronutrients		
Iron, mg	2.433 ±0.212	24.3/13.5**
Zinc, mg	1.345 ±0.191	11.2

Note: * RDA – recommended daily requirement for over 18 of age following MR 2.3.1.0253-21 (Norms of physiological requirements in energy and food substances for different population groups of the Russian Federation); ** values RDA for men/women.

The new types of chopped semi-finished products have a high balance of essential amino acids. The generalised desirability criterion for essential amino acids of the product is close to the reference value equal to one. The indicators in Table 8 indicate a high biological value of chopped meat and pastry semi-finished products.

Table 8 Indicators of nutrient balance, nutritional and energy value of meat and vegetable semi-finished products.

Name of indicators	Values
Generalized desirability criterion for essential amino acids **[14]**, percentage of units.	0.96
Minimum amino acid score, %	88.1
Comparable excess ratio **[14]**, g/100g protein	4.5
Coefficient of difference of amino acid score **[14]**, %	0.31
Biological value of protein **[14]**, %	99.7
EFAs, g/100 g lipids	25.1
MUFAs, g/100 g lipids	37.1
PUFAs, g/100 g lipids	25.7

Note: The values of the indicators were calculated using the Etalon computer program.

The combination of meat, vegetable raw materials, and functional ingredients made it possible to most significantly provide the developed food product with protein, PUFAs, vitamins, micro-, and macroelements, where the ratio of calcium and phosphorus is close to optimal. At the same time, the ratio of calcium and phosphorus in the developed semi-finished products is close to the optimal (1:1) for calcium assimilation **[53]** (Table 9).

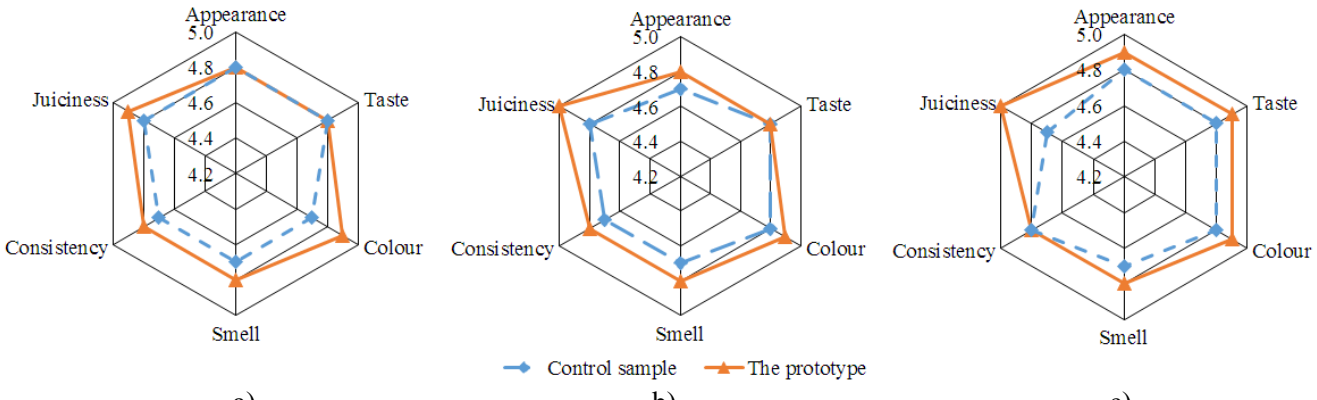
Table 9 The content of micro-, macroelements, and vitamins of meat and vegetable semi-finished products.

Nutrient	Content in 100 g in the semi-finished products	% of the RDA*
Vitamins		
Vitamin A, retinol equivalent, mg	0.138 ±0.011	15.4/17.3**
Vitamin B1, thiamine, mg	0.065 ±0.010	4.3
Vitamin B2, riboflavin, mg	0.095 ±0.021	5.3
Vitamin B3, niacin, mg	2.089 ±0.113	10.5
Vitamin B5, pantothenic acid, mg	0.341 ±0.042	6.8
Vitamin B6, pyridoxine, mg	0.113 ±0.020	5.7
Vitamin B12, cobalamin, mcg	8.297 ±0.473	8.3
Vitamin C, mg	0.16 ±0.03	1.1
Vitamin D, calciferol, mcg	0.665 ±0.040	4.4
Macronutrients		
Potassium, mg	198.50 ±21.84	5.7
Calcium, mg	153.82 ±17.68	15.4
Magnesium, mg	39.44 ±5.62	9.4
Phosphorus, mg	144.34 ±8.48	18.0
Micronutrients		
Iron, mg	0.733 ±0.098	7.3/4.1**
Zinc, mg	1.012 ±0.154	8.4

Note: *RDA – recommended daily requirement by over 18 of age by MR 2.3.1.0253-21 (Norms of physiological requirements in energy and food substances for different population groups of the Russian Federation); ** values RDA for men/women.

Following TR CU 022/2011 **[52]**, the developed chopped meat and vegetable semi-finished products are a source of micro- and macroelements and vitamins, namely calcium and phosphorus in their recommended ratio and vitamin A. They also contain most B vitamins, vitamin C, fat-soluble vitamins D and E, macronutrients potassium and magnesium, and trace elements iron and zinc.

Including dry milk molasses with lactulose "LactuVet-1" in the formulation of the developed meat products made it possible to obtain products with a more pronounced aroma, juiciness, and uniform colour shade (Figure 2).



a) b) c)

Figure 2 The profile of organoleptic parameters of the developed meat products with the inclusion of dry milk molasses with lactulose "LactuVet-1" in the formulation (the prototype) and without it (control sample): a) boiled sausage; b) liver pate; c) chopped semi-finished products.

The appearance of the developed meat and vegetable semi-finished products, boiled sausages, and liver paste is shown in Figure 3.

Figure 3 The appearance of the developed fortified meat products.

The use in formulations of fortified meat products of milk molasses with lactulose "LactuVet-1" allowed for the increase of organoleptic characteristics and provided a pronounced health-preventive effect of finished products at a reduced cost. This effect is due to the presence of molasses of lactulose – prebiotic No. 1 in the world **[11]** and dry milk sugar – lactose **[54]**, as well as a complex of minerals. Dry milk molasses with lactulose as a functional ingredient has a low cost **[10]**. In addition, in the course of our research, it was noted that dry milk molasses with lactulose provides a reduction in thermal losses and an increase in the yield of finished products.

CONCLUSION

The results of the presented studies developed fortified cooked sausages, liver pate, and chopped meat and vegetable semi-finished products demonstrate the following advantages. Combination of meat, vegetable raw materials, and functional ingredients, including dry milk molasses with lactulose "LactuVet-1", allowed to provide finished meat products with high-grade protein, vitamins, micro-, macroelements to achieve prebiotic action, high organoleptic characteristics and reduce the cost price, which is also important for young people. In the developed meat products, 100 g contains 20-35.5% of the minimum recommended daily intake of the lactulose prebiotic. The products are balanced in essential amino acids. They have consistently high nutritional and biological value while being enriched in a natural form of calcium and other minerals that are easily available for assimilation by the body. The values of the general criterion for the desirability of the amino acid composition of protein for the proposed meat products range from 0.67 to 0.96 units. They are rated “good” and “excellent” on the Harrington Desirability Scale. The biological value of protein is 87.7% for liver pate, 92.8% for boiled sausages, and 99.7% for chopped semi-finished products. Cooked sausage products developed by TR CU 022/2011 are a source of vitamin A, calcium, phosphorus, and selenium and contain about 45 mcg of iodine per 100 g product. Liver pate has a high content of vitamins A, B2, and B12, the amount of which in 100 g of product is approximately 3.47 mg, 0.78 mg, and 19.94 mcg, respectively. Pate is a source of iron, calcium, phosphorus, and vitamins E, B3 and B5. Chopped meat and vegetable semi-finished products contain most B vitamins, vitamin C, fat-soluble vitamins D and E, macroelements potassium and magnesium, and

microelements iron and zinc. Semi-finished products are a source of calcium, phosphorus, and vitamin A, which in 100 g of product is approximately 153.82 mg, 144.34 mg, and 0.14 mg, respectively.

Introducing the developed meat products into the diet of the target youth group of consumers aims to reduce the imbalance of the main nutrients identified during the study and analysis of their nutritional status. It is recommended that clinical studies be conducted with confirmation by an additional array of experimental data.

REFERENCES

1. Cafer, A. M. (2014). Food and Society: Principles and Paradoxes, by Amy E. Guptill, Denise A.Copelton, and BetsyLucal, Malden, MA: Polity Press, 2013. 232 pp. ISBN: 978-0-7456-4282-6. In Rural Sociology (Vol. 79, Issue 3, pp. 414–416). Wiley. https://doi.org/10.1111/ruso.12048_2
2. Downs, S. M., & Fox, E. L. (2021). Uneven decline in food system inequality. In Nature Food (Vol. 2, Issue 3, pp. 141–142). Springer Science and Business Media LLC. https://doi.org/10.1038/s43016-021-00247-3
3. Perez-Escamilla, R., Bermudez, O., Buccini, G. S., Kumanyika, S., Lutter, C. K., Monsivais, P., & Victora, C. (2018). Nutrition disparities and the global burden of malnutrition. In BMJ (p. k2252). BMJ. https://doi.org/10.1136/bmj.k2252
4. Tahseen, F. M. (2016). Functional Food – A Review. In European Academic Research (Vol. 4, Issue 6, pp. 5695–5702). Bridge Center.
5. Rana, A. (2022). Categories and Management of Functional Food. In Current Topics in Functional Food. IntechOpen. https://doi.org/10.5772/intechopen.104664
6. Kochetkova, A. A., Vorobyeva, V. M., Sarkisyan, V. A., Vorobyeva, I. S., Smirnova, E. A., & Shatnyuk, L. N. (2020). Dynamics of innovations in food technologies: from specialization to personalization. In Problems of Nutrition (Vol. 89, Issue 4, pp. 233–243). GEOTAR-Media publishing group. https://doi.org/10.24411/0042-8833-2020-10056
7. Olson, R., Gavin-Smith, B., Ferraboschi, C., & Kraemer, K. (2021). Food Fortification: The Advantages, Disadvantages and Lessons from Sight and Life Programs. In Nutrients (Vol. 13, Issue 4, p. 1118). MDPI AG. https://doi.org/10.3390/nu13041118
8. Eliseeva L. G., & Portnov, N. M. (2020). Assessment of the diet, taking into account the variability of the chemical composition of the products. In Nutrition Issues. https://doi.org/10.24411/0042-8833-2020-10018
9. Fern, E. B., Watzke, H., Barclay, D. V., Roulin, A., & Drewnowski, A. (2015). The Nutrient Balance Concept: A New Quality Metric for Composite Meals and Diets. In A. S. Wiley (Ed.), PLOS ONE (Vol. 10, Issue 7, p. e0130491). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0130491
10. Borisenko, A. A., Uzakov, Ya. M., Greseva, E. G., Borisenko, L. A., Borisenko, A. A., & Rudkovsky, A. V. (2023). Relevance and prospects of using a domestic lactose-lactulose product in the meat industry. In Vsyo o myase (Issue 3, pp. 20–25). The Gorbatov's All-Russian Meat Research Institute. https://doi.org/10.21323/2071-2499-2023-3-20-25
11. Ryabtseva, S., Khramtsov, A., Shpak, M., Lodygin, A., Anisimov, G., Sazanova, S., & Tabakova, Y. (2023). Biotechnology of Lactulose Production: Progress, Challenges, and Prospects. In Food Processing: Techniques and Technology (Vol. 53, Issue 1, pp. 97–122). Kemerovo State University. https://doi.org/10.21603/2074-9414-2023-1-2419
12. Aït-Aissa, A., & Aïder, M. (2013). Lactulose: production and use in functional food, medical and pharmaceutical applications. Practical and critical review. In International Journal of Food Science & Technology (Vol. 49, Issue 5, pp. 1245–1253). Wiley. https://doi.org/10.1111/ijfs.12465
13. Portnov, N. M., & Preobrazhenskaya, E. N. (2019). Diet development and system of monitoring nutrient status. In Informatics and education (Issue 3, pp. 63–67). Publishing House Education and Informatics. https://doi.org/10.32517/0234-0453-2019-34-3-63-67
14. Borisenko, A. A., Bratsikhin, A. A., Borisenko, L. A., & Borisenko, A. A. (2019). Computer modeling of nutrient composition of multicomponent food products as a way of their transfer to the segment of healthy nutrition. In Vsyo o myase (Issue 3, pp. 54–57). The Gorbatov's All-Russian Meat Research Institute. https://doi.org/10.21323/2071-2499-2019-3-54-57
15. Borisenko, A. A., Bratsikhin, A. A., Saricheva, L. A., Borisenko, A. A., Slyusarev, G. V., & Chebotarev, E. A. (2018). Meat and plant products designing based on the multilevel modeling method. In Journal of Hygienic Engineering and Design (Vol. 24, pp. 75–79). Consulting and Training Center KEY.
16. Borisenko, A. A., Saricheva, L. A., Borisenko, A. A., Oleshkevich, O. I., Mamay, D. S., Kostenko, E. G., & Savchenko, A. A. (2023). Methodology of multilevel modeling of food systems. In the international conference on battery for renewable energy and electric vehicles (ICB-REV) 2022. the international

conference on battery for renewable energy and electric vehicles (ICB-REV) 2022. AIP Publishing. https://doi.org/10.1063/5.0151741
17. GOST 9793-2016 Meat and meat products. Methods for determining moisture.
18. FSSAI, lab. manual 6., Manual of methods of analysis of foods, meat and meat products&fish and fish products, Food safety and standards authority of India, Government of India, New Delhi, 2012, Total Protein – Kjeldahl method, p. 23-24.
19. Manirakiza, P., Covaci, A., & Schepens, P. (2001). Comparative Study on Total Lipid Determination using Soxhlet, Roese-Gottlieb, Bligh & Dyer, and Modified Bligh & Dyer Extraction Methods. In Journal of Food Composition and Analysis (Vol. 14, Issue 1, pp. 93–100). Elsevier BV. https://doi.org/10.1006/jfca.2000.0972
20. Pérez-Palacios, T., Ruiz, J., Martín, D., Muriel, E., & Antequera, T. (2008). Comparison of different methods for total lipid quantification in meat and meat products. In Food Chemistry (Vol. 110, Issue 4, pp. 1025–1029). Elsevier BV. https://doi.org/10.1016/j.foodchem.2008.03.026
21. Ismail, B. P. (2017). Ash Content Determination. In Food Analysis Laboratory Manual (pp. 117–119). Springer International Publishing. https://doi.org/10.1007/978-3-319-44127-6_11
22. GOST 34134-2017. Meat and meat products. Determination of free carbohydrates.
23. GOST R 55482-2013. Meat and meat products. Method for determination the water-soluble vitamins.
24. GOST 32307-2013. Meat and meat products. Determination of fat-soluble vitamins by high performance liquid chromatography.
25. GOST 55484-2013. Meat and meat products. Determination of sodium, potassium, magnesium and manganese by flame atomic absorption.
26. GOST 30178-96. Raw material and food-stuffs. Atomic absorption method for determination of toxic elements.
27. GOST R 55573-2013. Meat and meat products. Determination by calcium atomic absorption and titrimetric methods.
28. GOST 9794-2015. Meat products. Methods for the determination of total phosphorus content.
29. M 04-33-2004 (edition of 2013) Food products and food raw materials, feed and feed feeds. Methods of measuring the mass fraction of selenium by the fluorimetric method on the fluid analyzer «Fluorat-02».
30. Methodological guidelines 4.1.1106-02. Determination of the mass fraction of iodine in food products and raw materials by the titrimetric method: Methodological guidelines—Moscow: Federal Center for State Sanitary and Epidemiological Supervision of the Ministry of Health of the Russian Federation, 2002,— 15 p.
31. Ayimbila, F., & Keawsompong, S. (2023). Nutritional Quality and Biological Application of Mushroom Protein as a Novel Protein Alternative. In Current Nutrition Reports (Vol. 12, Issue 2, pp. 290–307). Springer Science and Business Media LLC. https://doi.org/10.1007/s13668-023-00468-x
32. Kryzhova, Y., Antonuk, M., Stabnikov, V., & Stabnikova, O. (2021). Stability of selenium and iodine in the functional meat products prepared with seaweeds under different cooking procedures. In Ukrainian Food Journal (Vol. 10, Issue 1, pp. 136–144). National University of Food Technologies. https://doi.org/10.24263/2304-974x-2021-10-1-12
33. Khramtsov, A. G., Dykalo, N. Y., Shkola, S. S., Eremina, A. I., Anisimov, G. S., & Rudkovskii, A. V. (2022). Lactuvet – the food supplement of the future. In Agrarian-And-Food Innovations (Vol. 17, pp. 17–29). Volga Region Research Institute of Manufacture and Processing of Meat-and-Milk Production. https://doi.org/10.31208/2618-7353-2022-17-17-29
34. Aakre, I., Solli, D. D., Markhus, M. W., Mæhre, H. K., Dahl, L., Henjum, S., Alexander, J., Korneliussen, P.-A., Madsen, L., & Kjellevold, M. (2021). Commercially available kelp and seaweed products – valuable iodine source or risk of excess intake? In Food & Nutrition Research (Vol. 65). SNF Swedish Nutrition Foundation. https://doi.org/10.29219/fnr.v65.7584
35. Matos, Â. P., Novelli, E., & Tribuzi, G. (2022). Use of algae as food ingredient: sensory acceptance and commercial products. In Frontiers in Food Science and Technology (Vol. 2). Frontiers Media SA. https://doi.org/10.3389/frfst.2022.989801
36. Khramcov, A. G., Lodygin, A. D., Anisimov, G. S., Shkola, S. S., Dykalo, N. Y., Eremina, A. I., & Dinyakov, V. A. (2021). Regularities of the transition of substances from permeate to molasses in the process of lactose recovery. In IOP Conference Series: Earth and Environmental Science (Vol. 677, Issue 3, p. 032082). IOP Publishing. https://doi.org/10.1088/1755-1315/677/3/032082
37. Ryabtseva, S. A., Khramtsov, A. G., Budkevich, R. O., Anisimov, G. S., Chuklo, A. O., & Shpak, M. A. (2020). Physiological effects, mechanisms of action and application of lactulose. In Problems of Nutrition

(Vol. 89, Issue 2, pp. 5–20). GEOTAR-Media publishing group. https://doi.org/10.24411/0042-8833-2020-10012
38. Yartseva, N., Dolganova, N., Aleksanian, I., & Nugmanov, A. (2020). Prebiotic «Lactulose Premium» as a Promising Functional Additive in Minced Fish. In Food Industry (Vol. 5, Issue 3, pp. 25–34). Ural State University of Economics. https://doi.org/10.29141/2500-1922-2020-5-3-3
39. Oganesyants, L., Semipyatniy, V., Galstyan, A., Vafin, R., Khurshudyan, S., & Ryabova, A. (2020). Multi-criteria food products identification by fuzzy logic methods. In Foods and Raw Materials (Vol. 8, Issue 1, pp. 12–19). Kemerovo State University. https://doi.org/10.21603/2308-4057-2020-1-12-19
40. Montet, D., & Ray, R. C. (Eds.). (2017). Food Traceability and Authenticity. CRC Press. https://doi.org/10.1201/9781351228435
41. Reis, F. S., Barros, L., Martins, A., & Ferreira, I. C. F. R. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. In Food and Chemical Toxicology (Vol. 50, Issue 2, pp. 191–197). Elsevier BV. https://doi.org/10.1016/j.fct.2011.10.056
42. Barros, L., Cruz, T., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. In Food and Chemical Toxicology (Vol. 46, Issue 8, pp. 2742–2747). Elsevier BV. https://doi.org/10.1016/j.fct.2008.04.030
43. Guillamón, E., García-Lafuente, A., Lozano, M., D´Arrigo, M., Rostagno, M. A., Villares, A., & Martínez, J. A. (2010). Edible mushrooms: Role in the prevention of cardiovascular diseases. In Fitoterapia (Vol. 81, Issue 7, pp. 715–723). Elsevier BV. https://doi.org/10.1016/j.fitote.2010.06.005
44. See Toh, C. J. Y., Bi, X., Lee, H. W., Yeo, M. T. Y., & Henry, C. J. (2023). Is mushroom polysaccharide extract a better fat replacer than dried mushroom powder for food applications? In Frontiers in Nutrition (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fnut.2023.1111955
45. Victor L Fulgoni III, T. A. N. (2013). Mushroom Intake is associated with Better Nutrient Intake and Diet Quality: 2001-2010 National Health and Nutrition Examination Survey. In Journal of Nutrition & Food Sciences (Vol. 03, Issue 05). OMICS Publishing Group. https://doi.org/10.4172/2155-9600.1000229
46. Brown, P., & Hart, S. (2010). Soybean Oil and Other n-6 Polyunsaturated Fatty Acid-Rich Vegetable Oils. In Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds (pp. 133–160). CRC Press. https://doi.org/10.1201/9781439808634-c5
47. Herting, D. C., & Drury, E.-J. E. (1963). Vitamin E Content of Vegetable Oils and Fats. In The Journal of Nutrition (Vol. 81, Issue 4, pp. 335–342). Elsevier BV. https://doi.org/10.1093/jn/81.4.335
48. Valk, & Hornstra, G. (2000). Relationship Between Vitamin E Requirement and Polyunsaturated Fatty Acid Intake in Man: a Review. In International Journal for Vitamin and Nutrition Research (Vol. 70, Issue 2, pp. 31–42). Hogrefe Publishing Group. https://doi.org/10.1024/0300-9831.70.2.31
49. Jahreis, G., & Schäfer, U. (2011). Rapeseed (Brassica napus) Oil and its Benefits for Human Health. In Nuts and Seeds in Health and Disease Prevention (pp. 967–974). Elsevier BV. https://doi.org/10.1016/b978-0-12-375688-6.10114-8
50. Möllers, C. Potential and future prospects for rapeseed oil. In F. D. Gunstone (Ed.), Rapeseed and Canola Oil - Production, Processing, Properties and Uses (pp. 186-217). Oxford: Blackwell Publishing.
51. Probst, Y. (2009). Nutrient values for Australian and overseas chicken meat. In Nutrition & Food Science (Vol. 39, Issue 6, pp. 685–693). Emerald. https://doi.org/10.1108/00346650911003011
52. TR CU 022/2011 Food products in terms of its labeling / approved by Decision of the Customs Union Commission of December 9, 2011 № 880. Retrieved from https://docs.cntd.ru/document/902320347
53. Kraft, M. D. (2014). Phosphorus and Calcium. In Nutrition in Clinical Practice (Vol. 30, Issue 1, pp. 21–33). Wiley. https://doi.org/10.1177/0884533614565251
54. Romero-Velarde, E., Delgado-Franco, D., García-Gutiérrez, M., Gurrola-Díaz, C., Larrosa-Haro, A., Montijo-Barrios, E., Muskiet, F. A. J., Vargas-Guerrero, B., & Geurts, J. (2019). The Importance of Lactose in the Human Diet: Outcomes of a Mexican Consensus Meeting. In Nutrients (Vol. 11, Issue 11, p. 2737). MDPI AG. https://doi.org/10.3390/nu11112737

Funds:

The work was carried out with the financial support of the Ministry of Science and Higher Education of the Russian Federation within the framework of the implementation of a comprehensive project for the creation of high-tech production on the topic: "Creation of Russia's first high-tech production of prebiotic lactulose and functional dairy ingredients for import substitution in medicine, veterinary medicine, infant nutrition, production of therapeutic and preventive products for humans and animals" (Agreement on granting a subsidy from the federal budget for the development of cooperation between a state scientific institution and an

organization of the real sector of the economy for the purpose of implementing a complex project for the creation of high-tech production No. 075-11-2022-021 dated 04/07/2022) within the framework of the Resolution of the Government of the Russian Federation dated 9 April 2010 No. 218 on the basis of the FSAEI HE "North-Caucasus Federal University".

Acknowledgments: -

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Aleksander Borisenko, North-Caucasus Federal University; Faculty of Food Engineering and Biotechnology named after Academician A. G. Khramtsov, Department of Food Technology and Engineering, 1, Pushkin st., Stavropol, 355009, Russian Federation,
E-mail: alaborisenko@ncfu.ru
ORCID: https://orcid.org/0000-0003-0002-7871

Yassin Uzakov, Almaty Technological University, Department of Technology of Food products, 100, Tole Bi street, 050010, Almaty, Republic of Kazakhstan,
E-mail: uzakm@mail.ru
ORCID: https://orcid.org/0009-0001-2878-7170

Ekaterina Greseva, North-Caucasus Federal University; Faculty of Food Engineering and Biotechnology named after Academician A.G. Khramtsov, Department of Food Technology and Engineering, 1, Pushkin st., Stavropol, 355009, Russian Federation,
E-mail: kgreseva@bk.ru
ORCID: https://orcid.org/0009-0002-2838-4184

***Victoria Razinkova**, North-Caucasus Federal University; Faculty of Food Engineering and Biotechnology named after Academician A. G. Khramtsov, Department of Food Technology and Engineering, 1, Pushkin st., Stavropol, 355009, Russian Federation,
E-mail: viktoriya.atakishieva@mail.ru
ORCID: https://orcid.org/0000-0002-3394-584X

Aleksey Borisenko, North-Caucasus Federal University; Faculty of Food Engineering and Biotechnology named after Academician A. G. Khramtsov, Department of Food Technology and Engineering, 1, Pushkin st., Stavropol, 355009, Russian Federation,
E-mail: aborisenko@ncfu.ru
ORCID: https://orcid.org/0000-0003-3287-0644

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 25.6.2024
Revised: 26.7.2024
Accepted: 1.8.2024
Published: 20.8.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 697-718
https://doi.org/10.5219/1998
ISSN: 1337-0960 online
www.potravinarstvo.com

Substantiation of wild plants used as functional ingredients in the technology of crisp grain bread

Assel Izembaeva, Zilikha Moldakulova, Asemgul Abdreeva, Galiya Iskakova, Meruert Baiysbaeva, Makpal Atyhanova

ABSTRACT

Research on the nutritional content of wild plant fruits in Kazakhstan, including Hawthorn fruit (*Crataegus laevigata*), rosehip fruit (*Rose canina* L. variety), sea buckthorn fruit (*Hippophae rhamnoides* - Altai variety), and black chokeberry fruit (*Aronia melanocarpa*), has determined that these fruits possess a combination of properties essential for maintaining human health, attributed to the presence of bioactive substances (BAS) and other components. The technological process for obtaining extracts and concentrates from wild-growing raw material fruit has developed. The following extraction modes were proposed: ultrasonic wave frequency 40 kHz, extraction time 30 minutes, temperature 500C, and concentration of the obtained extracts was carried out by vacuum evaporation method, using IKA RV-10 apparatus at 40-50°C and pressure 800 mbar.

Found that extracts and concentrates have a higher concentration of nutritional substances when compared to the fruits themselves. Thus, the vitamin C content in hawthorn fruits was 27.8 mg, while in the extract, it was 47.84 mg, and in the concentrate – 62.19 mg. The vitamin C content in rosehip fruits, extracts, and concentrates was 578.01 mg, 811.8 mg, and 1101.3 mg, respectively. The vitamin C content in sea buckthorn fruits, extracts, and concentrates was 285.05 mg, 518.8 mg, and 640.9 mg, respectively. The vitamin C content in black chokeberry fruits, extracts, and concentrates was 86.2 mg, 128.8 mg, and 160.5 mg, respectively. A similar increase was observed for the content of vitamin E and other components.

Furthermore, the obtained concentrates meet the safety parameters required by regulatory documents. A technology for producing grain crispbread using the extrusion method has developed, incorporating whole grains, groats, flavouring additives, and concentrates derived from wild fruits such as hawthorn, rosehip, sea buckthorn, and black chokeberry into the recipe.

Keywords: wild plants, extracts, concentrates, chemical composition, food safety, functional foods

INTRODUCTION

Ready-to-eat breakfast cereals dominate the entire market due to the convenient solution they provide for consumers. The market is primarily propelled by individuals with active lifestyles, as these products are processed cereal formulations suitable for consumption without further preparation. Ready-to-eat giants find millennials and the younger generation are the most accessible targets, as they allocate a significant portion of their income to such food products. Consequently, the high level of convenience associated with these products contributes to the overall growth of the market **[1]**.

Accumulated data in the field of nutritional science testify that in contemporary conditions of human life, it is challenging to adequately meet the body's needs for all necessary nutrients and minor biologically active components solely through traditional nutrition. Worldwide, there is a growing emphasis on seeking safer alternative sources, including wild plants characterised by an increased content of biologically active substances known for their ecological purity and high efficiency. Wild plants serve as abundant sources of essential nutrients

such as vitamins, carbohydrates, fats, proteins, organic acids (citric, malic, and so on), aromatic substances, and antioxidants, fulfilling crucial needs for the human body **[1]**, **[2]**, **[3]**.

In connection with the above, research aimed at solving significant problems associated with creating qualitatively new food products enriched with biologically active components isolated from environmentally safe wild plants is relevant.

As non-traditional sources of plant raw materials in the production of breakfast cereals, fruits of various wild fruit and berry plants are used. These plants serve as a direct food source and technological raw material for processing traditional food products, resulting in unique flavours and maximising benefits **[4]**. Thus, utilising raw materials from wild plants can improve the flavour range of products and enrich them with biologically active substances. Among wild fruit plants, the fruits of rosehip, hawthorn, sea buckthorn, and mountain ash are particularly noteworthy, as they not only exhibit high taste properties but also offer a diverse range of physiological benefits attributed to a rich set of biologically active substances, including those with antioxidant properties.

Rosehip (*Rosa L.*) is widely used as a medicinal and food component and is represented by approximately 200 wild species worldwide **[5]**. Rosehip (*Rosa canina L.*) is a fruit member of the Rosaceae family that contains high amounts of phenolic compounds, carotenoids, tocopherols, flavonoids, and vitamin C.

Due to its rich bioactive compounds, rosehip generally prevents and treats diseases such as colds, cardiovascular diseases, gastrointestinal disorders, infections, and diabetes **[6]**. It is also recommended as a general tonic **[7]**.

According to the content of vitamin C (4.8% in seeds and up to 8.5% in pulp), it stands unrivalled among fruit and berry crops. Additionally, rose hips contain P-active compounds (up to 9%), vitamin E (6-10 mg/100 g), B_1, B_2, B_9, carotene, tannins, pectin, nitrogen compounds, flavonoids, sugar, organic acids, fats, and various trace elements crucial for hematopoiesis such as Fe, Mg, Ca, K, Cu, Zn. The seeds also comprise up to 12% of fatty acids, rubixanthin, gazaniaxanthin, β-cryptoxanthin, zeaxanthin, and phenolic compounds such as quercetin, ellagic acid, quercetin glycosides, hydroxycinnamic acids, proanthocyanidin, and aglycones **[8]**.

Rosehip fruits and seeds contain a substantial amount of crucial dietary antioxidants. The increased antioxidant activity is primarily due to ascorbic acid, and the content in rosehip fruits ranges from 6.0 to 8.2 mg g-1 in fresh weight (FW) **[9]**. Phytochemical studies have shown that Rosa species contain a wide range of chemical compounds, including quercetin, kaempferol, catechin, citronellol, limonene, lycopene, carvacrol, thymol, rosmarinic acid, etc. **[10]**. With antiviral and antitumor effects, rosehip preparations can inhibit the proliferation of cancer cells **[11]**.

One promising source of biologically active substances is a unique plant - sea buckthorn. Various bioactive substances are present in all parts of sea buckthorn, and these are used traditionally as raw materials for health foods and as nutritional supplements **[12]**, **[13]**, **[14]**. Research has identified 24 compounds in the seeds and 16 compounds in the pomace, including phenolic acids, flavonoids, and tannins. Sea buckthorn extracts demonstrated in vitro antidiabetic and anti-obesity potential by inhibiting α-glucosidase enzymes (71.52-99.31%) and pancreatic lipase (15.80-35.61%), respectively **[15]**. Additionally, the extracts showed antibacterial activity against *S. aureus*, *B. cereus*, and *P. aeruginosa* **[16]**.

The chemical composition of sea buckthorn fruit represents the lower sugars at 3.5-6.0 % (glucose and fructose), organic acids (oxalic, tartaric, malic, citric and caffeic acids), including fatty acids **[17]**.

The four sea buckthorn varieties (Klara, Dora, Kora, Mara) cultivated in the Republic of Moldova had varying contents of organic acids (malic acid 5.8–13.4 mg/100 g, citric acid 0.08–0.321 mg/100 g, succinic acid 0.03–1.1 mg/100 g), titratable acidity (2.15–8.76%), and pH values (2.73–3.00) **[18]**. The content of phenolic-type antioxidants (phenolic compounds, flavonoids, phenolic acids) and vitamin C was studied. The greatest variability of water-soluble antioxidants in sea buckthorn is associated with vitamin C content, ranging from 82 to 297 mg/100 g, depending on the botanical variety. The total phenolic compounds content was 600-795 mg/100 g, while flavonoids and phenolic acids were 265-346 and 105-170 mg/100 g, respectively. The content of fat-soluble antioxidants in sea buckthorn fruits of different varieties, including vitamin E and carotenoids, was also studied, which amounted to 6.9-8.3 and 10.7-14.9 mg/100 g, respectively **[19]**, **[20]**.

Hawthorn belongs to the genus Crataegus and is widely distributed in the northern temperate zone of the world, counting about 280 species. It has long been used in folk medicine to treat diseases such as heart (cardiovascular disorders), central nervous system, immune system, eyes, reproductive system, liver, kidneys and others. The multifunctional therapeutic role of hawthorn extracts in treating various chronic and degenerative diseases is emphasized, primarily focusing on flavonoids **[21]**. Treatment with hawthorn extracts may be associated with improvements in the complex pathogenesis of various liver and cardiovascular diseases **[22]**. Polysaccharides of *Crataegus pinnatifida* possess diverse biological activities, including antitumor, immunomodulatory,

hypoglycemic, cardioprotective, and antioxidant activities etc. **[23]**. Furthermore, assessments have demonstrated that hawthorn is non-toxic **[24]**.

The carbohydrates in hawthorn fruits consist of sugars, starch, pectin substances, and other compounds. So the content of glucose is 2.02 mg/g, fructose - 2.21 mg/g, sucrose - 0.23 mg/g, arabinose - 1.82 mg/g, xylose - 3.88 mg/g, mannose - 4.25 mg/g, galactose - 1.31 mg/g. Seeds contain from 27.5 to 39.2% fat. The content of organic matter in hawthorn fruits varies widely. Of the organic acids in hawthorn, fruits contain citric acid, oleic acid, ursolic acid, crotegus acid, caffeic acid, and chlorogenic acid **[25]**, **[26]**.

Black chokeberry is a source of many bioactive compounds with a broad spectrum of health-promoting properties. Its ripe and ripe berries are a vibrant source of polyphenolic compounds. Polyphenols are biofactors that determine the high bioactivity of chokeberries, some of the richest sources of polyphenols, which include anthocyanins, proanthocyanidins, flavonols, flavanols, proanthocyanidins, and phenolic acids **[27]**, **[28]**. A total of 27 polyphenolic compounds, comprising seven anthocyanins, 11 flavonols, five phenolic acids, three flavan-3-ols, and one flavanone, were identified in the fruits **[29]**. The highest content of total polyphenols (up to 20 g/100 g dry weight), procyanidins (10-15 g/100 g dry weight), and flavonoids (7-11 g/100 g dry weight), as well as the highest antioxidant activity (up to 100 mmol Trolox/100 g dry weight), was observed in unripe fruits. The content of procyanidins increased at later stages of ripening **[30]**. Proanthocyanidins contribute the most to the antioxidant activity of black chokeberry, being the most potent antimicrobial compound in these fruits **[31]**. Chokeberry fruit stands out as a promising food component due to its exceptionally high levels of antioxidants **[32]**, **[33]**.

It should also be emphasised that berries are rich in biologically active components such as polyphenolic compounds, vitamins (vitamin C and vitamin E), mineral elements (potassium, calcium, and magnesium), carotenoids, pectins, organic acids, and carbohydrates, which are present in smaller quantities **[34]**.

The overall content of key ingredients, polyphenolic compounds, influences many well-documented effects of chokeberry, such as antioxidant, anti-inflammatory, hypotensive, antiviral, anti-aggregatory, anti-diabetic, and anti-atherosclerotic activities, respectively **[35]**. It is possible to classify black chokeberry as a natural medicine. Chokeberry has positive effects in treating cardiovascular and gastrointestinal diseases due to its high antioxidant activity **[36]**. It should be noted that consuming chokeberry products may prevent oxidative-antioxidant imbalance induced by cadmium **[37]**, **[38]**. Black chokeberry can potentially inhibit the development of various types of cancers, including leukaemia, breast and intestinal cancer, and cancer stem cells **[39]**, **[40]**.

Wild plants like rosehip, sea buckthorn, hawthorn and chokeberry contain a diverse set of biologically active substances, including natural antioxidants and other valuable micro-ingredients, which have high antioxidant activity, which gives reason to evaluate them as promising ingredients of food products that can increase the nutritional value and expand the range of healthy food products.

Depending on growing conditions and other factors, more scientific information is needed regarding wild-harvested raw materials' chemical composition and functional-technological properties. Therefore, research on local wild plants' quality, chemical composition, and safety is relevant and in demand. This research aims to expand the raw material base and explore its application in producing functional dry breakfasts (such as crispbreads, snacks, slices, and chips).

Scientific Hypothesis

Extracts extend the raw material base and could be applied to the technology of functional breakfast cereals, particularly bread.

MATERIAL AND METHODOLOGY

Samples

The following wild plants of Kazakhstan have been selected for experimental research: Hawthorn fruit (*Crataegus laevigata*), rosehip fruit (*Rose canina* L. variety), sea buckthorn fruit (*Hippophae rhamnoides* - Altai variety), and black chokeberry fruit (*Aronia melanocarpa*). Extracts are products obtained from their processing (Figure 1).

Hawthorn fruit (*Crataegus laevigata*)	Rosehip fruit (Rose *canina* L. variety)	Sea buckthorn fruit (*Hippophae rhamnoides* - Altai variety)	Black chokeberry fruit (*Aronia melanocarpa*)

Figure 1 Photos of wild plant fruit samples for experimental research.

Raw materials (rose hips, sea buckthorn, black chokeberry, hawthorn) were purchased for experimental studies in a research project. Wild plants (rosehip, sea buckthorn, mountain ash, hawthorn) were collected according to norms approved by local maslikhats, ensuring the preservation of their populations, communities, and growth habitats.

The addition of concentrate from wild fruits (rosehip, sea buckthorn, chokeberry, hawthorn) in the recipe of grain bread carried out by the technical regulation of the Customs Union "Safety requirements for food additives, flavourings and technological auxiliaries" (TR CU 029/2012).

Chemicals

All reagents were of analytical grade and purchased from Laborfarm (Kazakhstan)and Sigma Aldrich (USA).

Animals and biological material

This research did not use animal or biological materials.

Instruments

Obtaining processing products—extracts and concentrates. Extracts from hawthorn, sea buckthorn, rosehip, and black chokeberry fruits were obtained by ultrasonic method. The obtained extracts were concentrated by vacuum evaporation using the IKA RV-10 apparatus.

Ultrasonic extraction (ultrasonic extraction) extracts bioactive compounds from different sources, including plants, fruits and vegetables, by applying ultrasonic waves to the extraction medium. The mechanism of ultrasonic extraction involves the generation of acoustic cavitation, which creates microbubbles in the extraction medium. These microbubbles then collapse, creating shock waves and micro-jets that increase the mass transfer between the sample and solvent, resulting in increased extraction efficiency. Ultrasonic extraction can use extraction methods, including solid-liquid extraction, liquid-liquid extraction, and supercritical fluid extraction.

Laboratory Methods

Determination of quality indicators of plant raw materials was carried out according to the following methods: protein content by the Kjeldahl method, carbohydrate content - by the permanganatometric method, mass fraction of fat - by the Soxhlet method, the content of organic acids - according to GOST 32771-2014, dietary fibres - according to GOST 34844-2022, mass fraction of ash - according to GOST 25555.4-91, the content of vitamin A - according to GOST R 54635-2011, vitamin B5 - according to GOST 32040-2012, vitamin C - according to GOST 24556-89, vitamin E - according to GOST EN 12822-2014, β-carotene - according to GOST EN 12823-2-2014, the content of minerals Mn, Cu, Si, Mo, K, Fe, Zn was determined according to GOST 56372-2015, Se - according to GOST 31707-2012.

The colourimetric method determined the content of heavy metals (cadmium, lead, arsenic, mercury) according to GOST 26927-86.

Aflatoxin B_1 content1 was determined by GOST 33780-2016.

The content of pesticides (α, β и γ-HCH, DDT and its metabolites, heptachlor) was determined by gas-liquid chromatography according to GOST 32689.2-2014.

The quantity of mesophilic aerobic and facultative anaerobic microorganisms was determined according to GOST 10444.15-94. Determination of the number of bacteria in the Escherichia coli group (coliform bacteria) was carried out according to GOST 31747-2012.

Description of the Experiment

The research aims to justify the choice (rosehip, sea buckthorn, hawthorn, mountain ash) based on the study of quality, chemical composition, and safety. It also aims to expand the raw material base and the possibility of using them in the technology of functional breakfast cereals and exceptionally crisp bread.

The following tasks were set to attain the intended aims:

- to substantiate the choice of wild plants of Kazakhstan (rosehip, sea buckthorn, hawthorn, black chokeberry) based on the study of quality and chemical composition;
- to obtain products by processing wild-growing raw materials, providing minimal losses of biologically active substances;
- to carry out a comprehensive assessment of quality (physicochemical parameters, food and biological value, safety indicators) of wild plants of Kazakhstan, extracts and concentrates from them;
- to develop the technology of crisp bread using products that process wild-growing raw materials.

Number of samples analyzed: We analyzed three crisp bread samples.

Number of repeated analyses: All tests were performed in triplicate.

Number of experiment replications: Experiments were carried out in two repetitions

Design of the experiment:

At the beginning of the experiment, work was carried out to prepare fruits of rosehip (variety *Rose Canina* L.), sea buckthorn (variety *Altai*), hawthorn (*Crataeguslaevigata*), and black mountain ash for laboratory analysis. The content of proteins, fats, carbohydrates, organic acids, dietary fibre, vitamins and minerals was determined in the fruits. Subsequently, extracts and concentrates from the fruits were obtained, and a regression model was constructed to evaluate the degree and nature of the relationship between the yield of extractive substances from hawthorn fruits and the effects; the qualitative characteristics of extracts and concentrates were determined. The technology of grain bread by extrusion method was developed, the recipe of which includes whole grains, groats, flavouring additives, and concentrate from wild fruits (hawthorn, rosehip, sea buckthorn and mountain ash).

Statistical Analysis

The experimental data were processed using STATISTICA 13 (TIBCO Software, Palo Alto, CA, USA) and Microsoft Excel 2019 (Microsoft, Lemoyne Township, PA, USA) application programmes.

A General Discriminant Analysis (GDA) model was applied in the mathematical processing of the research experiment analysis, specifically the hypothesis decomposition - Sigma-constrained parameterization. The Sigma-constrained model uses limited sigma-coding to represent the effects of categorical predictor variables in general and generalized linear models. In this method, discriminant functions are considered as a general multidimensional linear model, where the categorical dependent variable (extract yield, %) is represented by vectors with codes indicating different groups (such as high, medium, and low extract yield).

In the GDA method, these values are encoded as vectors (1,0,0), (0,1,0), (0,0,1). Independent quantitative predictor variables used include: the amount of extractant and the duration of extraction.

RESULTS AND DISCUSSION

Determination of the quality of wild plants

The chemical composition of wild fruits depends on genetic and ecological factors. Water composition, soil structure, the range of mineral substances present, the diversity of microorganism species, and climatic conditions condition the latter. Therefore, indicators of chemical composition are not absolute but can serve as a source of preliminary information for researchers working on creating functional food products **[41]**, **[42]**.

This research established the scatter of values in the chemical composition of mountain ash fruits depending on the harvest and variety of cultivars **[43]**.

The fruits of Crataegus oxyacantha L. (prickly hawthorn) have been studied. They collected in areas with low anthropogenic load (landscape-recreational zones). It found that under minimal exposure to factors of anthropogenic origin, C. oxyacantha fruits can accumulate phenolic compounds (up to 15.9 mg/g), leucoanthocyanins (up to 1.5 mg/g), tannins (up to 6.5 mg/g), catechins (up to 4.1 mg/g), flavonoids (up to 6. 0 mg/g), fructose (up to 14.1%), pectin substances (up to 11.6%), organic acids (1.45%), ascorbic acid (up to 49.3 mg%,), macronutrient calcium (up to 12.12 mg/g), trace element zinc (up to 39.12 mg/kg), have higher antioxidant activity (up to 9.7 mg/g) **[44]**.

Scientific research on the chemical composition of rose hips from the genus Rosa L. has shown that rosehip biomass has potential as a source of biologically active substances. It has been established that rosehips contain

vitamins such as C, P, K, and B1 and organic acids, pectin, polysaccharides, tannins, saponins, amino acids, and macro- and microelements **[45]**.

We have studied the quality of local wild plants: rosehip (Rose CaninaL. variety), sea buckthorn (Altai variety), hawthorn (Crataegus laevigata), and black chokeberry. Data on the content of the leading food substances in wild fruits are shown in Table 1.

Table 1 Quality parameters of fruits of wild plants.

Parameter	Hawthorn berries	Rosehip berries	Sea buckthorn berries	Black chokeberry berries
Physico-chemical parameters				
Protein (g)	1.5±0.02	4.0±0.2	3.2±0.1	1.85±0.02
Fats (g)	1.85±0.02	1.53±0.02	4.7±0.2	2.46±0.05
Carbohydrates (g)	11.57±0.06	13.46±0.8	1.05±0.2	12.75±0.7
Organic acids (g)	0.29±0.8	2.81±0.05	1.8±0.05	1.98±0.02
Dietary fibre (g)	7.2±0.3	12.28±0.7	2.24±0.05	4.56±0.02
Ash (g)	2.01±0.5	3.02±0.1	1.01±0.02	0.91±0.8
Vitamins				
A (mg)	-	0.411	0.279	1.708
B_5 (mg)	-	0.91	0.14	-
C (mg)	27.8	578.01	285.05	86.2
E (mg)	7.8	1.8	3.18	1.1
K(mg)	-	0.023	-	-
β-carotene (mg)	9.27	2.7	2.13	6.5
Minerals				
Mn (mg)	-	0.97	0.47	-
Cu (mg)	-	0.127	0.31	-
Si (mg)	-	-	5.21	-
Mo (mg)	-	-	0.009	-
K (mg)	14.72	26.18	197.18	190.12
Fe (mg)	0.05	1.7	1.14	5.6
Zn (mg)	0.08	0.23	0.004	-

Note: content in 100g.

The Table 1 shows the content of vitamin C in hawthorn fruit: 27.8 mg; rosehip fruit: 578.01 mg; sea buckthorn fruit: 285.05 mg; and black chokeberry fruit: 86.2 mg per 100 g of product. The content of vitamin E is 7.8; 1.8; 3.18, and 1.1 mg; β-carotene: 9.27; 2.7; 2.13, and 6.5 mg; and dietary fibre: 7.2; 12.28; 2.24, and 4.56 g, respectively.

Plants are a significant source of several minerals in an easily digestible form. Cobalt, copper, iron and manganese stimulate natural immunity factors. The combined presence of copper, cobalt and chromium provides P-vitamin activity. They also contribute to the accumulation of flavonoids in the fruit. Potassium maintains water-electrolyte balance and osmotic pressure in the cell **[46]**. Table 1 shows that the potassium content of hawthorn fruit is 14.72 mg, rosehip fruit - 26.18 mg, sea buckthorn fruit - 197.18 mg, and black rowan12 mg per 100 g of product. The iron content is respectively 0.05; 1.7; 1.14, and 5.6 mg, Zn - 0.08; 0.23; 0.004 and 0 mg, etc.

Preparation of extract and concentrate from wild plants fruits

In recent years, ultrasonic extraction has attracted attention due to its ability to increase extraction efficiency, reduce extraction time, and minimise the use of solvents, making this method environmentally friendly and cost-effective. Researchers often focus on extracting phenolic compounds due to their highly diverse pharmacological activity. Recently, ultrasound extraction has gained attention due to its ability to enhance extraction efficiency, reduce extraction time, and minimise solvent usage, making this method environmentally friendly and cost-effective. Researchers often focus on extracting phenolic compounds and antioxidant activity due to their highly diverse pharmacological activities **[47]**, **[48]**. Additionally, recent studies indicate a direct correlation between the

antioxidant activity of various plant extracts and their anti-cancer **[49]**, anti-diabetic **[50]**, **[51]**, and antibacterial **[52]** activities.

According to work, UAE was the more effective method to extract the total phenolics (54.4 mg GAE/g), with high antioxidant activity and a 33% time savings compared with MAE **[53]**.

The extraction process of plant material is influenced by several factors that must be considered when selecting extraction conditions: anatomical structure, nature or degree of grinding of plant material, concentration difference, temperature regime and duration of extraction, viscosity and nature of extractant, surfactants and hydrodynamic layer of plant material. Some authors found the influence of parameters such as the nature of the solvent, solvent volume, temperature and time **[54]**. It was proved that the applied techniques allowed for a reduction in solvent consumption and extraction time, and the extraction yield of analyses was equal to or to some higher extent than that of traditional techniques.

In studying the effects of ultrasound extraction parameters on yield and composition, as well as their influence on the antioxidant, anticancer, and antimicrobial properties of phenolic extracts, it was found that higher extraction temperatures above 50°C destroy polyphenols in the extracts, lower frequencies in the ultrasound power range below 40 kHz are most effective; the yield of polyphenols generally increases with increasing power, but there is a threshold beyond which no significant increase is observed; higher ultrasound power leads to the formation of free hydroxyl radicals, which destroy polyphenols, especially in the presence of high water content **[55]**.

The effect of process variables such as extraction temperature (30–50°C), power of ultrasound (20–40 W), extraction time (10–45 min) and solid-liquid ratio (1:10–1:20 g/ml) is studied. Multiple regression analysis was done on the experimental data to develop second-order polynomial models with a high coefficient of determination value ($R^2 > 0.99$). The optimal conditions were determined based on both individual and combinations of all process variables (extraction temperature of 50°C, ultrasound power of 20 W, extraction time of 45 min and solid-liquid ratio of 1:18.6 g/ml) **[56]**. Besides flavonoids, phenolic acids in sea buckthorn are noteworthy. It has been reported that gallic acid exhibits a broad spectrum of biological activity **[57]**, **[58]**.

The results of the biochemical analyses of the extracts showed that extraction by double infusion, given optimal conditions such as ethanol concentration in the extractant, raw material-to-extractant ratio, and the best infusion time selected, ensured an average 55-60% yield of nutrients from the studied fruit raw materials **[59]**.

The optimal composition of extracting mixtures, including the alcohol-to-water ratio of 1:1 by volume and the raw material-to-extractant ratio of 1:7 by mass, was established along with the ideal extraction duration. The fruits of rosehip (*Rosae*), hawthorn (*Crataegus*) and common rowan (*Sorbus aucuparia*) have been used as plant raw materials for obtaining extracts **[60]**.

The sequence and parameters of technological operations of pectin concentrate productions from pumpkin squeeze of the "Karina" variety have been substantiated. As a result of research, the secure storage period of pectin-containing concentrates at a temperature of 25°C is 7 months, and at a temperature of 80°C for 10 months **[61]**.

The technological process of obtaining alcoholic extract and concentrate from fruits consisted of the following stages: preparation of plant raw materials and extractant, grinding and sieving, extraction in an ultrasonic extractor, filtration, purification, concentration, pasteurisation and packaging.

Preparation of raw materials and extractant involves several key steps. The following procedures should be carried out at this stage: identifying the raw material and determining its integrity and compliance with established requirements. Different concentrations of ethanol (30%, 40%, 50%, 60%, 70%, and 96%) were employed as extractants to study their impact on extractive yield. Based on the data obtained, the highest extractive yield was achieved with 40% of ethanol. Based on the obtained data, the highest yield of extractive substances from rosehip fruit was achieved using 40% ethyl alcohol as the extractant, with an extraction duration of 30 minutes. For extracts from hawthorn fruit, the highest yield of extractive substances was achieved using 70% ethyl alcohol as the extractant, with an extraction duration of 45 minutes. When extracts from sea buckthorn and mountain ash fruits were obtained, the yield of extractive substances was with 70 % ethyl alcohol for 45 minutes and 30 % ethyl alcohol for 45 minutes, respectively. Therefore, these concentrations were chosen as the main extractant for further experiments.

Grinding and sieving: Rosehip, sea buckthorn, hawthorn, and black rowan fruits were crushed by using a laboratory mill. The resulting intermediate product was passed through a sieve.

Raw materials should be crushed to facilitate mass exchange in the process of extraction of BAS from wild plants. Because under the action of binding surface layers between the particles of raw materials, the extractant increases the surface layer of mass exchange. However, it is essential to consider that excessive grinding of raw materials can result in the inactivation of BAS and the destruction of cell walls, which leads to the flow into the extract of many accompanying substances that contaminate it. The optimal size for grinding raw materials is as

follows: up to 3-5 mm for leaves, flowers, and herbs; up to 1-3 mm for roots, stems, and bark; up to 0.3-0.5 mm for fruits and seeds.

Extraction. Prepared raw materials were placed in a flask, and ethanol was added using a funnel. The obtained solutions were left to wet for 60 minutes. Sample extraction was conducted under the following conditions: ultrasonic wave frequency of 40 kHz, extraction time of 30 minutes, and a temperature of 50ºC. Subsequently, the flask was securely sealed with cork and immersed in the ultrasonic bath.

Filtration. The filtration was held using filter paper and a glass funnel and continued for 60 minutes.

Purification - is a process that removes unwanted impurities or contaminants from an extract.

The obtained extracts were concentrated by vacuum evaporation using the IKA RV-10 apparatus at 40-500 C and 800 mbar pressure.

Pasteurisation. The obtained products were pasteurised at 75°C for 30 minutes.

Packaging. The extract from the raw material was carefully packed in containers that guarantee safe and effective extract storage.

Figure 2 shows the technological scheme for obtaining alcoholic extract and concentrate from rosehip and black rowan fruits by solvent extraction in an ultrasonic extractor.

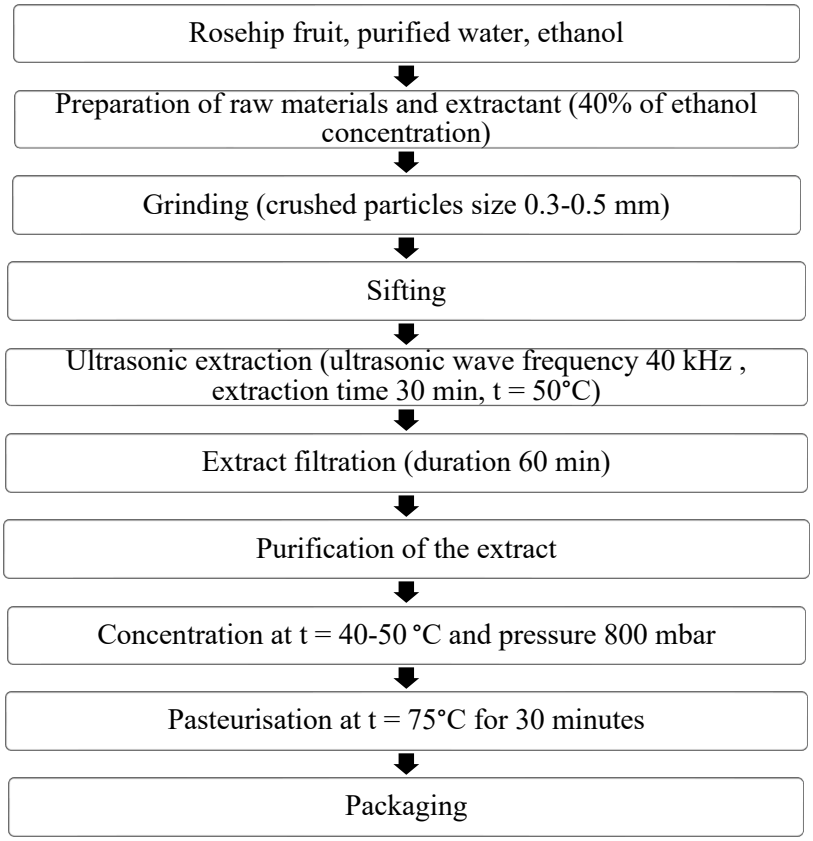


Figure 2 Technological scheme for obtaining extracts and concentrates from rosehip fruit.

To study the degree and nature of the relationship between the extract yield from hawthorn berries and the effects, a regression model was constructed to determine the influence of independent variables (amount of extractant and extraction duration) on the dependent variable (extract yield, %). Table 2 presents the univariate significance criterion for extractive yield,% (experiment) Sigma-limited parameterisation - Hypothesis decomposition used to assess the degree and nature of the relationship between the response (extractive yield, %) and effects (extractant amount and extraction duration) in the regression model.

Table 2 Results of the experiment.

Effect	Univariate significance criterion for yield, % (experience) Sigma-limited parameterisation Hypothesis decomposition				
	SS	**Freedoms degrees**	**MS**	**F**	**p**
St. member	31547.71	1	31547.71	671227.8	0.00
extractant,%	5099.82	6	849.97	18084.5	0.00
Time, min	205.41	4	51.35	1092.6	0.00
extractant,% time, min	38.28	24	1.59	33.9	0.00
Mistake	3.29	70	0.05		

The Table 2 shows that all the effects are statistically significant as the significance levels of Fisher's p criterion are less than 0.05. The effect "Extractant, %" makes the largest contribution to the overall linear model, as the SS statistic of 5099.82 takes the highest value, followed by the effect "time, min", and the combination of effects "Extractant, %*time, min".

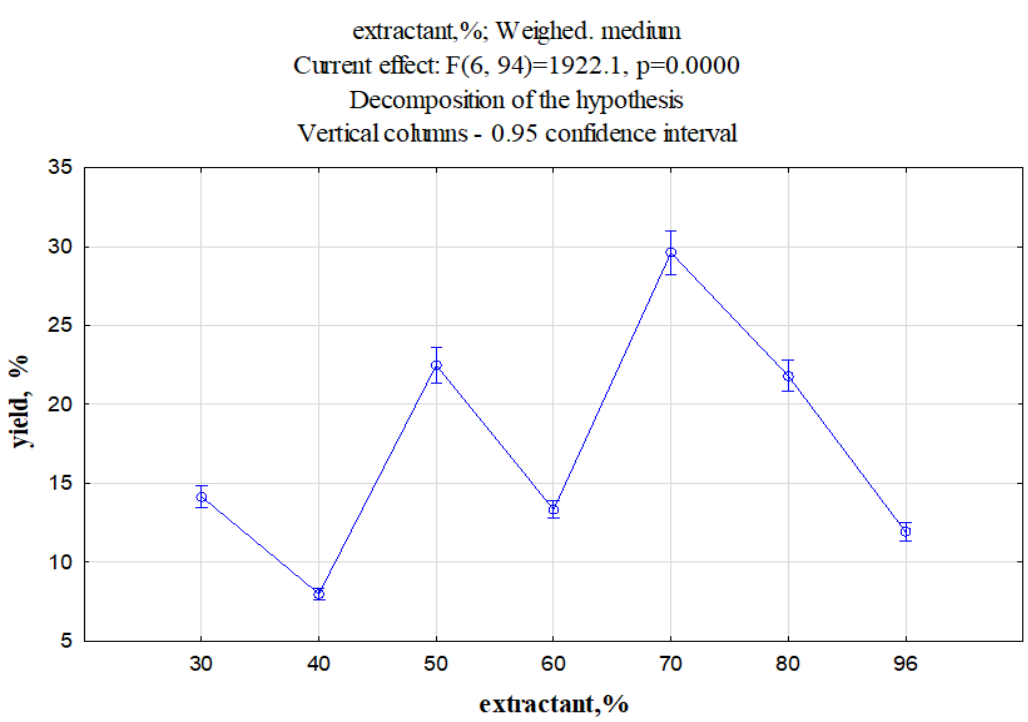


Figure 3 Weighted averages for Y 1 (extractive yield from hawthorn fruit), as a function of the dosage of extractant used.

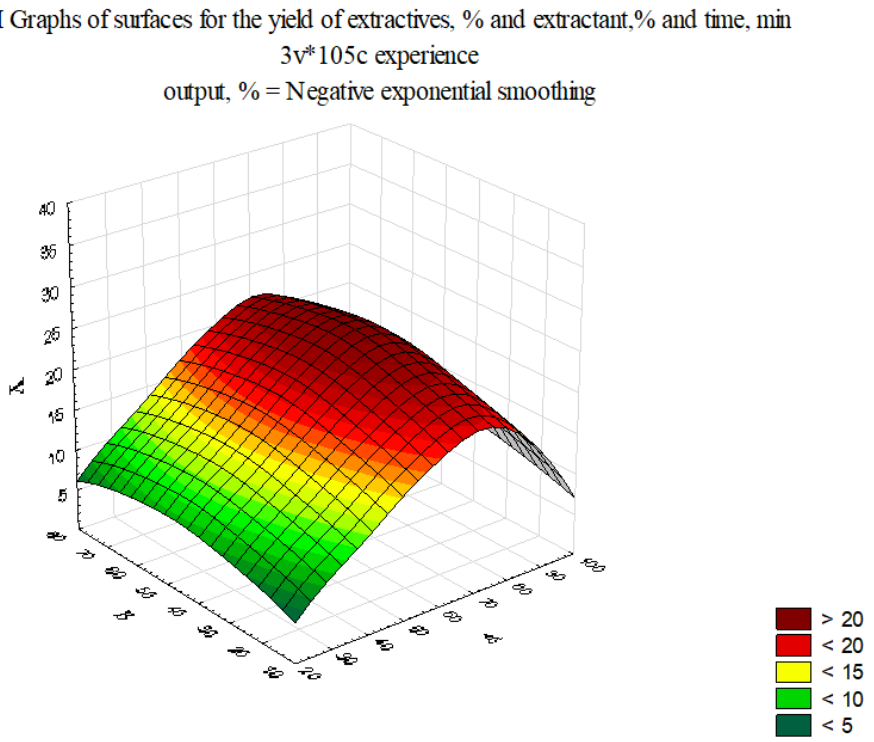


Figure 4 3M surface plot showing the dependence of extractive yield from hawthorn fruit on extractant and extraction duration.

As shown in Figure 4, the highest yield of extractive substances from hawthorn is observed at 70% extractant dosage and 30 minutes of extraction time.

Longest processing and increasing the dosage of extractant are not reasonable, as they hurt the yield of extractive substances and can lead to a significant increase in the cost of the extraction technological process.

A regression model was constructed to evaluate the degree and nature of the relationship between the extractive yield from rosehip fruit and its effects. Table 3 presents the univariate significance criterion for extractive yield, % (experiment). Sigma-limited parameterisation—Hypothesis decomposition was used to assess the degree and nature of the relationship between the response (extractive yield, %) and effects (extractant amount and extraction duration) in the regression model.

Table 3 Results of the experiment.

Effect	Univariate significance criterion for yield, % (experience) Sigma-limited parameterisation Hypothesis decomposition				
	SS	Freedoms degrees	MS	F	p
St. member	32943.26	1	32943.26	216273.9	0.00
extractant,%	3549.66	6	591.61	3883.9	0.00
Time, min	1333.87	4	333.47	2189.2	0.00
extractant,% time, min	149.85	24	6.24	41.0	0.00
Mistake	10.66	70	0.15		

The table shows that all the effects are statistically significant as the significance levels of Fisher's p criterion are less than, 0.05. The effect "Extractant, %" contributes the most to the overall linear model, as the SS statistic of 3549.66 takes the highest value, followed by the effect "time, min", and the combination of effects "Extractant, %*time, min".

Figure 5 shows the weighted averages for Y_1 (extractive yield from rosehip fruit), depending on the dosage of extractant used.

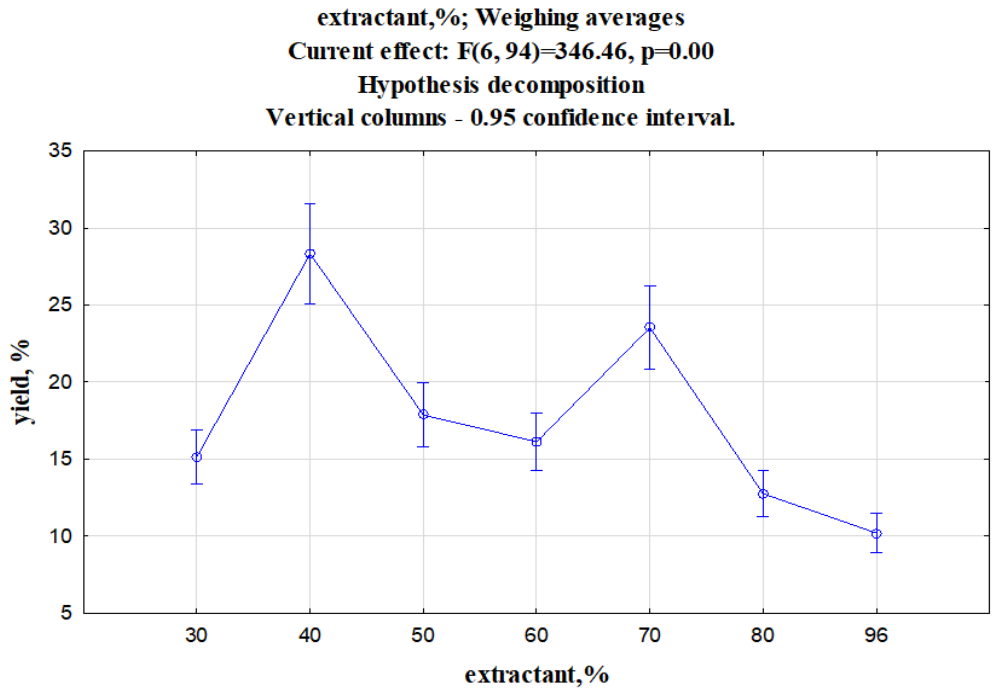


Figure 5 Weighted averages for Y 1 (extractive yield from rosehip fruit), depending on the dosage of extractant used.

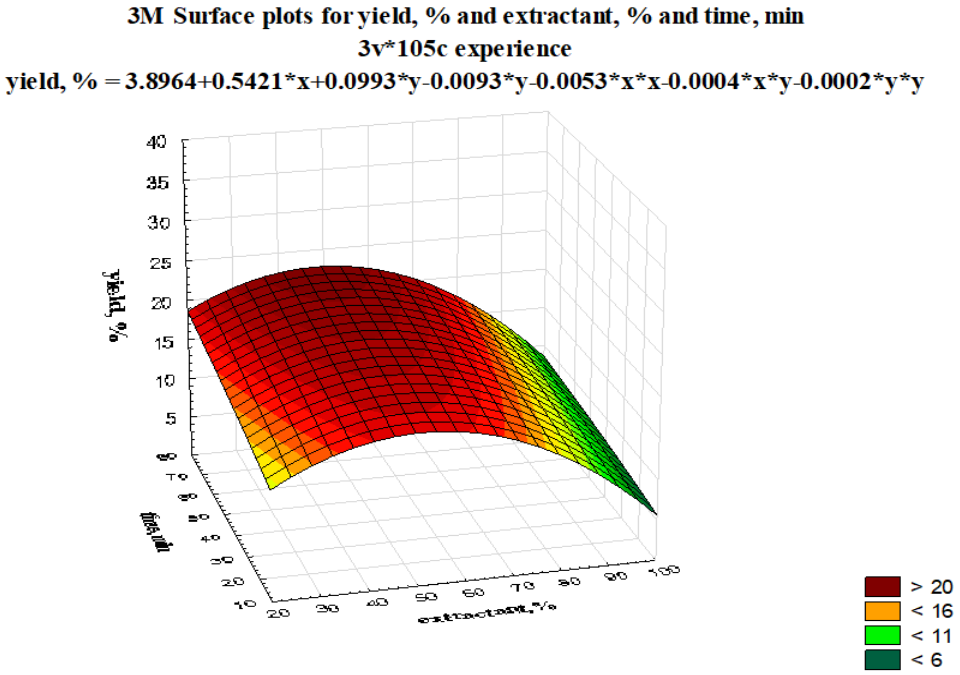


Figure 6 3M surface plot showing the dependence of extractive yield from rosehip fruit on extractant and extraction duration.

As can be seen from Figure 6, the highest yield of extractive substances from rosehip is observed at 40% of the extractant dosage at 45 minutes of extraction time. Longer processing and increasing the dosage of extractant are not reasonable, as they hurt the yield of extractive substances and can lead to a significant increase in the cost of the technological process of extraction.

Quality and safety of obtained extracts and concentrate

A comprehensive evaluation of extracts and concentrates from hawthorn, sea buckthorn, rosehip, and black rowan fruits was carried out, which included determining physicochemical parameters, nutritional value, and safety parameters. The data obtained during the determination are summarised in Table 4 and figures 6, 7, 8, and 9. These results provide a crucial insight into the chemical composition of the extracts and concentrates, highlighting the presence of various nutritional substances that may be relevant for numerous research applications.

Table 4 Chemical composition of extracts and concentrates from fruits of wild plants.

Parameter	hawthorn extract	hawthorn concentrate	rosehip extract	rosehip concentrate	sea buckthorn extract	sea buckthorn concentrate	black rowan extract	black rowan concentrate
Physico-chemical *parameters*								
Protein (g)	0.87	1.28	0.98	2.11	0.58	1.12	0.68	1.31
Fats (g)	-	-	-	-	0.8	1.2	-	-
Carbohydrates (g)	2.98	5.01	2.76	4.79	0.36	0.62	2.06	3.77
Organic acids (g)	0.48	0.59	4.11	5.08	2.91	3.59	3.31	4.21
Dietary fibre (g)	11.08	14.51	20.9	25.99	4.08	5.51	6.98	8.91
Antioxidants (mg)	24.12	31.98	19.32	26.11	19.08	26.02	21.02	29.21
Ash (g)	3.14	4.08	4.89	5.94	1.58	2.17	1.48	2.01
Vitamins								
A (mg)	-	-	0.748	0.98	0.72	0.91	2.72	3.99
B_5 (mg)	-	-	1.37	1.81	0.21	0.31	-	-
C (mg)	47.84	62.19	811.8	1101.3	518.8	640.9	128.8	160.5
E (mg)	12.08	15.17	2.12	2.91	5.02	6.48	1.52	2.09
K (mg)	-	-	0.035	0.048	-	-	-	-
Minerals								
Mn (mg)	-	-	1.05	1.41	0.65	0.82	-	-
Cu (mg)	-	-	0.21	0.29	0.39	0.52	-	-
Si (mg)	-	-	-	-	8.91	11.75	-	-
Mo (mg)	-	-	-	-	0.014	0.02	-	-
K (mg)	22.98	29.41	35.12	47.18	298.5	370.2	248.5	311.25
Fe (mg)	0.09	0.12	2.29	3.01	2.02	2.69	10.09	13.41
Zn (mg)	0.13	0.16	0.29	0.36	0.04	0.09	-	-
Se (mg)	0.012	0.018	-	-	-	-	-	-

Note: content in 100g of extract and concentrate.

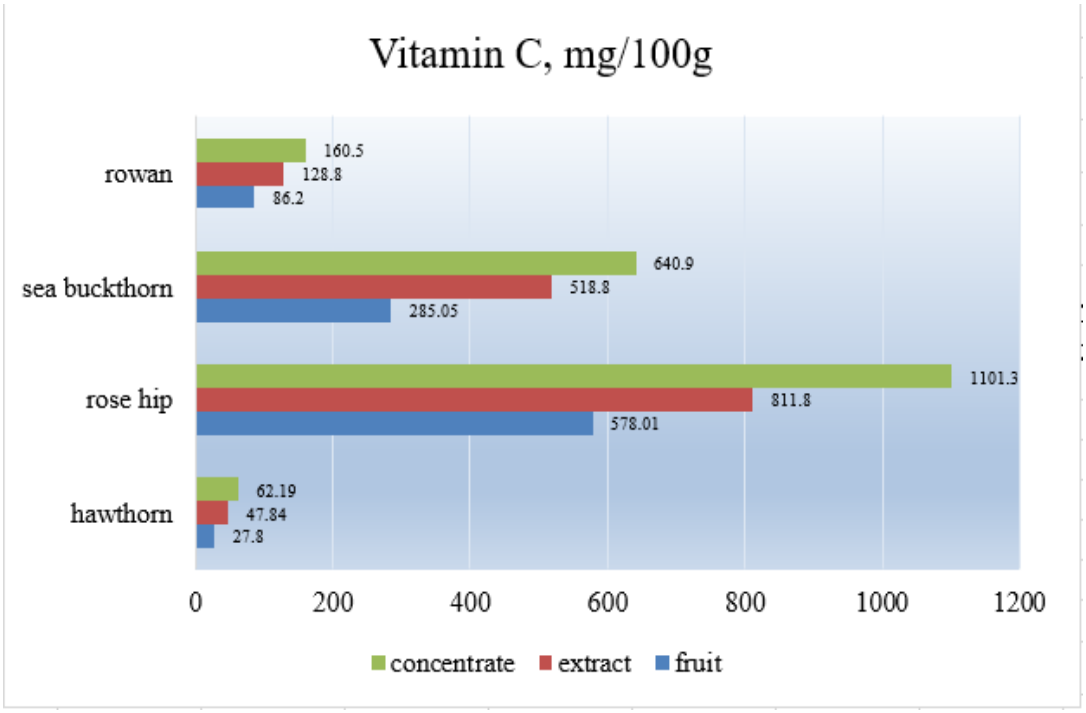


Figure 7 Vitamin C content in fruits and their processed products.

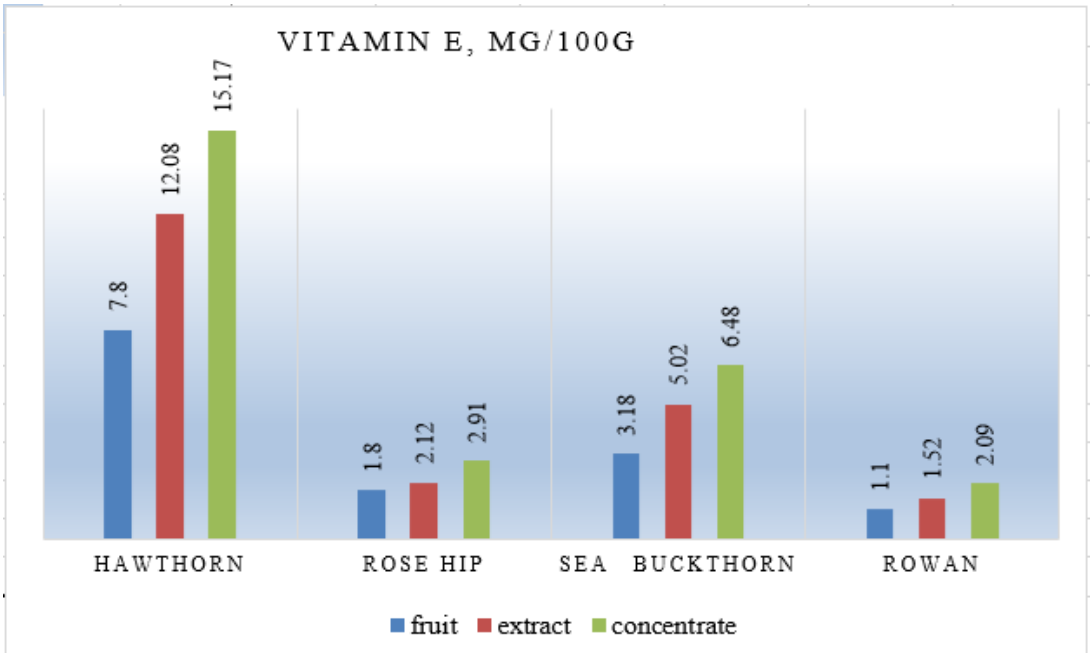


Figure 8 Vitamin E content in fruits and their processed products.

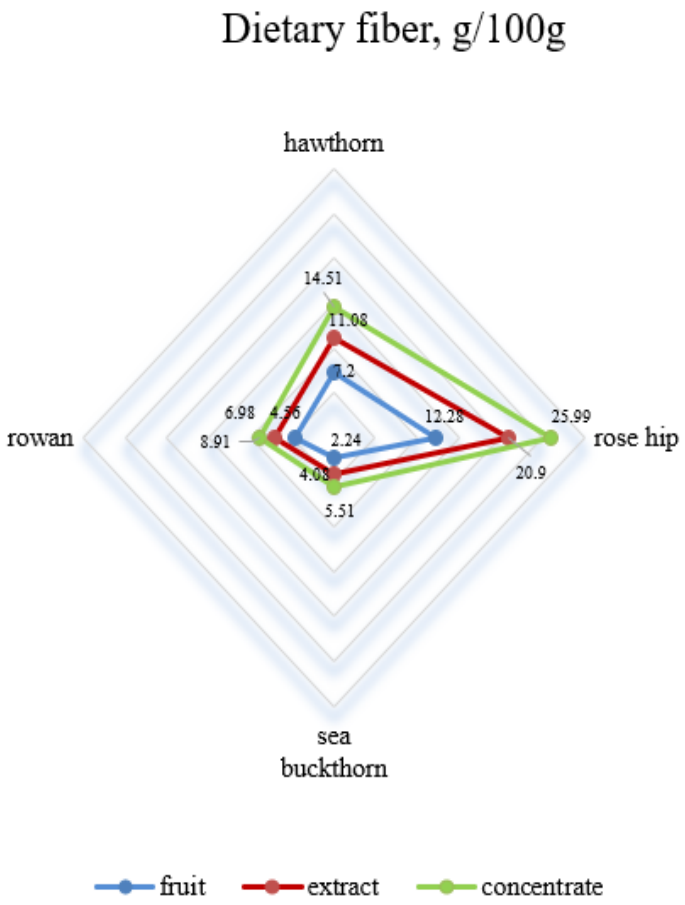


Figure 9 Dietary fibre content in fruits and their processed products.

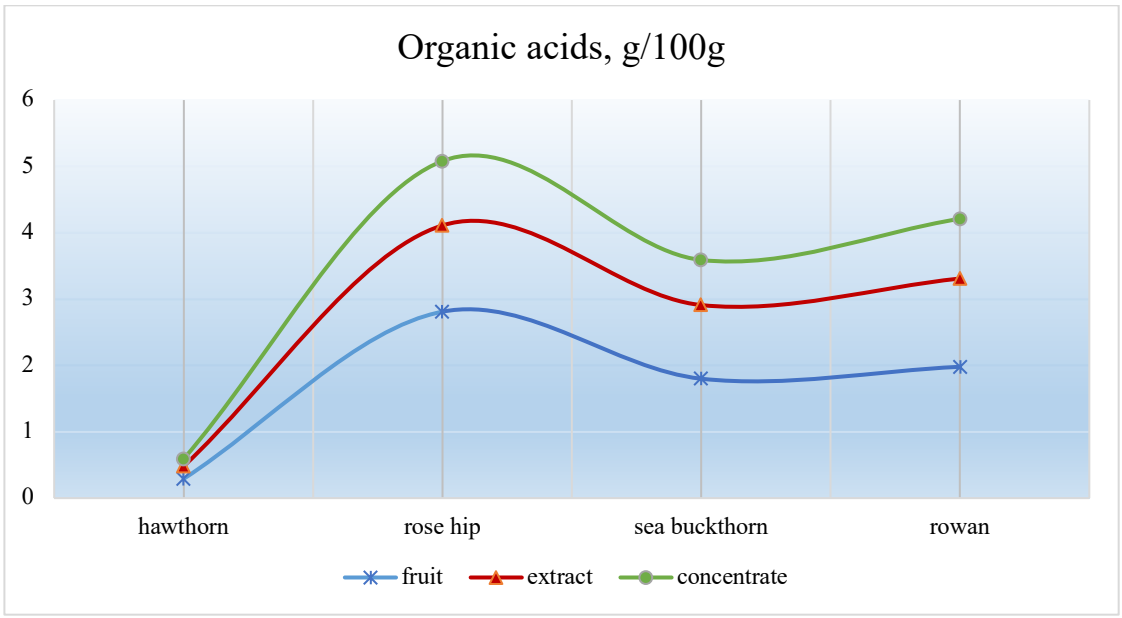


Figure 10 Organic acid content in fruits and their processed products.

From Tables 1, 4, and Figures 7 and 8, it can be seen that the vitamin C content in hawthorn berries was 27.8 mg, whereas in the extract, it was 47.84 mg, and in the concentrate, it was 62.19 mg. For rosehip, the vitamin C content in the fruits, extracts, and concentrates was 578.01 mg, 811.8 mg, and 1101.3 mg, respectively. The vitamin C content in the fruits, extracts, and concentrates for sea buckthorn was 285.05 mg, 518.8 mg, and 640.9 mg, respectively. For black chokeberry, the vitamin C content in the fruits, extracts, and concentrates was 86.2 mg, 128.8 mg, and 160.5 mg, respectively. A similar increase is observed for vitamin E content.

In addition to proteins, fats, carbohydrates, and vitamins, essential components of food include mineral substances. Each mineral element performs a specific function in the body. A comparative analysis of Tables 1 and 4 showed a trend of increased mineral content in extracts and concentrates compared to the fruits.

As shown in Figures 9 and 10, the content of dietary fibre and organic acids was higher in concentrates than in fruits.

We will use concentrates of hawthorn, rosehip, sea buckthorn, and black chokeberry in the future production of snacks, bread, and slices. Therefore, we researched the safety of concentrates from wild plants. Table 5 shows the safety parameters of concentrates from wild fruits.

Table 5 Safety parameters of concentrates from fruits of wild plants.

Parameter	Hawthorn concentrate	Rosehip concentrate	Sea buckthorn concentrate	Black chokeberry concentrate
		Microbiological parameters		
QMAFAnM,CFU/cm^3 (g), notmore	2×10^1	2×10^1	1×10^1	1×10^1
E. coli group g, cm3 in 1.0 g of product	ND	ND	ND	ND
Heavy metals, mg/kg:				
- lead	0.0017±0.0004	0.0027±0.0006	0.0007±0.0003	0.0011±0.0004
- cadmium	0.0009±0.0002	0.0007±0.0001	0.0012±0.0004	0.0002±0.0001
- mercury	ND	ND	ND	ND
- arsenic	ND	ND	ND	ND
		Pesticides, mg/kg		
HCH ($\alpha-, \beta-, \gamma-$ *isomers*)	ND	ND	ND	ND
- Heptachlor	ND	ND	ND	ND
- DDT and its metabolites	ND	ND	ND	ND
		Mycotoxins, mg/kg		
- aflatoxin B_1	ND	ND	ND	ND

The research results on the safety of hawthorn, rosehip, sea buckthorn and black chokeberry concentrates showed their compliance with the requirements of the Technical Regulations of the Customs Union 021/2011 and confirmed their safety.

Development of technology production of crispbreads by extrusion method

From both scientific and technological perspectives, significant efforts are focused on developing new alternative and adapted technological processes for food production. These processes aim to meet consumer sensory and functional requirements while simultaneously addressing the needs of producers for value-added food products from both technological and economic standpoints **[62]**.

Extrusion products have high consumer appeal, good digestibility, low microbial contamination, and increased oxidation resistance, making them suitable for various of populations.

Extrusion is considered one of the most suitable methods for bread production, as the formation of starch rather than gluten causes the formation of textural and consumer properties in extruded products such as breakfast cereals, snacks, and crisp bread.

The primary purpose of the study was the possibility of introducing concentrate from wild plants (*rosehip, hawthorn, mountain ash, sea buckthorn*) in the production of new breads and the influence of its dosage on the technological and consumer properties of finished products.

A technology for cereal crisp bread has been developed. The recipe includes groats, flavouring additives, and concentrate from wild fruits (*hawthorn, rosehip, sea buckthorn and black chokeberry*). The technological process begins with grain mixture preparation by cleaning it of impurities and moistening it with water. Moisture of raw

materials brought to 18 - 25%. The prepared grain mixture is fed to the loading hopper, then to the dosing compartment and then to the sintering chamber, which has a rectangular shape (this is the reason for the appearance of crisp bread). The created temperature difference causes the grain and groats to explode due to the instantaneous boiling of the moisture in its composition. As a result, the groats become voluminous and porous and fill the entire space of the chamber. The machine was used to produce grain crisp bread that we purchased for the project (Figure 11).

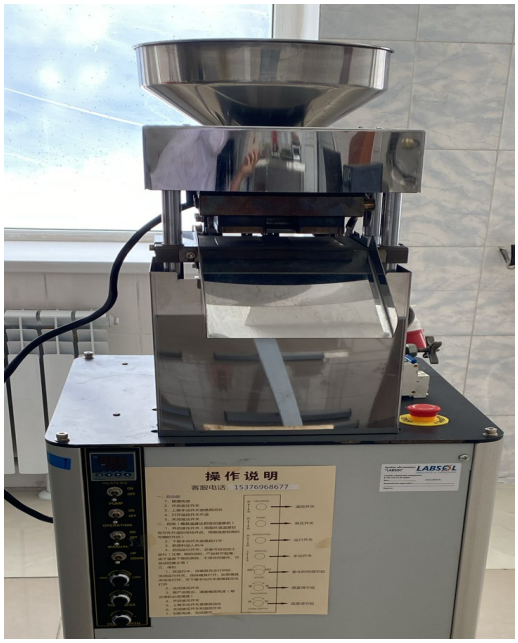


Figure 11 - Machine for the production of grain crisp bread

Crisp bread had a regular shape, and the thickness of the tiles was uniform around the perimeter, without dents, with even edges. The surface of the bread was slightly rough, without bloating, cracks and stains. The colour of the products is uniform light cream with dotted flecks of dark colour. Products are brittle, not stiff, easily breakable with a crunch, well loosened, and porous with a uniform structure. Taste and odour correspond to used raw materials, with the extruded product exhibiting its inherent smell without any extraneous taste or odour.

Thus, developing technologies for grain crisp bread using extracts, contributing to reducing human body weight, is relevant and aimed at improving public health.

CONCLUSION

The analysis of scientific publications on the researched topic has shown that wild plants contain a different set of biologically active substances, and our research confirms this. The study of the chemical composition of fruits of wild plants of Kazakhstan - rose hips (variety Rose Canina L), sea buckthorn (variety Altai), hawthorn (Crataeguslaevigata), black chokeberry has established that they contain a diverse set of biologically active substances, which gives reason to evaluate them as promising components of food products that can increase the nutritional value and expand the range of healthy food products. It has been established that the vitamin C content in hawthorn fruits is 27.8 mg, in rosehip fruits is 578.01 mg, in sea buckthorn fruits is 285.05 mg, and in black chokeberry fruits is 86.2 mg per 100 g of the product. The vitamin E content is 7.8 mg, 1.8 mg, 3.18 mg, and 1.1 mg, respectively. The dietary fibre content is 7.2 g, 12.28 g, 2.24 g, and 4.56 g; potassium content is 14.72 mg, 26.18 mg, 197.18 mg, and 190.12 mg; iron content is 0.05 mg, 1.7 mg, 1.14 mg, and 5.6 mg, Zn content is 0.08 mg, 0.23 mg, 0.004 mg, and 0 mg, respectively, and so on. The technological scheme has been developed to obtain extracts and concentrates from fruits of wild-growing raw materials. The modes of extraction of samples were proposed: ultrasonic wave frequency 40 kHz, extraction time 30 minutes, temperature 500C, and *concentration of* the extracts obtained were carried out by vacuum evaporation, using IKA RV-10 apparatus at 40-50^{0}C and pressure 800 mbar. Compared to other extraction methods, ultrasonic extraction has gained attention for its mild temperature conditions, ability to increase extraction efficiency, shorter extraction times, and reduced solvent use, making it an environmentally friendly and cost-effective method.

The mathematical analysis of the research experiment was conducted using hypothesis decomposition - Sigma-restricted parameterisation.

Based on the analysis of the chemical composition of extracts and concentrates from hawthorn, sea buckthorn, rosehip, and black chokeberry fruits and comparison with the composition of fruits, it was found that the content of nutritional substances in extracts and concentrates is higher compared to fruits. Thus, the vitamin C content in hawthorn fruits was 27.8 mg, while in the extract, it was 47.84 mg, and in the concentrate – 62.19 mg. The vitamin C content in rosehip fruits, extracts, and concentrates was 578.01 mg, 811.8 mg, and 1101.3 mg, respectively. The vitamin C content in sea buckthorn fruits, extracts, and concentrates was 285.05 mg, 518.8 mg, and 640.9 mg, respectively. The vitamin C content in black chokeberry fruits, extracts, and concentrates was 86.2 mg, 128.8 mg, and 160.5 mg, respectively. A similar increase was observed for the content of vitamin E and other components.

The obtained concentrates meet the requirements of TR CU 021/2011 Technical Regulations of the Customs Union "On the safety of food products" regarding safety parameters. The technology of grain bread by extrusion method has been developed, and the recipe includes groats, flavouring additives, and concentrate from wild fruits (hawthorn, rosehip, sea buckthorn, and chokeberry). Extrusion is one of the most important methods for processing plant raw materials into food products. This process increases the digestibility of many components of plant raw materials and reduces the content of anti-nutritional factors. The extrusion process occurs with high temperatures and moisture levels of raw material. However, high values for these parameters can lead to a decrease in valuable nutrient content. Therefore, scientific research should focus on identifying the optimal extrusion parameters to maintain a balance between the desired properties of the extrudate and the preservation of nutrients.

The subject of extruding plant raw materials for food production is promising but requires additional research.

REFERENCES

1. Analyze North America breakfast cereals market size and share - growth trends and forecasts (2023-2028). Available at: https://www.mordorin telligence.com/en/industry-reports/north-america-breakfast-cereals-market
2. Azembaev A.A., Tegisbaev N.E., Kusnieva A.E., Baimurzina M.A., Adibaeva G.L., 2015. Medicinal plants of Kazakhstan used in oriental and academic medicine. – Almaty: “Nur-Print” Publishing House, pp-178.
3. Gemejieva N.G., Karjaubekova J.J., Lesova J.T. et al., 2016. Analysis of species diversity and study of useful plants in the Baltic region. Uspekhi sovremennoi naukh nostvosnaniya. No.1, pp. 66-70. https://s.natural-sciences.ru/pdf/2016/1/35743.pdf
4. B.Zh., B., N.E., T., Z.M., K., & L.A., K. (2021). Wild plants of Pavlodar region as potential sugar substitutes. In Biological sciences of Kazakhstan (Vol. 3, pp. 8–15). Non-Profit Limited Company Pavlodar Pedagogical University. https://doi.org/10.52301/1684-940x-2021-3-8-15
5. Korkmaz, M., & Dogan, N. Y. (2018). Analysis of Genetic Relationships Between Wild Roses (Rosa L. Spp.) Growing in Turkey. In Erwerbs-Obstbau (Vol. 60, Issue 4, pp. 305–310). Springer Science and Business Media LLC. https://doi.org/10.1007/s10341-018-0375-9
6. Saricaoglu, F. T., Atalar, I., Yilmaz, V. A., Odabas, H. I., & Gul, O. (2019). Application of multi pass high pressure homogenization to improve stability, physical and bioactive properties of rosehip (Rosa canina L.) nectar. In Food Chemistry (Vol. 282, pp. 67–75). Elsevier BV. https://doi.org/10.1016/j.foodchem.2019.01.002
7. Mármol, I., Sánchez-de-Diego, C., Jiménez-Moreno, N., Ancín-Azpilicueta, C., & Rodríguez-Yoldi, M. (2017). Therapeutic Applications of Rose Hips from Different Rosa Species. In International Journal of Molecular Sciences (Vol. 18, Issue 6, p. 1137). MDPI AG. https://doi.org/10.3390/ijms18061137
8. Muranets, A. P., Yessimseitova, A. K., Dyussembekova, D. A., Nurtaza, A. S., Kalybayev, K. R., Kozhanov, K. Z., & Kakimzhanova, A. A. (2022). Study of the biodiversity of wild plants of the genus Rosa L. in Kazakhstan and their molecular-genetic identification. In Bulletin of the L.N. Gumilyov Eurasian National University. Bioscience Series (Vol. 139, Issue 2, pp. 44–60). L. N. Gumilyov Eurasian National University. https://doi.org/10.32523/2616-7034-2022-139-2-44-60
9. Peña, F., Valencia, S., Tereucán, G., Nahuelcura, J., Jiménez-Aspee, F., Cornejo, P., & Ruiz, A. (2023). Bioactive Compounds and Antioxidant Activity in the Fruit of Rosehip (Rosa canina L. and Rosa rubiginosa L.). In Molecules (Vol. 28, Issue 8, p. 3544). MDPI AG. https://doi.org/10.3390/molecules28083544

10. Ayati, Z., Ramezani, M., Amiri, M. S., Sahebkar, A., & Emami, S. A. (2021). Genus Rosa: A Review of Ethnobotany, Phytochemistry and Traditional Aspects According to Islamic Traditional Medicine (ITM). In Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health (pp. 353–401). Springer International Publishing. https://doi.org/10.1007/978-3-030-64872-5_23
11. Ayati, Z., Amiri, M. S., Ramezani, M., Delshad, E., Sahebkar, A., & Emami, S. A. (2019). Phytochemistry, Traditional Uses and Pharmacological Profile of Rose Hip: A Review. In Current Pharmaceutical Design (Vol. 24, Issue 35, pp. 4101–4124). Bentham Science Publishers Ltd. https://doi.org/10.2174/1381612824666181010151849
12. Olas, B. (2018). The beneficial health aspects of sea buckthorn (Elaeagnus rhamnoides (L.) A.Nelson) oil. In Journal of Ethnopharmacology (Vol. 213, pp. 183–190). Elsevier BV. https://doi.org/10.1016/j.jep.2017.11.022
13. Hasenova A.B., Aralbaeva A.N., Utegalieva R.S., Mamataeva A.T., Murzakhmetova M.K., (2020). Buckthorn (Hippophae Rhamnoides L.) - a source of bioactive substances. Bulletin of Almaty Technological University. No.1. pp.82-88. https://www.vestnik-atu.kz/jour/article/view/259/0
14. Bittová, M., Krejzová, E., Roblová, V., Kubáň, P., & Kubáň, V. (2014). Monitoring of HPLC profiles of selected polyphenolic compounds in sea buckthorn (Hippophaë rhamnoides L.) plant parts during annual growth cycle and estimation of their antioxidant potential. In Open Chemistry (Vol. 12, Issue 11, pp. 1152–1161). Walter de Gruyter GmbH. https://doi.org/10.2478/s11532-014-0562-y
15. Danielski, R., & Shahidi, F. (2024). Phenolic composition and bioactivities of sea buckthorn (Hippophae rhamnoides L.) fruit and seeds: an unconventional source of natural antioxidants in North America. In Journal of the Science of Food and Agriculture (Vol. 104, Issue 9, pp. 5553–5564). Wiley. https://doi.org/10.1002/jsfa.13386
16. Criste, A., Urcan, A. C., Bunea, A., Pripon Furtuna, F. R., Olah, N. K., Madden, R. H., & Corcionivoschi, N. (2020). Phytochemical Composition and Biological Activity of Berries and Leaves from Four Romanian Sea Buckthorn (Hippophae Rhamnoides L.) Varieties. In Molecules (Vol. 25, Issue 5, p. 1170). MDPI AG. https://doi.org/10.3390/molecules25051170
17. Tagibergenova G.G., Omarov M.M., (2020). Biotechnological aspects of the use of sea buckthorn in the production of marmalade.Young Scientist. № 3 (293). pp. 84-86.
18. Netreba, N., Sandulachi, E., Macari, A., Popa, S., Ribintev, I., Sandu, I., Boestean, O., & Dianu, I. (2024). A Study on the Fruiting and Correlation between the Chemical Indicators and Antimicrobial Properties of Hippophae rhamnoides L. In Horticulturae (Vol. 10, Issue 2, p. 137). MDPI AG. https://doi.org/10.3390/horticulturae10020137
19. Rozhnov, E. (2021). Antioxidant Potential of Sea Buckthorn Fruits and Products of Its Processing. In Food Industry (Vol. 6, Issue 1, pp. 23–30). Ural State University of Economics. https://doi.org/10.29141/2500-1922-2021-6-1-3
20. Nilova, L. P., & Malyutenkova, S. M. (2021). Antioxidant complexes of sea buckthorn (Hippopha? rhamnoides L.) of northwest Russia. In Proceedings of the Voronezh State University of Engineering Technologies (Vol. 83, Issue 1, pp. 108–114). FSBEI HE Voronezh State University of Engineering Technologies. https://doi.org/10.20914/2310-1202-2021-1-108-114
21. Martinelli, F., Perrone, A., Yousefi, S., Papini, A., Castiglione, S., Guarino, F., Cicatelli, A., Aelaei, M., Arad, N., Gholami, M., & Salami, S. (2021). Botanical, Phytochemical, Anti-Microbial and Pharmaceutical Characteristics of Hawthorn (Crataegus monogyna Jacq.), Rosaceae. In Molecules (Vol. 26, Issue 23, p. 7266). MDPI AG. https://doi.org/10.3390/molecules26237266
22. Kim, E., Jang, E., & Lee, J.-H. (2022). Potential Roles and Key Mechanisms of Hawthorn Extract against Various Liver Diseases. In Nutrients (Vol. 14, Issue 4, p. 867). MDPI AG. https://doi.org/10.3390/nu14040867
23. Sun, Y., Meng, X., Chen, M., Li, D., Liu, R., & Sun, T. (2024). Isolation, structural properties and bioactivities of polysaccharides from Crataegus pinnatifida. In Journal of Ethnopharmacology (Vol. 323, p. 117688). Elsevier BV. https://doi.org/10.1016/j.jep.2023.117688

24. Cui, M., Cheng, L., Zhou, Z., Zhu, Z., Liu, Y., Li, C., Liao, B., Fan, M., & Duan, B. (2024). Traditional uses, phytochemistry, pharmacology, and safety concerns of hawthorn (Crataegus genus): A comprehensive review. In Journal of Ethnopharmacology (Vol. 319, p. 117229). Elsevier BV. https://doi.org/10.1016/j.jep.2023.117229

25. Karomatov Inomjon, Zhalilov Nabijon., 2019. Chemical composition and therapeutic properties of hawthorn. Biology and Integrative Medicine. No.1. pp.109-141.

26. Kazuma, K., Isobe, Y., Asahina, H., Nehira, T., Satake, M., & Konno, K. (2016). Crataegusins A and B, New Flavanocoumarins from the Dried Fruits of Crataegus pinnatifida var. major (Rosaceae). In Natural Product Communications (Vol. 11, Issue 7, p. 1934578X1601100). SAGE Publications. https://doi.org/10.1177/1934578x1601100724

27. Sidor, A., & Gramza-Michałowska, A. (2019). Black Chokeberry Aronia Melanocarpa L.—A Qualitative Composition, Phenolic Profile and Antioxidant Potential. In Molecules (Vol. 24, Issue 20, p. 3710). MDPI AG. https://doi.org/10.3390/molecules24203710

28. Jurendić, T., & Ščetar, M. (2021). Aronia melanocarpa Products and By-Products for Health and Nutrition: A Review. In Antioxidants (Vol. 10, Issue 7, p. 1052). MDPI AG. https://doi.org/10.3390/antiox10071052

29. Oszmiański, J., & Lachowicz, S. (2016). Effect of the Production of Dried Fruits and Juice from Chokeberry (Aronia melanocarpa L.) on the Content and Antioxidative Activity of Bioactive Compounds. In Molecules (Vol. 21, Issue 8, p. 1098). MDPI AG. https://doi.org/10.3390/molecules21081098

30. Wawer, Katarzyna Zawada, M. G., Iwona. (2019). Aronia melanocarpa berries: phenolics composition and antioxidant properties changes during fruit development and ripening. In Emirates Journal of Food and Agriculture (p. 214). Pensoft Publishers. https://doi.org/10.9755/ejfa.2019.v31.i3.1921

31. Denev, P., Číž, M., Kratchanova, M., & Blazheva, D. (2019). Black chokeberry (Aronia melanocarpa) polyphenols reveal different antioxidant, antimicrobial and neutrophil-modulating activities. In Food Chemistry (Vol. 284, pp. 108–117). Elsevier BV. https://doi.org/10.1016/j.foodchem.2019.01.108

32. Wang, L., Wang, R., Dong, J., Wang, Y., Huang, X., & Chen, C. (2023). Research on the extraction, purification and determination of chemical components, biological activities, and applications in diet of black chokeberry (Aronia melanocarpa). In Chinese Journal of Analytical Chemistry (Vol. 51, Issue 10, p. 100301). Elsevier BV. https://doi.org/10.1016/j.cjac.2023.100301

33. Sidor, A., Drożdżyńska, A., & Gramza-Michałowska, A. (2019). Black chokeberry (Aronia melanocarpa) and its products as potential health-promoting factors - An overview. In Trends in Food Science & Technology (Vol. 89, pp. 45–60). Elsevier BV. https://doi.org/10.1016/j.tifs.2019.05.006

34. Borowska, S., & Brzóska, M. M. (2016). Chokeberries (Aronia melanocarpa) and Their Products as a Possible Means for the Prevention and Treatment of Noncommunicable Diseases and Unfavorable Health Effects Due to Exposure to Xenobiotics. In Comprehensive Reviews in Food Science and Food Safety (Vol. 15, Issue 6, pp. 982–1017). Wiley. https://doi.org/10.1111/1541-4337.12221

35. Jurendić, T., & Ščetar, M. (2021). Aronia melanocarpa Products and By-Products for Health and Nutrition: A Review. In Antioxidants (Vol. 10, Issue 7, p. 1052). MDPI AG. https://doi.org/10.3390/antiox10071052

36. Yang, S.-Q., Wang, D., Gao, Y.-X. Advances in studies on the function and application of Aronia melanocarpa (2021) Food Res. Dev, 42, pp. 206-213.

37. Ren, Y., Frank, T., Meyer, G., Lei, J., Grebenc, J. R., Slaughter, R., Gao, Y. G., & Kinghorn, A. D. (2022). Potential Benefits of Black Chokeberry (Aronia melanocarpa) Fruits and Their Constituents in Improving Human Health. In Molecules (Vol. 27, Issue 22, p. 7823). MDPI AG. https://doi.org/10.3390/molecules27227823

38. Ruczaj, A., Brzóska, M. M., & Rogalska, J. (2024). The Protective Impact of Aronia melanocarpa L. Berries Extract against Prooxidative Cadmium Action in the Brain—A Study in an In Vivo Model

of Current Environmental Human Exposure to This Harmful Element. In Nutrients (Vol. 16, Issue 4, p. 502). MDPI AG. https://doi.org/10.3390/nu16040502

39. Sidor, A., Drożdżyńska, A., & Gramza-Michałowska, A. (2019). Black chokeberry (Aronia melanocarpa) and its products as potential health-promoting factors - An overview. In Trends in Food Science & Technology (Vol. 89, pp. 45–60). Elsevier BV. https://doi.org/10.1016/j.tifs.2019.05.006

40. Ren, Y., Frank, T., Meyer, G., Lei, J., Grebenc, J. R., Slaughter, R., Gao, Y. G., & Kinghorn, A. D. (2022). Potential Benefits of Black Chokeberry (Aronia melanocarpa) Fruits and Their Constituents in Improving Human Health. In Molecules (Vol. 27, Issue 22, p. 7823). MDPI AG. https://doi.org/10.3390/molecules27227823

41. Ovcharenko A.S., Rasulova E.A., Kondakova O.E., Ivanova O.V.(2017). Functional ingredients of wild fruits.Food industry. No.12. pp.53-57. https://bik.sfu-kras.ru/elib/view?id=PRSV-pipr/2017/12

42. B, V., & V, V. (2017). Biochemical characteristic of essential nutrients as a scientific basis of determination of functional properties of specialized products and mechanisms of their action on metabolic processes. In Human. Sport. Medicine (Vol. 17, Issue 2, pp. 5–19). FSAEIHE South Ural State University (National Research University). https://doi.org/10.14529/hsm170201

43. Ostroumov L.A., Krieger O.V., Karchin K.V., Shchetinin M.P. (2014). Study of the chemical composition of fruits of common chokeberry (Sorbus Aucuparia) growing in the Kemerovo region. Technics and technology of food production. № 4. pp. 38.-42. https://cyberleninka.ru/article/n/issledovanie-himicheskogo-sostava-plodov-ryabiny-obyknovennoy-sorbusaucuparia-proizrastayuschey-v-kemerovskoy-oblasti

44. Skrypnik, L. N., Melnichuk, I. P., & Koroleva, Y. V. (2020). Nutritional and biological value of fruits of Crataegus oxyacantha L. In chemistry of plant raw material (Issue 1, pp. 265–275). Altai State University. https://doi.org/10.14258/jcprm.2020015452

45. Petrova S.N., Ivkova A.V. (2014). Chemical composition and antioxidant properties of species of the genus Rosa. L. Chemistry of plant raw materials.. №2. - pp. 13-19. https://cyberleninka.ru/article/n/himicheskiy-sostav-i-antioksidantnye-svoystva-vidov-roda-rosa-l

46. Mogilny M.P., Balasanyan A.Y., Shaltumayev T.Sh. (2014). Rational use of sources of dietary fibre in the production of food products// New Technologies. - № 1. -pp. 28-33. https://cyberleninka.ru/article/n/sovremennye-napravleniya-ispolzovaniya-pischevyh-volokon-v-kachestve-funktsionalnyh-ingredientov

47. Elapov, A. A., Kuznetsov, N. N., & Marakhova, A. I. (2021). The Use of Ultrasound in the Extraction of Biologically Active Compounds from Plant Raw Materials, Used or promising for Use in Medicine (Review). In Drug development & registration (Vol. 10, Issue 4, pp. 96–116). Center of Pharmaceutical Analytics Ltd. https://doi.org/10.33380/2305-2066-2021-10-4-96-116

48. Madhu, B., Srinivas, M. S., Srinivas, G., & Jain, S. K. (2019). Ultrasonic Technology and Its Applications in Quality Control, Processing and Preservation of Food: A Review. In Current Journal of Applied Science and Technology (Vol. 32, Issue 5, pp. 1–11). Sciencedomain International. https://doi.org/10.9734/cjast/2019/46909

49. Stolarczyk, E. U., Strzempek, W., Łaszcz, M., Leś, A., Menaszek, E., & Stolarczyk, K. (2022). Thiogenistein—Antioxidant Chemistry, Antitumor Activity, and Structure Elucidation of New Oxidation Products. In International Journal of Molecular Sciences (Vol. 23, Issue 14, p. 7816). MDPI AG. https://doi.org/10.3390/ijms23147816

50. Li, X., Liu, J., Chang, Q., Zhou, Z., Han, R., & Liang, Z. (2021). Antioxidant and Antidiabetic Activity of Proanthocyanidins from Fagopyrum dibotrys. In Molecules (Vol. 26, Issue 9, p. 2417). MDPI AG. https://doi.org/10.3390/molecules26092417

51. Nisar, J., Ali Shah, S. M., Ayaz, S., Akram, M., Rashid, A., Mustafa, I., & Nisar, Z. (2022). In vitro comparative evaluation of Tamarix gallica extracts for antioxidant and antidiabetic activity. In Experimental Biology and Medicine (Vol. 248, Issue 3, pp. 253–262). Frontiers Media SA. https://doi.org/10.1177/15353702221139208

52. Toledo, A. G., Souza, J. G. de L. de, Santana, C. B., Mallmann, A. P., dos Santos, C. V., Corrêa, J. M., & Pinto, F. G. da S. (2023). Antimicrobial, antioxidant activity and phytochemical prospection of Eugenia involucrata DC. leaf extracts. In Brazilian Journal of Biology (Vol. 83). FapUNIFESP (SciELO). https://doi.org/10.1590/1519-6984.245753

53. Rodsamran, P., & Sothornvit, R. (2019). Extraction of phenolic compounds from lime peel waste using ultrasonic-assisted and microwave-assisted extractions. In Food Bioscience (Vol. 28, pp. 66–73). Elsevier BV. https://doi.org/10.1016/j.fbio.2019.01.017

54. Zoumpoulakis, P., Sinanoglou, V., Siapi, E., Heropoulos, G., & Proestos, C. (2017). Evaluating Modern Techniques for the Extraction and Characterisation of Sunflower (Hellianthus annus L.) Seeds Phenolics. In Antioxidants (Vol. 6, Issue 3, p. 46). MDPI AG. https://doi.org/10.3390/antiox6030046

55. Dzah, C. S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G., & Ma, H. (2020). The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. In Food Bioscience (Vol. 35, p. 100547). Elsevier BV. https://doi.org/10.1016/j.fbio.2020.100547

56. Prakash Maran, J., Manikandan, S., Vigna Nivetha, C., & Dinesh, R. (2017). Ultrasound assisted extraction of bioactive compounds from Nephelium lappaceum L. fruit peel using central composite face centered response surface design. In Arabian Journal of Chemistry (Vol. 10, pp. S1145–S1157). Elsevier BV. https://doi.org/10.1016/j.arabjc.2013.02.007

57. Ashrafizadeh, M., Zarrabi, A., Mirzaei, S., Hashemi, F., Samarghandian, S., Zabolian, A., Hushmandi, K., Ang, H. L., Sethi, G., Kumar, A. P., Ahn, K. S., Nabavi, N., Khan, H., Makvandi, P., & Varma, R. S. (2021). Gallic acid for cancer therapy: Molecular mechanisms and boosting efficacy by nanoscopical delivery. In Food and Chemical Toxicology (Vol. 157, p. 112576). Elsevier BV. https://doi.org/10.1016/j.fct.2021.112576

58. He, Y., Lin, Y., Li, Q., & Gu, Y. (2020). The contribution ratio of various characteristic tea compounds in antioxidant capacity by DPPH assay. In Journal of Food Biochemistry (Vol. 44, Issue 7). Hindawi Limited. https://doi.org/10.1111/jfbc.13270

59. Guseinova, B. M., & Mukailov, M. D. (2018). features of nutrient extraction from of mountain ash, sloe and dogrose fruits. In Izvestiâ Timirâzevskoj sel′skohozâjstvennoj akademii (Issue 1, pp. 109–117). Russian State Agrarian University - Moscow Timiryazev Agricultural Academy. https://doi.org/10.26897/0021-342x-2018-1-109-117

60. Bazarnova Yu.G., Ivanchenko O.B. (2016). Study of the composition of biologically active substances of extracts of wild plants. Nutrition Issues. Vol. 85, No. 5, pp. 100-107. https://cyberleninka.ru/article/n/issledovanie-sostava-biologicheski-aktivnyh-veschestv-ekstraktov-dikorastuschih-rasteni y

61. Iskakova, G., Kizatova, M., Baiysbayeva, M., Azimova, S., Izembayeva, A., & Zharylkassynova, Z. (2021). Justification of pectin concentrate safe storage terms by pectin mass ratio. In Eastern-European Journal of Enterprise Technologies (Vol. 4, Issue 11(112), pp. 25–32). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2021.237940

62. Özdemir, N., Pashazadeh, H., Zannou, O., & Koca, I. (2022). Phytochemical content, and antioxidant activity, and volatile compounds associated with the aromatic property, of the vinegar produced from rosehip fruit (Rosa canina L.). In LWT (Vol. 154, p. 112716). Elsevier BV. https://doi.org/10.1016/j.lwt.2021.112716

Funds:

The experiments were conducted within the framework of the funded project of the Ministry of Education and Science of the Republic of Kazakhstan No.AP19577363 “Development of technology of functional extrusion food products using wild plants of Kazakhstan”.

Acknowledgments:

The authors thank the Ministry of Education and Science of the Republic of Kazakhstan for financial support (grant for young scientists for 2023-2025 – No.AP19577363 " Development of technology of functional extrusion foods using wild plants of Kazakhstan ")

Conflict of interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Assel Izembayeva, Almaty Technological University, Faculty of Food Technology, Department of Technology of Bread Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstan, Tel.: +77073577850,
E-mail: asel_190183@mail.ru
ORCID: https://orcid.org/0000-0002-1246-2726

Zilikha Moldakulova, Almaty Technological University, Faculty of Food Technology, Department of Technology of Bread Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstan, Tel.: +7707-4403026,
E-mail: zliha_92_kz@mail.ru
ORCID: https://orcid.org/0000-0003-1398-3040

Asemgul Abdreeva, Almaty Technological University, Faculty of Food Technology, Department of Food Biotechnology, Tole bi 100, 050012, Almaty, Republic of Kazakhstan, Tel.: +77057757932,
E-mail: zliha_92_kz@mail.ru
ORCID: https://orcid.org/0000-0002-3567-8028

***Galiya Iskakova**, Almaty Technological University, Faculty of Food Technology, Department of Technology of Bread Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstan, Tel.: +77074028610,
E-mail: iskakova-611@mail.ru
ORCID: https://orcid.org/0000-0002-2077-8755

Meruyet Baiysbayeva, Almaty Technological University, Faculty of Food Technology, Department of Technology of Bread Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstan, Tel.: +77082200485,
E-mail: meruert_80@mail.ru
ORCID: https://orcid.org/0000-0002-1172-9281

Makpal Atyhanova, Almaty Technological University, Faculty of Food Technology, Department of Technology of Bread Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstan, Tel.: +7771-1288604,
E-mail: makpal_atyhanova@mail.ru
ORCID: https://orcid.org/0000-0003-3098-1340

Corresponding author: *

potravinárstvo®

OPEN ACCESS

Received: 19.6.2024
Revised: 16.7.2024
Accepted: 20.8.2024
Published: 20.8.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18 , 2024, p. 719-740
https://doi.org/10.5219/1995
ISSN: 1337-0960 online
www.potravinarstvo.com

Use of non-conventional raw materials in the production of gluten-free pasta – a review

Fariza Sagyntay, Baltash Tarabayev, Bayan Muldabekova, Auelbek Iztaev, Gaukhar Kossaliyeva, Fatima Dikhanbayeva, Ramza Berzhanova, Nazym Alzhaxina, Anar Kabylda

ABSTRACT

Currently, about 5% of the earth's population suffers from gluten-related disorders. Modern technologies for gluten-free diets and filling the protein deficit are aimed at manufacturing gluten-free (GF) pasta products using non-conventional plant raw materials with high biological value. GF grains and crops (rice, corn, buckwheat, amaranth, quinoa, etc.) are used to produce GF pasta products. However, there is a scarcity of studies that comprehensively understand GF flour addition on the nutritive, sensory and cooking properties. Therefore, the scope of this literature review covers the main types of non-conventional raw materials for GF pasta products and summarizes the research on pasta products made from them. Results indicate that the rheological and sensory attributes of pasta made from pure GF flours such as rice or corn still requires a deeper study of technological processes in producing GF pasta. Incorporating nutrient-dense ingredients such as amaranth, quinoa, sorghum, and chia flours not only enhances the nutritional profile of gluten-free pasta but also offers opportunities for diverse culinary applications. The improvement of sensory attributes in rice-, corn-, buckwheat-based pasta, coupled with the effective use of cooking enhancers like xanthan gum, transglutaminases, inulin, and alternative starches, paves the way for creating more palatable gluten-free options. As techniques such as high-temperature treatment, extrusion cooking, and starch pregelatinization become more refined, future developments may focus on optimizing these processes to further improve the texture, taste, and overall quality of gluten-free pasta. Continued research and innovation in ingredient selection and processing technologies will be crucial for meeting the growing demand for gluten-free products that do not compromise on culinary experience or nutritional value.

Keywords: gluten-free pasta; review; celiac disease; gluten-free flour

INTRODUCTION

Gluten is a protein found in wheat, rye, barley and other grains **[1]**. Most cereal grains are traditionally divided into glutenins, structural and biologically active proteins, and prolamins. Prolamines are mainly found in grains and flours as relatively simple and small molecules, while glutenins are rich in disulfide bonds and have significant molecular weight **[2]**.

Currently, about 5% of the earth's population suffers from gluten-related disorders **[3]**. In recent years, a new pathology, also associated with gluten intake but characterised by specific, different features from celiac disease, has attracted particular attention from researchers. Currently, it is called “gluten sensitivity” (GS) **[4]** or “non-celiac gluten intolerance” (NCGI) to be more reasonable **[5]**. Gluten intolerance, as well as celiac disease, is associated with eating foods containing gluten. The gluten-free diet should be followed by people suffering from hereditary metabolic disorders such as celiac disease, as well as in the case of individual intolerance to cereal protein. The consumption of gluten-containing foods manifests itself in these people by inflammation of the intestinal mucosa **[6]**. Also, scientific research has shown that if one adheres to a gluten-free diet, it benefits the body (helping to lose weight) and reduces the progression of chronic diseases **[7]**.

Pasta is one of the most popular food products of the population and is included in the list of everyday goods **[8]**. Modern technologies for gluten-free diet and filling the protein deficit are aimed at manufacturing gluten-free (GF) pasta products using non-conventional plant raw materials with high biological value **[9]**. Cereal and legume-based products still dominate the world's population's diet as the main vegetable protein supplier. GF grains and crops (rice, amaranth, buckwheat, quinoa, etc.) produce many commonly used products, including bread, pasta, snacks. They remain the most accessible to all categories of consumers due to their high nutritional value **[10]**. However, GF products are less likely to be found in some countries **[11]**.

Recently, many findings have been investigated to enhance organoleptic qualities and nutritional and practicality profiles to meet the prospering demand for high-quality gluten-free pasta products. However, when GF raw materials are used for pasta production, the GF pasta properties might also vary. There needs to be more studies that comprehensively understand GF flour addition on the nutritive, sensory and cooking properties. Therefore, the aim of this literature review covers the main types of non-traditional raw materials for pasta products and summarises the research on pasta products made from them.

Literature search and description of studies

A systematic scientific literature search about gluten-free pasta was performed to identify English papers using Web of Science database. The search was performed on the 16th of April, 2024. In addition, manual screening was performed to find other papers on GF pasta products using other sources (PubMed, Google Scholar). Figure 1 demonstrates the number of papers published between 2000-2023 on "gluten-free pasta" according to Web of Science database. A total of 628 research and review articles in English were found, with 74 articles published in 2019.

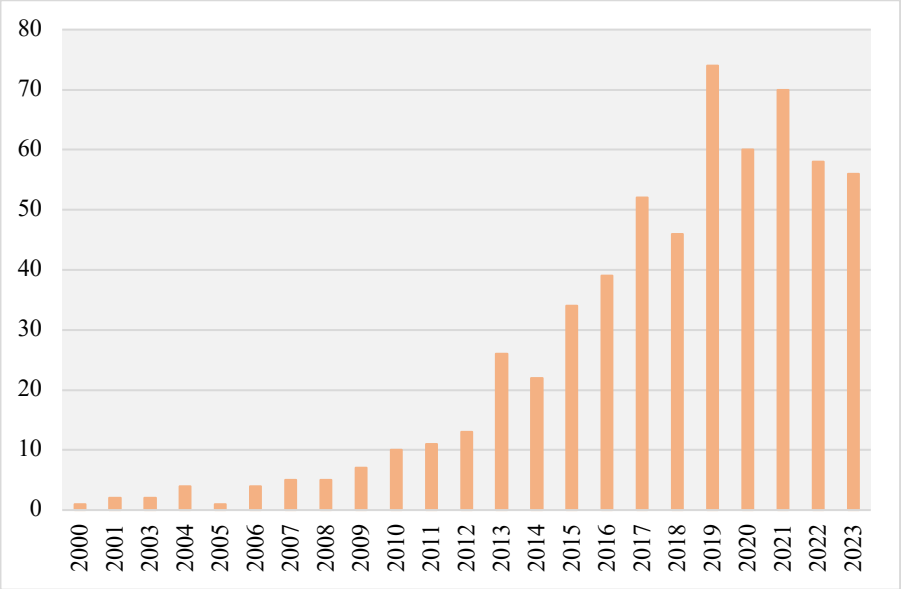


Figure 1 Number of papers published between 2000 and 2023 on the topic "gluten-free pasta", according to the Web of Science database.

TECHNOLOGICAL OPPORTUNITIES TO DEVELOP GLUTEN-FREE PASTA

Additives in GF pasta production

A gluten-free alternative will undoubtedly help in solving the problem of improving public health. When switching to a gluten-free diet, it is necessary to ensure the availability of all essential nutrients in sufficient quantities. Thus, an improvement in the nutritional or functional characteristics of the gluten-free paste is achieved by mixing different types of GF flours. Gluten gives wheat-based products their characteristic texture, integrity and other properties. Gluten serves such an important function, but unfortunately, its consumption can cause serious medical complications, such as celiac disease in a certain percentage of the population **[12]**. Most GF pasta products on the market exhibit poor cooking and sensory qualities compared to wheat flour-based pasta. The use of GF raw materials implies some changes in the production technology of gluten-free pasta **[13]**. The critical rheological properties of the paste are hardness, elasticity and stickiness, which may be affected by the lack of gluten.

Nowadays, hydrocolloids, starch and various proteins are used to improve the quality of GF pasta as potential gluten substitutes. The hydrocolloids can be thickeners, gel-formers, stabilisers and, in some cases, emulsifiers. Using hydrocolloids to produce GF pasta products allows significant progress in the functional and technical properties of finished products. Published studies of hydrocolloids have shown that their type considerably affects the characteristics of fresh and cooked noodles. At the same time, the degree of their influence depends on the level of hydration used in pasta making. For example, 0.5% xanthan gum in GF tiger nut noodles and adjusting the amount of water showed low cooking losses and high elasticity **[14]**. The hydrocolloids, including xanthan gum (XG), guar gum (GG), and sodium alginate (SA) were found to enhance the

elasticity and viscosity of the pasta from the GF proso-millet pasta, with 2% XG showing the highest elasticity **[15]**. Another study showed that the best overall quality, lower glycemic index, and increased water-insoluble fibre content was achieved when 2% carboxymethyl cellulose or chitosan was added to pasta based on corn and oat flour **[16]**. Furthermore, the mixture of amaranth flour with guar gum (1%) exhibited paramount peak viscosity and minimum cooking loss **[17]**. Xanthan gum concentration of 0.6% with cassava starch GF pasta reduced cooking losses and showed the lowest elasticity, cohesiveness, etc. **[18]**. Synergism of hydrocolloids 1% glycerol monostearate and 2% xanthan gum improved the texture, cooking properties and flavour of gluten-free brown rice pasta **[19]**. In addition, using 0.1% propylene glycol alginate and 0.5% fatty acid monoglycerides and gelatinisation of mixed flours resulted in GF pasta with the best cooking quality and texture **[20]**. Interestingly, alternative hydrocolloids such as gledis gum and bream gum used in chakpea pasta showed similar physiochemical characteristics to pasta made with traditional hydrocolloids. In contrast, pasta made with gledis gum was more favoured according to sensory analyses **[21]**.

Dietary fibre is often a process additive or secondary quality-enhancing ingredient **[22]**. Adding dietary fibre to the pasta can create products low in calories, fat and cholesterol. The dietary fibres that have been utilized in GF products are β-glucan, oligofructose, inulin, carob fibre, polydextrose, etc. **[23]**. Corn pasta with inulin at 5% and 7.5% concentrations demonstrated greater stretching, shear viscosity, and higher elasticity **[24]**. The presence of inulin in pasta increased optimum cooking time, cooking loss, swelling and water absorption index, and deterioration of textural characteristics **[25]**. Dietary fibre (inulin) was justified as an enrichment agent in instant pasta from non-traditional raw materials **[26]**. A study of high-fibre fenugreek, chickpea and tiger nut flours showed that the soluble and insoluble fibre composition was balanced in GF pasta **[27]**. Unripe plantain flour (UPF) and Hi-Maize 260 as a source of dietary fibre were added to GF pasta, and the results showed that spaghetti with UPF demonstrated shorter cooking time and less cooking loss **[28]**. 20% dietary fibre could form a stronger protein-starch network, thereby reducing swelling and cooking losses of pasta enriched with sweet potato **[29]**.

Starch plays a critical role in forming the structure. Glucose residues connected by glycosidic bonds represent starch molecules. Polymerisation of glucose molecules occurs by forming two polysaccharide structures: linear *amylose* and branching *amylopectin* **[30]**. Depending on the rate and degree of cleavage by amylolytic enzymes of the intestinal microflora, starch is divided into glycaemic, which are easily digested, partially resistant and resistant starch. The latter are not adsorbed in the human small intestine. However, once in the large intestine and rectum, they become available for fermentation by bacteria, thus performing the function of dietary fibre **[31]**. This is why they are currently considered among the most persistent foodstuff prebiotics. Rapid gelatinisation and good viscosity retention after gelatinisation are the main properties of starch, as high dough viscosity is essential for creating the structure of gluten-free food products.

Consequently, eliminating wheat flour also sheds starch from the product. Starches from alternative sources such as potato, tapioca, cassava, and corn have been added to GF recipes **[32]**, **[33]**. The supplementation of resistant starch (RS) considerably increased the finished pasta products' elasticity; the samples showed lower stickiness values at RS substitution levels above 100 g kg-1 **[34]**. Modified starches have also been analysed within gluten-free pasta. Modified banana starch (15%), modified potato starch (15%) and taro flour (70%) provided optimum cooking time, cooking loss and elongation **[35]**. Cervini et al. (2021) showed that 15 g/100 g of annealed sorghum starch in rice-based pasta resulted in the highest total dietary fibre and resistant starch **[36]**. Moreover, partially replacing rice flour with legume flour increased resistant starch content **[37]**. In addition, 30% green plantain flour in GF pasta demonstrated good cooking quality and high resistant strach content **[38]**. Ferreira et al. (2015) showed that pasta samples containing sorghum flour (40 %), rice flour (20 %) and potato starch (40%) showed better culinary qualities **[39]**.

Enzymes are often selected based on their ability to prompt the formation of cross-links between polymers in the product formulation, thereby inducing network formation identical to what would occur if gluten were present in the product formulation **[40]**. Enzymes such as glucose oxidase, transglutaminase, tyrosinase, and laccase have been studied recently. It was shown that higher TG concentration and prolonged kneading increased macaroons' elasticity, cohesiveness and chewability. In contrast, TG concentration of 1.5% and 10 minutes of kneading significantly increased the content of phenolic compounds **[41]**. In another study, transglutaminase in gluten-free corn noodles showed a reduction in cooking losses and total organic matter **[42]**. Supplementing the oxidative enzyme POx to quinoa paste with 12% rice or pea protein improves elasticity **[43]**. The best sensory evaluation and cooking properties were obtained with 140 mg kg-1 TG enzyme in yellow pea noodles **[44]**. Transglutaminase and egg protein in taro-based pasta demonstrated a positive effect on cooking time and increased weight of cooked pasta while hurting pasta firmness **[45]**. Adding TG to millet pasta increased digestibility, cooking loss, and water absorption **[46]**.

Physical modifications

A technology using high-temperature treatments was proposed to improve the properties of GF pasta. It is proved that the thermal treatment of rice and chickpea flours decreased their moisture-absorbing and swelling ability **[47]**, **[48]**. Extruded amaranth and barnyard millet provided higher final viscosity **[49]**. In addition, the germination process is considered the most effective procedure to enhance the nutritional and bioactive potential of lentils **[50]**.

The extrusion-cooking approach is a distinct technology appropriate for producing gluten-free pasta products because it involves the unification of the pre-gelatinization and moulding stages **[51]**. Extrusion cooking of pasta based on rice and amaranth flours before pasta preparation gave the best results in terms of textural characteristics **[52]**. Bouasla et al. (2019) investigated the optimum effect of extrusion cooking parameters (30% moisture content, 120 °C temperature and 80 rpm screw speed) to obtain high-quality rice-buckwheat pasta **[53]**. The extrusion cooking at 30% moisture content of dough at 80 rpm is suitable for producing rice-yellow pea pre-cooked pasta with great phenolic content and proper quality **[54]**. However, extruded maise and sweet potato pasta with orange flesh showed lower beta-carotene levels but had higher

antioxidant properties and shorter cooking time **[55]**. In addition, extrusion cooking improved the nutritional composition, reduced cooking losses and improved the texture of brown rice- and maise flour- based pasta products **[56]**.

Pregelatinisation is a type of physical modification of starch by heating and mechanical shear. Pregelatinised starch is obtained by sufficient heating, subsequent drying and grinding. The addition of 30% pre-gelatinized native grain plantain flour or drum-dried green banana flour improved the functional properties of the paste **[38]**. Pregelatinised cassava starch and bagasse, amaranth flour and cassava starch resulted in fewer solids in cooking water and optimum cooking time **[57]**. The gelatinisation of mixed GF flours (buckwheat, maise, rice) produced a gluten-free pasta with good nutritional and culinary properties **[20]**. Pregelatinised flour from macerated carioca and black bean flour demonstrated increased protein and dietary fibre content compared to semolina pasta **[58]**. Pregelatinised rice flour combined with germinated chickpea flour in GF noodles significantly increased crude protein, crude fibre, amylose, antioxidant activity, reduced glycemic index and improved cooking quality **[59]**. Although the extrusion process can improve important quality parameters of GF pasta, the characteristics of wheat pasta still need to be achieved.

Furthermore, annealing is often involved in starch as a physical treatment to alter its physicochemical properties. Heat-moisture treatment of rice starch decreases the swelling power and solvability of starch **[60]**. Wang et al. (2018) revealed that the replacement of 40% of native rice starch with annealed rice starch led to increased cooking quality and sensory and texture properties of rice noodles **[61]**. Annealed sorghum starch has been proven to positively influence the optimum cooking loss, cooking time, elasticity and stickiness of rice-based pasta samples **[36]**. However, the annelisation process requires specialised equipment and careful control of temperature and moisture levels, making it more expensive to produce gluten-free pasta.

Moreover, it has been suggested that kneading in boiling water increases the bound water content and the elasticity of oat dough **[62]**. In contrast, the redox activity associated with lipoxygenase, which leads to dough kneading and extrusion deterioration, can be delayed or even prevented using low-temperature kneading or thermal pre-treatment of the legume flour **[63]**. Proper pasta drying is key to a superior quality finished product **[64]**. Pre-heating at 40 °C before drying and evaporating at elevated temperatures under high humidity enhanced the maximum hardness of GF pasta **[65]**. D'Amico et al. (2015) studied the effects of high-temperature drying (80 and 100 °C) on the texture and culinary characteristics of amaranth/quinoa/wheat or millet/white bean pasta. Moreover, the 57 °C at an operating speed of 3 m/s was suitable for minimising hot-air drying time to obtain cassava flour with a progressive water-holding capacity **[66]**. However, exposing gluten-free pasta to high temperatures for extended periods of time can lead to a loss of nutrients and natural flavours in the pasta.

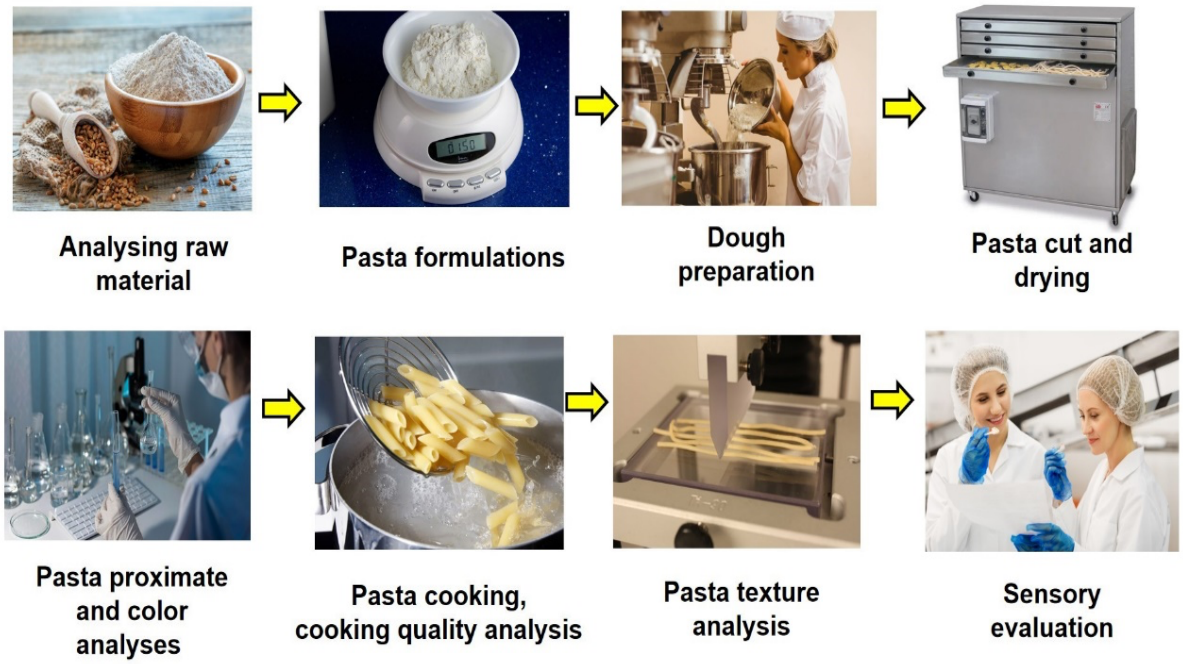


Figure 2 General technical road of studying gluten-free pasta.

NON-TRADITIONAL RAW MATERIALS FOR GF PASTA PRODUCTION

Amaranth (Amaranthus)

In recent years, a new source of raw materials for a whole range of functional foods has appeared on the world market is an ancient grain amaranth. Amaranth grain originates in Central and South America, but India also has a large distribution and variety of amaranths **[67]**. The most valuable feature of amaranth is the absence of gluten in its protein. The amaranth grain contains carbohydrates 68.1%, fat 6.04%, ash 2.04%, protein 14.6% and dietary fiber 6.7% **[68]**. Amaranth contains minerals, vitamins, and microelements, considerably enhancing its antioxidant features **[69]**, **[70]**. Mystkowska et al. (2024)

reported that amaranth flour, along with other GF flours, contained more Ca, Mg, Fe, Zn compared to gluten-containing flours obtained from the local market in Poland **[71]**. Amaranth grain has also been shown to be a source of amino acids such as histidine, leucine, isoleucine, lysine, valine, threonine etc. **[72]**. Besides, albumin is the major protein fraction in amaranth protein **[73]**.

To date, amaranth-based fresh/dried pasta has yet to be discovered. Recently, Lux et al. (2023) studied the use of amaranth flour (1:4) and water (1:6) with alginate (1.5%) resulted in good properties for dough processing and cooking **[74]** (Table 1). Furthermore, it has been shown that pasta with amaranth flour and dried amaranth leaves exhibited higher amount of protein, minerals and antioxidant capacity compared to semolina **[75]**. In addition, protein, dietary fibre, β-carotene, iron and zinc were significantly increased in cassava pasta with the addition of amaranth leaf powder **[76]**. The study's results on amaranth, buckwheat and quinoa pasta showed that amaranth-based pasta had decreased texture elasticity and cooking time. In contrast, quinoa-based pasta had increased cooking loss, but buckwheat pasta exhibited the least negative effects **[77]**. Thus, the use of amaranth in pasta manufacture still needs improvement to acquire pasta with good sensory features.

Buckwheat (*Fagopyrum esculentum*)

The buckwheat crop originated in Central and North-East Asia. Buckwheat cultivation occurred in China, from where it was introduced to Europe via Turkey and Russia **[78]**. Buckwheat flour is a unique product with exceptional flavour properties and useful qualities **[79]**. Buckwheat, unlike wheat, contains more vitamins (B3 (niacin) – 6.2-18.0 mg/100g, B6 (pyridoxine) – 0.6 mg/100g, etc.) and minerals (Mg – 231.0-390.0 mg/100g, K – 450.0-460.0 mg/100g, etc.) necessary for the human body **[80]**, is completely gluten-free and a unique source protein with digestibility of ~80% **[81]**.

Oniszczczuk et al. (2019) revealed that buckwheat flour pasta with 32 % moisture content had a higher content of total free phenolic acids than pasta with 30 and 34 % moisture content **[82]**. Furthermore, a gelatinisation study on pasta made from buckwheat flour mixed with other gluten-free raw materials (maise and rice) showed that adding 49.2-99.4% buckwheat flour to pasta showed high protein and dietary fibre content. Using 0.1% PGA 0.5% MFA and gelatinisation of mixed flours (buckwheat, maise, rice) also displayed good cooking properties **[20]**. Hydrothermal pre-treatment of wholegrain buckwheat flour pasta exhibited the potential to be implemented in buckwheat pasta production **[83]**. Interestingly, 100% buckwheat flour pasta showed the best culinary properties. However, it was the least acceptable colour than buckwheat-rice pasta, suggesting that it needs sensory properties improved **[84]**. It was revealed that the addition of buckwheat flour significantly affected the change in the pasta's textural properties and appeared more bitter than the wholegrain wheat samples **[85]**. Another interesting research revealed that supplementing buckwheat pasta with 5% silkworm powder reduced the carbohydrate content (from 59.5g to 57.3g), while enhancing protein content (from 26.2 to 28.2 g) in 100 g pasta. Although silkworm reduced the optimum preparation time and increased the acidity of the paste during storage, the addition of 10 g silkworm powder showed the highest overall acceptability **[86]**. Buckwheat-amaranth-quinoa flour-based pasta enriched with egg white powder provided high nutritional properties and low cooking losses **[87]**. In addition, sensory evaluation analysis showed that semolina pasta enriched with buckwheat showed a significant decrease in scores ($p < 0.05$) for the appearance, colour, and odour of the pasta **[89]**. Therefore, the results indicate that buckwheat pasta fortified with amaranth, quinoa, and rice, along with the addition of egg powder, can result in nutritious and consumer-acceptable pasta products.

Chickpea (*Cicer arietinum*)

Chickpea flour is a favorable raw substance for the production of gluten-free products. The analysis of literature data on chickpea flour's chemical composition and nutritional value showed that it could be used in this way. The chemical composition of chickpeas is characterised by the predominance of carbohydrates (62.95 g), protein (21.07%), and fat (2.7-6.48%) **[88]**.

The paste prepared from chickpea flour (15%) with the supplementation of maise flour had low elasticity and increased firmness, and using guar resulted in a lower glycemic index **[89]**. Also, the dough matrix and protein content were significantly improved by combining teff flour, chickpea flour, and xanthan gum in addition to buckwheat **[90]**. Moreover, the GF pasta prepared using chickpea flour (65.7%) and egg whites (34.3%) contained less fat, more protein and dietary fibre, and had good texture and taste **[91]**. Germinated chickpea flour enhanced crude protein levels, crude fibre, amylose, antioxidant activity, and total phenolic acid **[59]**. In addition, pasta made from chickpea flour, maize flour, and unripe plantain showed less total starch than in control pasta, an essential nutritional issue to consider when developing GF pasta **[92]**. Increasing the concentration of chickpeas in barley-based pasta produced pasta with increased protein and fibre content and improved colour characteristics and texture compared to control barley pasta **[93]**. Pasta enriched with two different chickpea hulls showed higher antioxidant activity, improved colour intensity, lower cooking loss and higher elasticity compared to control rice-based GF pasta, indicating that chickpeas can be used for preparing GF pasta **[94]**. In contrast, supplementation of durum wheat semolina pasta with chickpea flour and its protein isolate increased cooking losses, moisture content and swelling index **[95]**.

Chia (Salvia hispanica)

The nutritional profile and functional applications of chia seeds (*Salvia hispanica*) have garnered significant attention within both the nutritional science community and the culinary arts. Chia seeds are a good source of phenolic compounds, protein (16.5-24.2 g/100g), and dietary fibre (30.2-34.4) **[96]**. Previous research reported that partially deoiled chia flour contained more protein, fibre, and minerals compared to wheat flour **[97]**.

Replacing rice flour with 10% chia mucilage or seeds has been reported to increase dietary fibre, protein, and phenolic acids **[98]**. Enrichment of chia seed flour (20%) with diacetyl tartaric acid esters of mono (and di) glycerides (DATEM) can be used to refine the sensory properties and nutritional values of GF pasta. However, the addition of 30% chia seed flour particularly reduced the appearance of raw noodle, surface smoothness, and the chewability of cooked noodle samples **[99]**. A recent study showed that the addition of chia seed flour (15%) to quinoa flour (85%) resulted in good cooking, sensory

and textural qualities as well as increased chemical composition **[100]**. Overall, there is a lack of research on recipes using chia seed flour, which could be considered for future research.

Corn (Zea mays)

Cornbread, pasta and flour have become a familiar alternative to wheat products. Corn loses out on the content of useful substances to other cereals, but it is also a baseline of vitamins B and E, copper, selenium, iron, and antioxidants, it has low-calorie content and easy digestibility **[101]**. Corn starch contains 0.38-7.70 (g/100g) crude protein, 0.32-7.13 (g/100g) crude fat, 0.32-0.62 ash **[102]**.

Combining GF maise/corn paste with other raw materials such as legumes and additives and using different processing methods shows good nutritional, culinary, and sensory characteristics. Bresciani et al. (2021) reported that 25% high-amylose corn flour displayed a rational compromise between high starch stability (4.2%) and good culinary properties in pasta with high-amylose corn in combination with conventional corn **[103]**. Furthermore, 40% corn meal and 60% brown rice flour exhibited better texture evaluations (sensory and instrumental) **[56]**. A mixture of cassava starch and corn resulted in a higher percentage of total dietary fibre, high protein digestibility and slowly digestible starch **[104]**. Broad bean and quinoa-based gluten-free corn-pasta-like products greatly increased net protein utilisation and decreased digestibility **[105]**. A previous study showed that adding 15% whey powder and 1% grape peel resulted in a good sensory profile of corn-based GF pasta **[106]**. Furthermore, it has been demonstrated that different processing methods affect corn-based pasta quality. Also, pumpkin flour (25%) influences corn pasta colour, texture and sensory properties **[107]**. Moreover, adding corn starch to corn flour changes rheological properties during the cooking stage of gluten-free pasta **[108]**. Fortification of corn and rice pasta with tempeh flour enhanced protein content (6.30-9.07%) and altered whiteness level (66.08-71.97%) **[109]**.

The optimum extraction conditions, including barrel temperature (80 ºC), rotation screw (25 rpm) and expansion ratio (1.38) with optimising cooking time (3.3 min), cooking yield (196%), swelling (210%) and cooking losses (16.3%) were obtained for corn-based GF pasta **[110]**. The hydrothermal-treated corn-field bean semolina mixture pasta displayed good cooking and texture parameters **[111]**. In addition, using extrusion (80%) and cooking (70%), GF blue maise pasta retained the greatest total phenolic content. It showed a definite second peak in viscosity due to the rearrangement of starch components during cooling **[112]**. Maise flour and oat bran enriched with additives such as carboxymethylcellulose and chitosan (2%) provided the best overall quality **[113]**. A recent study revealed that hard-grained, high-hectoliter-weight maise genotypes produce flours with elevated solvent retention due to increased damaged starch content. Consequently, extruded and cooked pasta from these flours exhibits lower water retention and absorption and greater firmness **[114]**.

Rice (Oryza sativa)

Rice, an inherently gluten-free staple grain, possesses a remarkable combination of attributes that render it an exceptional dietary choice for various individuals, particularly those with gluten sensitivities or celiac disease. This cereal is characterised by its lack of gluten, which makes it an ideal component for gluten-free diets **[115]**. In enhancing the technological attributes of rice flour products, incorporating a protein source emerges as a viable strategy, potentially leading to significant improvements in both functional and nutritional qualities. For instance, the study reported that the supplementation of soybean flour increases crude fibre (0.8-1.3%), protein (6.7-12.1%), energy value (379-389 kcal/100 g), and 15% soybean flour is the optimum amount for rice pasta to get better acceptability to consumers **[116]**. Furthermore, it has been proven that adding egg albumin provides short cooking time, low cooking losses (4.38–4.63%) and better firmness to GF pasta **[117]**. In addition, another study revealed that replacing rice flour with defatted soy flour (DSF) at a ratio of 90:10 increased the protein content of the pasta. Also, sensory evaluations indicated that the rice spaghetti formulated with rice flour, DSF, and modified starch was comparable in quality to commercial spaghetti made from durum semolina **[118]**. Bouasla et al. (2017) revealed that fortifying rice pasta with legumes enhanced fibre, protein, and ash contents while decreasing the expansion coefficient, hardness and lightness **[53]**. Moreover, formulation with 20% gelatinised rice flour was reported to have optimal cooking quality and texture properties **[119]**. Utilising the high-protein white and brown rice flour with xanthan gum and canola oil had less cooking loss and greater protein and fat content **[120]**. Interestingly, adding 10% passion fruit peel flour increased nutritional value and flavour quality of rice and corn-based pasta **[121]**. 30% pre-gelatinised native green plantain flour or drum-dried green banana flour improved the functional properties of the rice paste **[38]**. It has been reported that corn starch, rice starch, and lentil flour pasta with additives, such as transglutaminase, hydroxypropyl methylcellulose and xanthan gum, positively affected GF pasta properties **[122]**.

In addition to the basic extrusion process, recent advancements have spotlighted its multifaceted utility in treating rice flour before its application in pasta production, facilitating many functional modifications. For instance, the extrusion process resulted in considerable alterations in the digestibility of rice flour, which led to enhanced resistant starch content and lowered predicted glycemic index **[123]**. It was also reported that harsher extrusion conditions lead to low cold paste viscosity, hot paste viscosity and low final extrudate viscosity **[124]**. In addition, the low amylose rice extrudate showed a lower sticking profile and apparent viscosity, indicating a higher starch cleavage level than high-amylose rice **[125]**.

Quinoa (Chenopodium quinoa Willd.)

Quinoa seeds are a versatile enrichment agent in producing a broad range of foodstuffs, as they are characterised by a high content of essential amino acids **[126]**. The amino acid composition of quinoa proteins is close to milk proteins **[127]**, allowing the crop to compete with recognised high-protein raw materials such as barley, buckwheat, and amaranth.

In comparison to raw quinoa flour, germinated quinoa flour had more pronounced adverse effects on the cooking quality of pasta. However, it significantly enhanced the nutritional and functional attributes **[128]**. Demir & Bilgiçli, (2021) reported that gluten-free pasta with quinoa flour improved mean protein values from 8.1 to 12.7 %, total phenolic content from 0.7 mg GAE/g to 1.5 mg GAE/g, antioxidant activity from 13.4 to 28.8 % **[129]**. An increase in mineral content was also observed in gluten-free pasta at all quinoa flour addition ratios. Several studies proved that the use of quinoa flour with egg white and

yucca flour might be a potential formulation to get extruded pasta **[130]**, **[131]**. For instance, Torres Vargas et al. (2021) found that the score of sensory attributes was acceptable in GF pasta prepared using extruded quinoa flour **[130]**. The pasta made with quinoa flour, cassava starch, water, and a constant egg-white factor, exhibits favourable sensory characteristics. This product shows great market potential due to its appealing taste and high protein content, making it well-accepted by consumers **[131]**. More recent, Itusaca-Maldonado et al. (2024) obtained a 100% quinoa paste with high nutritional value, cooking quality, water absorption, swelling index and minimal carbohydrates **[132]**. Hyper-protein-defatted quinoa flour positively influenced the viscosity of rice-corn-quinoa flour-based GF pasta **[133]**. It was shown that zein protein added to quinoa pasta hurt the texture of the pasta, whereas quinoa flour resulted in greater protein content and reasonable textural features **[134]**. In addition, the inclusion of hyperprotein-defatted quinoa flour enhanced the technical and functional attributes of the dough **[135]**.

Sorghum (Sorghum bicolor)

In Southwest Asia, Equatorial and Southern Africa, grain sorghum is the main bread plant manufacturing bakery and flour confectionery products. However, the protein quality of sorghum could be better because it lacks essential amino acids **[136]**. Sorghum contains 8-15% protein, 60-77% starch, 1–3.4% crude fibre, and 2.1-7.6% fat **[137]**.

GF pasta with white and brown sorghum flour was high in dietary fibre, protein, and antioxidant activity, while the greatest eGI was detected in rice (69.8) and then in corn-based pasta (66.4) **[138]**. Moreover, white sorghum flour showed greater sensory satisfaction than brown sorghum flour pasta **[139]**. Furthermore, a combination of sorghum 40%, rice 40% and potato flour 40% was found to be best regarding cooking quality **[39]**. Other recipes have also been developed that combine sorghum paste with other types of GF flours **[140], [141]**. For instance, GF is prepared using toasted soy, channa, and sorghum flour with additives comparable to the control in all quality parameters **[140]**. Gluten-free pasta was developed by combining high-protein, non-gluten flours like soy, chickpea, and sorghum flour with gums as additives to meet protein needs while avoiding allergic reactions **[141]**.

Limitations and future perspectives

It is necessary to ensure the nutritional safety of celiac disease patients, prevent disease relapses, ensure gradual social adaptation of patients and improve their quality of life **[142]**. Nowadays, GF pasta products are in a higher price category than conventional products due to the complexity of technological processes. This is further complicated by the fact that supermarkets have a rather limited range of these products. Therefore, it is advisable to establish gluten-free pasta production, which will allow the production and sale of products at a low price. Besides, many products may contain “hidden” gluten, and there may be no labelling on these products, which may cause unintentional dietary infringement **[143]**. “Gluten-free” labelling is available for products that only partially remove gluten. This amount of remaining gluten in products varies from country to country.

Currently, few recipes have been developed due to the difficulties of working with gluten-free raw materials. Although the visco-elastic properties of dough can be formed by heat treatment, this decreases the finished product's nutritional value, dietary fibre and vitamin content. Therefore, studies must be continued to evolve new recipes that respond to these issues. Rice and corn flour could be a raw material that could proficiently restore wheat- or barley-based pasta, but using 100% buckwheat, chia, quinoa or sorghum led to less promising results. On the one hand, using non-starch polysaccharides makes it possible to obtain products with high nutritional value and balanced composition. Still, the properties of these polysaccharides have yet to be sufficiently studied. On the other hand, additives such as xanthan gum, egg or whey protein powder can improve the properties of GF paste.

All the results of studies on GF pasta development indicate that the rheological and sensory properties still cannot reach the level of pasta made from conventional raw materials. The biggest challenge remains sensory characteristics such as flavour, colour, and overall appearance. Since GF is of great importance for people suffering from gluten intolerance, it requires a deeper study of technological processes in producing specialised foods.

Table 1. Studies on gluten-free pasta using different non-conventional raw materials

The main GF material	Pasta formulations	Additives	Effects	Sources
Amaranth	Whole amaranth flour	sodium alginate	Had good dough processing properties and cooking attributes.	[74]
	Dried amaranth leaves, amaranth seed flour, semolina	Carboxymethylcellulose, fresh egg	Exhibited higher content of protein, minerals and antioxidant capacity.	[75]
	Amaranth flour	gum tragacanth, guar gum, gum acacia	The best overall quality of gluten-free paste was when 1.0% guar gum was added.	[17]
	Amaranth and barnyard millet	refined oil (5%)	Extrusion led to higher final viscosities; preheating at 40 °C before drying at high temperatures under high humidity conditions increased the firmness.	[49]
	Cassava and amaranth flour	carboxymethyl cellulose	Moisture content, hardness and protein content improved, but earthy flavour was a major problem for consumer satisfaction.	[66]
	Amaranth flour, cassava bagasse, cassava starch	-	Pregelatinised bagasse flour, cassava starch, and amaranth flour resulted in less solids being lost to cooking water and optimum cooking time.	[57]
	Amaranth and rice flour	-	Extrusion cooking before pasta making gave good results in terms of textural features.	[52]
Buckwheat	Rice: buckwheat 50:50	-	Pasta from mixture of rice and buckwheat showed low cooking attributes and acceptable sensory characteristics.	[9]
	Buckwheat, maise, rice flour	monoglycerides of fatty acids (MFA), propylene glycol alginate (PGA)	gelatinisation of mixed flours with 0.1% of PGA and 0.5% of MFA showed good nutritional and cooking quality.	[20]
	Buckwheat flour: water 30%, 32%, 34%	-	32% moistened buckwheat pasta showed a higher total content of free phenolic acids	[82]
	Wholegrain buckwheat flour	-	hydrothermally treated buckwheat flour was more liked by consumers.	[83]
	100% buckwheat or replaced with 5, 10 and 15%, amaranth or rice flour	eggs, water and 2.0% xanthan gum	pasta made from 100% buckwheat flour was less accepted in terms of the colour, while combined pasta showed better acceptance.	[84]
	Buckwheat flour	bombyx moria powder 5-10g	the supplementation of silkworm powder enhanced the nutritional value of buckwheat pastereduced the optimum cooking time, and added 10 g silkworm powder showed the highest overall acceptability.	[86]
	Buckwheat, amaranth, quinoa	egg white powder, emulsifier	This resulted in reasonable texture firmness and low cooking loss, high cooking stability	[77]
	Buckwheat flour; rice flour	Pre-gelatinized rice starch; whole egg product; methylcellulose; eggs;	Adding 30% egg to the pasta formulation results in well-textured GF fresh pasta with improved nourishing features. The use of pre-cooked rice flour and methylcellulose as well as heat treatment, improved the texture of the products.	[87]

	Amaranth-quinoa-buckwheat 20:20:60 (AQB), millet-white bean 70:30 (MW)	Egg white powder, emulsifier	At a higher drying temperature, protein solubility and cooking loss reduced, but the elasticity and protein solubility did not reach the level of wheat pasta.	**[65]**
Chickpea	The pre- gelatinized maize flour and chickpea flour (5%, 10%, 15%, 20%, 25% and 30%)	Monoglycerides, hydrocolloids (pectin , guar flour and agar)	15% chickpea flour addition showed low elasticity and increased firmness; use of guar resulted in reduced glycemic index.	**[89]**
	Chickpea, buckwheat, teff flour	xanthan gum	The dough matrix and protein content were significantly improved.	**[90]**
	Chickpea flour	Egg white	GF pasta contained less fat (37.3%), more protein (53.8%) and dietary fibre (166.5%), and had good texture and taste	**[91]**
	Hydrothermal treated white rice (Oryza sativa L.) and germinated desi chickpea (Cicer arietinum L.)	guar gum	germinated chickpea flour enhanced crude protein level, crude fibre, amylose, antioxidant activity, total phenolic acids.	**[59]**
	chickpea, maize, unripe plantain	carboxymethyl cellulose 0.5%	Cooking losses were higher in gluten-free products with acceptance limits; total starch in GF pasta was lower than in control.	**[92]**
	chickpeas (10%, 20%, 30%, 40%) and hullless barley	and 1.5% xanthan gum	Enhanced total phenolics and flavonoids	**[93]**
	Two different chickpea hulls, rice flour, carob seed flour	Xanthan gum and	Resulted in lower cooking loss and the higher water absorption capacity and firmness	**[94]**
Chia	Milled chia seeds (5-10%) and chia mucilage (5-19%)	-	10% of chia mucilage or seeds in rice pasta increases protein, dietary fiber and phenolic acid amount.	**[98]**
	Chia *Salvia hispani*ca L. (0, 10, 20 and 30%), corn starch, rice flour	diacetyl tartaric acid esters of mono and DATEM, whole egg	Chia flour supplementation significantly increased mineral contents; DATEM enrichment (30%) showed lower cooking loss; Chia seed flour (20%) with DATEM can be used to improve sensory properties.	**[99]**
	Chia seed and quinoa flour	-	The addition of chia seed flour (15%) to quinoa flour (85%) resulted in good cooking, sensory and textural qualities as well as increased protein, dietary fibre, etc.	**[100]**
Corn/maize	High-amylose 25% and conventional corn 50%	mono- and di-glycerides of fatty acids (0.3%)	25% high amylose shows a rational compromise between high starch stability (4.2%) and certain culinary properties	**[103]**
	Corn and brown rice	-	The proportion of 40:60 CM:BR had better texture evaluations (sensory and instrumental).	**[56]**
	Corn flour, cassava starch	vegetable fat, xanthan gum, whole milk powder	a mixture of cassava starch and corn resulted in a higher percentage of total dietary fibre, high protein digestibility and slowly digestible starch.	**[104]**
	Corn:broad bean 30%:quinoa 20%	-	GF pasta demonstrated increased net protein utilisation and decreased digestibility.	**[105]**
	Hydrothermal treated corn-field bean semolina mixture 2/1 (w/w)	-	Hydrothermal-treated pasta displayed good cooking and texture parameters.	**[48]**

	maize flour	inulin	corn pasta with inulin (5-7.5%) exhibited higher elongation and shear viscosity and higher elasticity.	**[24]**
	Maise flour, oat bran enriched 22% β-glucans	egg white, 2% hydrocolloids	carboxymethylcellulose and chitosan (2%) provides the best overall quality.	**[113]**
	nixtamalised white corn flour, grape peels powder	unhydrolysed sweet commercial whey powder	The amount of 15% whey powder and 3% grape peels allows acceptable textural, physical, and sensory features.	**[106]**
	Corn flour and starch	dry egg, locust has been gums, dry egg-white, xanthan and sunflower oil	Alters in rheological attributes during cooking.	**[108]**
	wholegrain maize flours,	egg albumin, pregelatinized maize starch	Exhibited the lowest swelling index and water absorption values. The open-pollinated flint variety C6006 received a greater number of top preference selections	**[114]**
	White and blue maize, unripe plantaiոճ chickpea	carboxymethylcellulose	blue maise flour reserved the superlative total phenolic content after extrusion (80%) and cooking (70%), showed a definite second peak in viscosity due to the rearrangement of starch components during cooling.	**[84]**
	Cassava starch and corn flour	whole milk powder, egg, xanthan gum, vegetable fat	XG concentration of 0.6% showed the greatest potential for enhancing the paste-forming ability.	**[18]**
Rice	Rice flour	-	Extrusion conditions induced by the harsh parboiling process of paddy rice favoured the formation of effective starch networks in the flour.	**[51]**
	Oryza sativa rice, soy, tapioca starch	-	Increased protein, ash, energy value, crude fibre	**[116]**
	Organic rice bran, rice flour	egg albumen, whey and soy protein concentrates, emulsifier (distilled monoglyceride)	The addition of egg albumin provided short cooking time, low cooking losses (4.38–4.63%) and better firmness.	**[117]**
	Rice flour, chickpea flour, yellow pea flour, lentil flour (10 g/100 g, 20 g/100 g, 30 g/100 g)	glycol	improved the dietary fibre, protein, and ash; decreased expansion ratio, increased water absorption capacity as well as cooking loss without exceeding 6%.	**[53]**
	Rice flour, defatted soy flour (DFS)	Modified cross-linked cornstarch	the rice spaghetti formulated with rice flour, DSF, and modified starch was comparable in quality to commercial spaghetti made from durum semolina	**[118]**
	Broken rice kernels (*O. sativa* ssp. Indica and Japonica)	rice flour gel	50:50 rice gel:rice flour ratio, 20% gelatinised rice flour gave optimal cooking quality and texture properties	**[119]**
	Rice flour, green plantain and drum-dried green banana flour	Monoglyceride, egg albumen	30% pregelatinised native green plantain flour or drum dried green banana flour improved the functional properties of the paste.	**[38]**
	High-protein white and brown rice flour,	xanthan gum, canola oil	high-protein white and brown rice pasta had less cooking loss; greater protein and fat content.	**[120]**

	commercial white and brown rice flour			
	Red rice flour, tapioca flour, white quinoa flour	psyllium husk, transglutaminase (TG)	treatment with 1.5% TG and 10 minutes of kneading considerably increased the phenolic content.	**[41]**
	Rice flour	propylene glycol alginate resistant starch distilled monoglycerides	using resistant starch markedly enhanced the elasticity of the finished pasta products.	**[34]**
	Rice flour, corn flour, passion fruit peel flour	egg, xanthan gum, oil	the addition of 10% passion fruit peel flour increased nutritional value and flavour quality	**[121]**
	Corn starch, rice starch, lentil	whey and egg white powder, xanthan gum, hydroxypropyl methylcellulose, transglutaminase	The addition of additives affected different pasta properties.	**[122]**
Quinoa	Raw and germinated quinoa seeds, semolina	-	Germinated quinoa flour had more pronounced adverse effects on the cooking quality of pasta; while significantly enhanced the nutritional and functional attributes	**[128]**
	Rice-corn semolina; Quinoa flour	guar gum	quinoa flour enhanced the protein and total phenolic content, antioxidant activity	**[129]**
	Quinoa grains	egg white, yucca starch	increased protein amount and decreased the carbohydrate value, decreased in tensile strength and an increase in water absorption and swelling.	**[130]**
	Quinoa flour, starch	egg white, yucca starch	the score of sensory attributes were acceptable	**[131]**
	100% quinoa flour	-	Optimizing the production conditions obtained a GF pasta with high nutritive value, satisfactory cooking conditions, and satisfactory technological peculiarities.	**[132]**
	Rice-corn semolina, hyperprotein quinoa flour, cassava starch, defatted high protein quinoa flour	-	hyper-protein defatted quinoa flour had positive influence on viscosity.	**[133]**
	Quinoa, lupine whole flour, potato starch, rice and pea protein	POx enzyme (pyranose-2-oxidase)	Adding the oxidative enzyme POx to quinoa paste with 12% rice or pea protein significantly improves elasticity.	**[43]**
	Quinoa flour, corn starch, corn flour	dry egg-white, dry egg, locust bean gum, xanthan, zein protein	zein protein had an adverse effect on the texture of the pasta, whereas quinoa flour resulted in greater protein content and reasonable textural features.	**[134]**
	High protein quinoa flour, hyperprotein defatted quinoa flour (HHPD), rice and maize flour, modified cassava starch	-	The inclusion of HHPD enhances the technical and functional attributes of the dough.	**[135]**
Sorghum	White and brown sorghum	egg powder, egg albumen, xanthan gum, pregelatinized corn starch	high protein, dietary fibre, polyphenol, antioxidant activity.	**[138]**
	Sorghum flour	Egg albumen, egg powder, xanthan gum, pregelatinized corn starch	white sorghum flour pasta exhibited better sensory features compared to brown pasta.	**[139]**

Sorghum, rice or corn 10–20% and potato starch 1	eggs, oil	proportion of sorghum 40%, rice 40% and potato flour 40% was found to be best regarding cooking quality.	**[39]**
Sorghum flours of five hybrids, rice flour	potato starch, psyllium, egg white	brown sorghum resulted the best otcomes for bioactive compounds.	**[140]**
Toasted soy, channa, whey protein concentrate (WPC), sorghum flour	guar gum, xanthan gum, hydroxypropylmethylcellulose	GF paste with added gum was similar to the control regarding all properties.	**[141]**

CONCLUSION

Overall, rice and maize-based pasta is the most common, but it requires the addition of nutrient-rich flours such as amaranth, quinoa, sorghum, etc., to increase the nutritional value. Despite the potential of buckwheat flour in GF pasta formulations, sensory attributes such as texture and taste still need improvement. Key additives like xanthan gum, transglutaminases, and inulin have been widely recognised for enhancing the cooking properties and structure of GF pasta. Similarly, starches from alternative sources, including potato, cassava, and corn, have shown promising results in increasing the resistant starch content, which is vital for improving GF products' nutritional and functional qualities. Finally, using high-temperature treatment of raw materials, extrusion cooking and starch gelatinisation proved to be the most suitable approaches for producing gluten-free pasta with good culinary properties. By addressing existing challenges and exploring innovative solutions, the gluten-free pasta industry has the potential to grow significantly and meet the evolving needs of consumers seeking both quality and nutrition in their dietary choices.

REFERENCES

1. Biesiekierski, J. R. (2017). What is gluten? In Journal of Gastroenterology and Hepatology (Vol. 32, Issue S1, pp. 78–81). Wiley. https://doi.org/10.1111/jgh.13703
2. Wieser, H. (2007). Chemistry of gluten proteins. In Food Microbiology (Vol. 24, Issue 2, pp. 115–119). Elsevier BV. https://doi.org/10.1016/j.fm.2006.07.004
3. Taraghikhah, N., Ashtari, S., Asri, N., Shahbazkhani, B., Al-Dulaimi, D., Rostami-Nejad, M., Rezaei-Tavirani, M., Razzaghi, M. R., & Zali, M. R. (2020). An updated overview of spectrum of gluten-related disorders: clinical and diagnostic aspects. In BMC Gastroenterology (Vol. 20, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12876-020-01390-0
4. Cascella, N. G., Kryszak, D., Bhatti, B., Gregory, P., Kelly, D. L., Mc Evoy, J. P., Fasano, A., & Eaton, W. W. (2009). Prevalence of Celiac Disease and Gluten Sensitivity in the United States Clinical Antipsychotic Trials of Intervention Effectiveness Study Population. In Schizophrenia Bulletin (Vol. 37, Issue 1, pp. 94–100). Oxford University Press (OUP). https://doi.org/10.1093/schbul/sbp055
5. Roszkowska, A., Pawlicka, M., Mroczek, A., Bałabuszek, K., & Nieradko-Iwanicka, B. (2019). Non-Celiac Gluten Sensitivity: A Review. In Medicina (Vol. 55, Issue 6, p. 222). MDPI AG. https://doi.org/10.3390/medicina55060222
6. Leonard, M. M., Sapone, A., Catassi, C., & Fasano, A. (2017). Celiac Disease and Nonceliac Gluten Sensitivity. In JAMA (Vol. 318, Issue 7, p. 647). American Medical Association (AMA). https://doi.org/10.1001/jama.2017.9730
7. Littlejohns, T. J., Chong, A. Y., Allen, N. E., Arnold, M., Bradbury, K. E., Mentzer, A. J., Soilleux, E. J., & Carter, J. L. (2021). Genetic, lifestyle, and health-related characteristics of adults without celiac disease who follow a gluten-free diet: a population-based study of 124,447 participants. In The American Journal of Clinical Nutrition (Vol. 113, Issue 3, pp. 622–629). Elsevier BV. https://doi.org/10.1093/ajcn/nqaa291
8. Littlejohns, T. J., Chong, A. Y., Allen, N. E., Arnold, M., Bradbury, K. E., Mentzer, A. J., Soilleux, E. J., & Carter, J. L. (2021). Genetic, lifestyle, and health-related characteristics of adults without celiac disease who follow a gluten-free diet: a population-based study of 124,447 participants. In The American Journal of Clinical Nutrition (Vol. 113, Issue 3, pp. 622–629). Elsevier BV. https://doi.org/10.1093/ajcn/nqaa291
9. Bouasla, A., & Wójtowicz, A. (2019). Rice-Buckwheat Gluten-Free Pasta: Effect of Processing Parameters on Quality Characteristics and Optimization of Extrusion-Cooking Process. In Foods (Vol. 8, Issue 10, p. 496). MDPI AG. https://doi.org/10.3390/foods8100496
10. Khairuddin, M. A. N., & Lasekan, O. (2021). Gluten-Free Cereal Products and Beverages: A Review of Their Health Benefits in the Last Five Years. In Foods (Vol. 10, Issue 11, p. 2523). MDPI AG. https://doi.org/10.3390/foods10112523
11. Lu, Z., Zhang, H., Luoto, S., & Ren, X. (2018). Gluten-free living in China: The characteristics, food choices and difficulties in following a gluten-free diet – An online survey. In Appetite (Vol. 127, pp. 242–248). Elsevier BV. https://doi.org/10.1016/j.appet.2018.05.007
12. Bascuñán, K. A., Vespa, M. C., & Araya, M. (2016). Celiac disease: understanding the gluten-free diet. In European Journal of Nutrition (Vol. 56, Issue 2, pp. 449–459). Springer Science and Business Media LLC. https://doi.org/10.1007/s00394-016-1238-5
13. Purwandari, U., Khoiri, A., Muchlis, M., Noriandita, B., Zeni, N., Lisdayana, N., Fauziyah, E. (2014). Textural, cooking quality, and sensory evaluation of gluten-free noodle made from breadfruit, konjac, or pumpkin flour. International Food Research Journal, (Vol. 21, pp. 1623-1627).
14. Gasparre, N., & Rosell, C. M. (2019). Role of hydrocolloids in gluten free noodles made with tiger nut flour as non-conventional powder. In Food Hydrocolloids (Vol. 97, p. 105194). Elsevier BV. https://doi.org/10.1016/j.foodhyd.2019.105194
15. Motta Romero, H., Santra, D., Rose, D., & Zhang, Y. (2017). Dough rheological properties and texture of gluten-free pasta based on proso millet flour. In Journal of Cereal Science (Vol. 74, pp. 238–243). Elsevier BV. https://doi.org/10.1016/j.jcs.2017.02.014
16. Padalino, L., Mastromatteo, M., De Vita, P., Maria Ficco, D. B., & Del Nobile, M. A. (2012). Effects of hydrocolloids on chemical properties and cooking quality of gluten-free spaghetti. In International Journal of Food Science & Technology (Vol. 48, Issue 5, pp. 972–983). Wiley. https://doi.org/10.1111/ijfs.12049
17. Chauhan, A., Saxena, D. C., & Singh, S. (2017). Effect of hydrocolloids on microstructure, texture and quality characteristics of gluten-free pasta. In Journal of Food Measurement and Characterization (Vol. 11, Issue 3, pp. 1188–1195). Springer Science and Business Media LLC. https://doi.org/10.1007/s11694-017-9495-4

18. Milde, L. B., Chigal, P. S., Olivera, J. E., & González, K. G. (2020). Incorporation of xanthan gum to gluten-free pasta with cassava starch. Physical, textural and sensory attributes. In LWT (Vol. 131, p. 109674). Elsevier BV. https://doi.org/10.1016/j.lwt.2020.109674
19. Udachan, I., Sahoo, A. (2017). Effect of hydrocolloids in the development of gluten free brown rice pasta. In International Journal of ChemTech Research (Vol. 10, Issue 6, pp. 407-415). CODEN (USA).
20. De Arcangelis, E., Cuomo, F., Trivisonno, M. C., Marconi, E., & Messia, M. C. (2020). Gelatinization and pasta making conditions for buckwheat gluten-free pasta. In Journal of Cereal Science (Vol. 95, p. 103073). Elsevier BV. https://doi.org/10.1016/j.jcs.2020.103073
21. Losano Richard, P., Steffolani, M. E., Barrera, G. N., & León, A. E. (2022). Effect of alternative hydrocolloids in gluten-free chickpea pasta. In International Journal of Food Science & Technology (Vol. 57, Issue 8, pp. 4887–4893). Wiley. https://doi.org/10.1111/ijfs.15905
22. Yazici, G. N., Yilmaz, I., Taspinar, T., & Ozer, M. S. (2023). Application of Inulin in Pasta: The Influence on Technological and Nutritional Properties and on Human Health—A Review. In Foods 2023. Foods 2023. MDPI. https://doi.org/10.3390/foods2023-14967
23. Rout, S., Gupta, R. K., Jadhav, H. B., Srivastav, P. P., & Annapure, U. (2024). Understanding the role of dietary fibers on gluten-free pasta's functional quality aspects. In Development of Gluten-Free Pasta (pp. 157–178). Elsevier. https://doi.org/10.1016/b978-0-443-13238-4.00012-5
24. Melilli, M. G., Buzzanca, C., & Di Stefano, V. (2024). Quality characteristics of cereal-based foods enriched with different degree of polymerization inulin: A review. In Carbohydrate Polymers (Vol. 332, p. 121918). Elsevier BV. https://doi.org/10.1016/j.carbpol.2024.121918
25. Foschia, M., Peressini, D., Sensidoni, A., Brennan, M. A., & Brennan, C. S. (2015). How combinations of dietary fibres can affect physicochemical characteristics of pasta. In LWT - Food Science and Technology (Vol. 61, Issue 1, pp. 41–46). Elsevier BV. https://doi.org/10.1016/j.lwt.2014.11.010
26. Nechaev, A. P., Tsyganova, T. B., Butova, S., Nikolaeva, J. V., Tarasova, V. V., & Smirnov, D. A. (2021). Development of a new generation instant pasta based on gluten-free raw materials and dietary fiber. In IOP Conference Series: Earth and Environmental Science (Vol. 640, Issue 2, p. 022006). IOP Publishing. https://doi.org/10.1088/1755-1315/640/2/022006
27. Llavata, B., Albors, A., & Martin-Esparza, M. E. (2019). High Fibre Gluten-Free Fresh Pasta with Tiger Nut, Chickpea and Fenugreek: Technofunctional, Sensory and Nutritional Properties. In Foods (Vol. 9, Issue 1, p. 11). MDPI AG. https://doi.org/10.3390/foods9010011
28. Agama-Acevedo, E., Bello-Perez, L. A., Pacheco-Vargas, G., Tovar, J., & Sáyago-Ayerdi, S. G. (2019). Unripe plantain flour as a dietary fiber source in gluten-free spaghetti with moderate glycemic index. In Journal of Food Processing and Preservation (Vol. 43, Issue 8). Hindawi Limited. https://doi.org/10.1111/jfpp.14012
29. Krishnan, J. G., Menon, R., Padmaja, G., Sajeev, M. S., & Moorthy, S. N. (2012). Evaluation of nutritional and physico-mechanical characteristics of dietary fiber-enriched sweet potato pasta. In European Food Research and Technology (Vol. 234, Issue 3, pp. 467–476). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-011-1657-8
30. Bangar, S. P., Ashogbon, A. O., Dhull, S. B., Thirumdas, R., Kumar, M., Hasan, M., Chaudhary, V., & Pathem, S. (2021). Proso-millet starch: Properties, functionality, and applications. In International Journal of Biological Macromolecules (Vol. 190, pp. 960–968). Elsevier BV. https://doi.org/10.1016/j.ijbiomac.2021.09.064
31. Witczak, M., Ziobro, R., Juszczak, L., & Korus, J. (2016). Starch and starch derivatives in gluten-free systems – A review. In Journal of Cereal Science (Vol. 67, pp. 46–57). Elsevier BV. https://doi.org/10.1016/j.jcs.2015.07.007
32. Padalino, L., Conte, A., & Del Nobile, M. (2016). Overview on the General Approaches to Improve Gluten-Free Pasta and Bread. In Foods (Vol. 5, Issue 4, p. 87). MDPI AG. https://doi.org/10.3390/foods5040087
33. Vieira, A., Silva, A., Albuquerque, A., Almeida, R., Rodrigues, T., Silva, L., Duarte, M., Cavalcanti-Mata, M., & Rocha, A. (2021). Effects of long-term frozen storage on the quality and acceptance of gluten-free cassava pasta. In Heliyon (Vol. 7, Issue 8, p. e07844). Elsevier BV. https://doi.org/10.1016/j.heliyon.2021.e07844
34. Foschia, M., Beraldo, P., & Peressini, D. (2016). Evaluation of the physicochemical properties of gluten-free pasta enriched with resistant starch. In Journal of the Science of Food and Agriculture (Vol. 97, Issue 2, pp. 572–577). Wiley. https://doi.org/10.1002/jsfa.7766
35. Sonia, S., Julianti, E., & Ridwansyah, R. (2020). The Characteristic of Taro Flour Based Pasta with Addition of Modified Starch and Hydrocolloids. In Indonesian Food and Nutrition Progress (Vol. 16, Issue 1, p. 27). Universitas Gadjah Mada. https://doi.org/10.22146/ifnp.45681

36. Cervini, M., Gruppi, A., Bassani, A., Spigno, G., & Giuberti, G. (2021). Potential Application of Resistant Starch Sorghum in Gluten-Free Pasta: Nutritional, Structural and Sensory Evaluations. In Foods (Vol. 10, Issue 5, p. 908). MDPI AG. https://doi.org/10.3390/foods10050908

37. Giuberti, G., Gallo, A., Cerioli, C., Fortunati, P., & Masoero, F. (2015). Cooking quality and starch digestibility of gluten free pasta using new bean flour. In Food Chemistry (Vol. 175, pp. 43–49). Elsevier BV. https://doi.org/10.1016/j.foodchem.2014.11.127

38. Sarawong, C., Rodríguez Gutiérrez, Z. C., Berghofer, E., & Schoenlechner, R. (2014). Gluten-free pasta: effect of green plantain flour addition and influence of starch modification on the functional properties and resistant starch content. In International Journal of Food Science & Technology (Vol. 49, Issue 12, pp. 2650–2658). Wiley. https://doi.org/10.1111/ijfs.12599

39. Ferreira, S. M. R., de Mello, A. P., de Caldas Rosa dos Anjos, M., Krüger, C. C. H., Azoubel, P. M., & de Oliveira Alves, M. A. (2016). Utilization of sorghum, rice, corn flours with potato starch for the preparation of gluten-free pasta. In Food Chemistry (Vol. 191, pp. 147–151). Elsevier BV. https://doi.org/10.1016/j.foodchem.2015.04.085

40. Mohammadi, M., Zoghi, A., & Mirmahdi, R. S. (2021). Impact of enzymes in development of gluten-free cereal-based products. In Journal of Food Processing and Preservation (Vol. 46, Issue 5). Hindawi Limited. https://doi.org/10.1111/jfpp.15295

41. Ramos, N. J. da S., Rocha, E. B. M., Gusmão, T. A. S., Nascimento, A., Lisboa, H. M., & de Gusmão, R. P. (2023). Optimizing gluten-free pasta quality: The impacts of transglutaminase concentration and kneading time on cooking properties, nutritional value, and rheological characteristics. In LWT (Vol. 189, p. 115485). Elsevier BV. https://doi.org/10.1016/j.lwt.2023.115485

42. Yalcin, S., & Basman, A. (2008). Quality characteristics of corn noodles containing gelatinized starch, transglutaminase and gum. In Journal of Food Quality (Vol. 31, Issue 4, pp. 465–479). Wiley. https://doi.org/10.1111/j.1745-4557.2008.00212.x

43. Linares-García, L., Repo-Carrasco-Valencia, R., Glorio Paulet, P., & Schoenlechner, R. (2019). Development of gluten-free and egg-free pasta based on quinoa (Chenopdium quinoa Willd) with addition of lupine flour, vegetable proteins and the oxidizing enzyme POx. In European Food Research and Technology (Vol. 245, Issue 10, pp. 2147–2156). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-019-03320-1

44. Takács, K., Némedi, E., Márta, D., Gelencsér, É., & Kovács, E. (2007). Use of the enzyme transglutaminase for developing glutenfree noodle products from pea flour. In Acta Alimentaria (Vol. 36, Issue 2, pp. 195–205). Akademiai Kiado Zrt. https://doi.org/10.1556/aalim.2007.0008

45. Campos, B. V. P., & Almeida, E. L. (2021). Gluten-free pasta elaborated with taro flour (Colocasia esculenta): a study of the employ of egg white and transglutaminase on the technological properties. In Research, Society and Development (Vol. 10, Issue 1, p. e52710111454). Research, Society and Development. https://doi.org/10.33448/rsd-v10i1.11454

46. Manoj Kumar, C. T., Sabikhi, L., Singh, A. K., Raju, P. N., Kumar, R., & Sharma, R. (2019). Effect of incorporation of sodium caseinate, whey protein concentrate and transglutaminase on the properties of depigmented pearl millet based gluten free pasta. In LWT (Vol. 103, pp. 19–26). Elsevier BV. https://doi.org/10.1016/j.lwt.2018.12.071

47. Kim, A.-N., Rahman, M. S., Lee, K.-Y., & Choi, S.-G. (2021). Superheated steam pretreatment of rice flours: Gelatinization behavior and functional properties during thermal treatment. In Food Bioscience (Vol. 41, p. 101013). Elsevier BV. https://doi.org/10.1016/j.fbio.2021.101013

48. Dib, A., Wójtowicz, A., Benatallah, L., Zidoune, M. N., Mitrus, M., Sujak, A. (2018). Optimization of rice-field bean gluten-free pasta improved by the addition of hydrothermally treated rice flour: Optimization of rice-field bean pasta [JB]. Italian Journal of Food Science, 30(2). https://doi.org/10.14674/IJFS-923

49. Rudra, S. G., Anand, V., Kaur, C., Bhooshan, N., & Bhardwaj, R. (2020). Hydrothermal Treatment to Improve Processing Characteristics of Flour for Gluten-Free Pasta. In Starch - Stärke (Vol. 72, Issues 9–10). Wiley. https://doi.org/10.1002/star.201900320

50. Gandhi, H., Toor, B. S., Kaur, A., & Kaur, J. (2022). Effect of processing treatments on physicochemical, functional and thermal characteristics of lentils (Lens Culinaris). In Journal of Food Measurement and Characterization (Vol. 16, Issue 6, pp. 4603–4614). Springer Science and Business Media LLC. https://doi.org/10.1007/s11694-022-01549-1

51. Marti, A., Caramanico, R., Bottega, G., & Pagani, M. A. (2013). Cooking behavior of rice pasta: Effect of thermal treatments and extrusion conditions. In LWT - Food Science and Technology (Vol. 54, Issue 1, pp. 229–235). Elsevier BV. https://doi.org/10.1016/j.lwt.2013.05.008

52. Cabrera-Chávez, F., Calderón de la Barca, A. M., Islas-Rubio, A. R., Marti, A., Marengo, M., Pagani, M. A., Bonomi, F., & Iametti, S. (2012). Molecular rearrangements in extrusion processes for the production of amaranth-enriched, gluten-free rice pasta. In LWT (Vol. 47, Issue 2, pp. 421–426). Elsevier BV. https://doi.org/10.1016/j.lwt.2012.01.040

53. Bouasla, A., Wójtowicz, A., & Zidoune, M. N. (2017). Gluten-free precooked rice pasta enriched with legumes flours: Physical properties, texture, sensory attributes and microstructure. In LWT (Vol. 75, pp. 569–577). Elsevier BV. https://doi.org/10.1016/j.lwt.2016.10.005

54. Sabbatini, S. B. (2014). Design of a Premix for Making Gluten Free Noodles. In International Journal of Nutrition and Food Sciences (Vol. 3, Issue 5, p. 488). Science Publishing Group. https://doi.org/10.11648/j.ijnfs.20140305.29

55. Baah, R. O., Duodu, K. G., & Emmambux, M. N. (2022). Cooking quality, nutritional and antioxidant properties of gluten-free maize – Orange-fleshed sweet potato pasta produced by extrusion. In LWT (Vol. 162, p. 113415). Elsevier BV. https://doi.org/10.1016/j.lwt.2022.113415

56. Silva, E. M., Ascheri, J. L., Ascheri, D. P. (2016). Quality assessment of gluten-free pasta prepared with a brown rice and corn meal blend via thermoplastic extrusion. LWT Food Science & Technology, (Vol. 68, pp. 698-706). https://doi.org/10.1016/j.lwt.2015.12.067

57. Fiorda, F. A., Soares Júnior, M. S., da Silva, F. A., Souto, L. R. F., & Grosmann, M. V. E. (2013). Amaranth flour, cassava starch and cassava bagasse in the production of gluten-free pasta: technological and sensory aspects. In International Journal of Food Science & Technology (Vol. 48, Issue 9, pp. 1977–1984). Wiley. https://doi.org/10.1111/ijfs.12179

58. Bento, J. A. C., Bassinello, P. Z., Morais, D. K., Souza Neto, M. A. de, Bataus, L. A. M., Carvalho, R. N., Caliari, M., & Soares Júnior, M. S. (2021). Pre-gelatinized flours of black and carioca bean by-products: Development of gluten-free instant pasta and baked snacks. In International Journal of Gastronomy and Food Science (Vol. 25, p. 100383). Elsevier BV. https://doi.org/10.1016/j.ijgfs.2021.100383

59. Sofi, S. A., Singh, J., Mir, S. A., & Dar, B. N. (2020). In vitro starch digestibility, cooking quality, rheology and sensory properties of gluten-free pregelatinized rice noodle enriched with germinated chickpea flour. In LWT (Vol. 133, p. 110090). Elsevier BV. https://doi.org/10.1016/j.lwt.2020.110090

60. Zavareze, E. da R., Storck, C. R., de Castro, L. A. S., Schirmer, M. A., & Dias, A. R. G. (2010). Effect of heat-moisture treatment on rice starch of varying amylose content. In Food Chemistry (Vol. 121, Issue 2, pp. 358–365). Elsevier BV. https://doi.org/10.1016/j.foodchem.2009.12.036

61. Wang, L., Zhang, C., Chen, Z., Wang, X., Wang, K., Li, Y., Wang, R., Luo, X., Li, Y., & Li, J. (2018). Effect of annealing on the physico-chemical properties of rice starch and the quality of rice noodles. In Journal of Cereal Science (Vol. 84, pp. 125–131). Elsevier BV. https://doi.org/10.1016/j.jcs.2018.10.004

62. Gu, Y., Qian, X., Sun, B., Wang, X., & Ma, S. (2023). Effects of gelatinization degree and boiling water kneading on the rheology characteristics of gluten-free oat dough. In Food Chemistry (Vol. 404, p. 134715). Elsevier BV. https://doi.org/10.1016/j.foodchem.2022.134715

63. Laleg, K., Cassan, D., Abecassis, J., & Micard, V. (2021). Processing a 100% legume pasta in a classical extruder without agglomeration during mixing. In Journal of Food Science (Vol. 86, Issue 3, pp. 724–729). Wiley. https://doi.org/10.1111/1750-3841.15604

64. Ahmed, M. W., Jothi, J. S., Saifullah, M., Hannan, Md. A., & Mohibbullah, Md. (2024). Impact of drying temperature on textural, cooking quality, and microstructure of gluten-free pasta. In Development of Gluten-Free Pasta (pp. 65–110). Elsevier. https://doi.org/10.1016/b978-0-443-13238-4.00010-1

65. D'Amico, S., Mäschle, J., Jekle, M., Tömösközi, S., Langó, B., & Schoenlechner, R. (2015). Effect of high temperature drying on gluten-free pasta properties. In LWT - Food Science and Technology (Vol. 63, Issue 1, pp. 391–399). Elsevier BV. https://doi.org/10.1016/j.lwt.2015.03.080

66. Ramírez, M., Tenorio, M., Ramírez, C., Jaques, A., Nuñez, H., Simpson, R., & Vega, O. (2019). Optimization of hot-air drying conditions for cassava flour for its application in gluten-free pasta formulation. In Food Science and Technology International (Vol. 25, Issue 5, pp. 414–428). SAGE Publications. https://doi.org/10.1177/1082013219828269

67. The Amaranth Genome. (2021). In D. Adhikary, M. K. Deyholos, & J. P. Délano-Frier (Eds.), Compendium of Plant Genomes. Springer International Publishing. https://doi.org/10.1007/978-3-030-72365-1

68. Sattar, M., Saeed, F., Afzaal, M., Rasheed, A., Asif, A., Sharif, S., Hussain, M., Asad Ur Rehman, H., Raza, M. A., Munir, H., & Al Jbawi, E. (2024). An overview of the nutritional and therapeutic properties of amaranth. In International Journal of Food Properties (Vol. 27, Issue 1, pp. 263–272). Informa UK Limited. https://doi.org/10.1080/10942912.2024.2304266

69. Baraniak, J., & Kania-Dobrowolska, M. (2022). The Dual Nature of Amaranth—Functional Food and Potential Medicine. In Foods (Vol. 11, Issue 4, p. 618). MDPI AG. https://doi.org/10.3390/foods11040618

70. Zhang, X., Shi, J., Fu, Y., Zhang, T., Jiang, L., & Sui, X. (2023). Structural, nutritional, and functional properties of amaranth protein and its application in the food industry: A review. In Sustainable Food Proteins (Vol. 1, Issue 1, pp. 45–55). Wiley. https://doi.org/10.1002/sfp2.1002

71. Mystkowska, I., Plażuk, E., Szepeluk, A., & Dmitrowicz, A. (2024). Gluten-containing flours and gluten-free flours as a source of calcium, magnesium, iron and zinc. In Scientific Reports (Vol. 14, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-024-65530-2

72. Zhu, F. (2023). Amaranth proteins and peptides: Biological properties and food uses. In Food Research International (Vol. 164, p. 112405). Elsevier BV. https://doi.org/10.1016/j.foodres.2022.112405

73. Zhang, X., Shi, J., Fu, Y., Zhang, T., Jiang, L., & Sui, X. (2023). Structural, nutritional, and functional properties of amaranth protein and its application in the food industry: A review. In Sustainable Food Proteins (Vol. 1, Issue 1, pp. 45–55). Wiley. https://doi.org/10.1002/sfp2.1002

74. Lux (née Bantleon), T., Spillmann, F., Reimold, F., Erdös, A., Lochny, A., & Flöter, E. (2023). Physical quality of gluten-free doughs and fresh pasta made of amaranth. In Food Science & Nutrition (Vol. 11, Issue 6, pp. 3213–3223). Wiley. https://doi.org/10.1002/fsn3.3301

75. Cárdenas-Hernández, A., Beta, T., Loarca-Piña, G., Castaño-Tostado, E., Nieto-Barrera, J. O., & Mendoza, S. (2016). Improved functional properties of pasta: Enrichment with amaranth seed flour and dried amaranth leaves. In Journal of Cereal Science (Vol. 72, pp. 84–90). Elsevier BV. https://doi.org/10.1016/j.jcs.2016.09.014

76. Lawal, O. M., van Stuijvenberg, L., Boon, N., Awolu, O., Fogliano, V., & Linnemann, A. R. (2021). Technological and nutritional properties of amaranth-fortified yellow cassava pasta. In Journal of Food Science (Vol. 86, Issue 12, pp. 5213–5225). Wiley. https://doi.org/10.1111/1750-3841.15975

77. Schoenlechner, R., Drausinger, J., Ottenschlaeger, V., Jurackova, K., & Berghofer, E. (2010). Functional Properties of Gluten-Free Pasta Produced from Amaranth, Quinoa and Buckwheat. In Plant Foods for Human Nutrition (Vol. 65, Issue 4, pp. 339–349). Springer Science and Business Media LLC. https://doi.org/10.1007/s11130-010-0194-0

78. Pirzadah, T. B., & Rehman, R. U. (2021). Buckwheat: Forgotten Crop for the Future. CRC Press. https://doi.org/10.1201/9781003089100

79. Huda, Md. N., Lu, S., Jahan, T., Ding, M., Jha, R., Zhang, K., Zhang, W., Georgiev, M. I., Park, S. U., & Zhou, M. (2021). Treasure from garden: Bioactive compounds of buckwheat. In Food Chemistry (Vol. 335, p. 127653). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.127653

80. Jha, R., Zhang, K., He, Y., Mendler-Drienyovszki, N., Magyar-Tábori, K., Quinet, M., Germ, M., Kreft, I., Meglič, V., Ikeda, K., Chapman, M. A., Janovská, D., Podolska, G., Woo, S.-H., Bruno, S., Georgiev, M. I., Chrungoo, N., Betekhtin, A., & Zhou, M. (2024). Global nutritional challenges and opportunities: Buckwheat, a potential bridge between nutrient deficiency and food security. In Trends in Food Science & Technology (Vol. 145, p. 104365). Elsevier BV. https://doi.org/10.1016/j.tifs.2024.104365

81. Jin, J., Ohanenye, I. C., & Udenigwe, C. C. (2020). Buckwheat proteins: functionality, safety, bioactivity, and prospects as alternative plant-based proteins in the food industry. In Critical Reviews in Food Science and Nutrition (Vol. 62, Issue 7, pp. 1752–1764). Informa UK Limited. https://doi.org/10.1080/10408398.2020.1847027

82. Oniszczuk, A., Kasprzak, K., Wójtowicz, A., Oniszczuk, T., & Olech, M. (2019). The Impact of Processing Parameters on the Content of Phenolic Compounds in New Gluten-Free Precooked Buckwheat Pasta. In Molecules (Vol. 24, Issue 7, p. 1262). MDPI AG. https://doi.org/10.3390/molecules24071262

83. Škrobot, D., Pezo, L., Tomić, J., Pestorić, M., Sakač, M., & Mandić, A. (2022). Insights into sensory and hedonic perception of wholegrain buckwheat enriched pasta. In LWT (Vol. 153, p. 112528). Elsevier BV. https://doi.org/10.1016/j.lwt.2021.112528

84. Rosa, C. S., Prestes, R. C., Tessele, K., Crauss, M. (2015). Influence of the different addition levels of amaranth flour and rice flour on pasta buckwheat flour. International Food Reserch Journal, (Vol. 22, pp. 691-698).

85. Sujka, K., Cacak-Pietrzak, G., Sułek, A., Murgrabia, K., & Dziki, D. (2022). Buckwheat Hull-Enriched Pasta: Physicochemical and Sensory Properties. In Molecules (Vol. 27, Issue 13, p. 4065). MDPI AG. https://doi.org/10.3390/molecules27134065

86. Biró, B., Fodor, R., Szedljak, I., Pásztor-Huszár, K., & Gere, A. (2019). Buckwheat-pasta enriched with silkworm powder: Technological analysis and sensory evaluation. In LWT (Vol. 116, p. 108542). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.108542

87. Alamprese, C., Casiraghi, E., & Pagani, M. A. (2006). Development of gluten-free fresh egg pasta analogues containing buckwheat. In European Food Research and Technology (Vol. 225, Issue 2, pp. 205–213). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-006-0405-y

88. Wang, J., Li, Y., Li, A., Liu, R. H., Gao, X., Li, D., Kou, X., & Xue, Z. (2021). Nutritional constituent and health benefits of chickpea (Cicer arietinum L.): A review. In Food Research International (Vol. 150, p. 110790). Elsevier BV. https://doi.org/10.1016/j.foodres.2021.110790

89. Padalino, L., Mastromatteo, M., Lecce, L., Spinelli, S., Conte, A., & Alessandro Del Nobile, M. (2014). Optimization and characterization of gluten-free spaghetti enriched with chickpea flour. In International Journal of Food Sciences and Nutrition (Vol. 66, Issue 2, pp. 148–158). Informa UK Limited. https://doi.org/10.3109/09637486.2014.959897

90. Güngörmüşler, M., Başınhan, İ., & Üçtuğ, F. G. (2020). Optimum formulation determination and carbon footprint analysis of a novel gluten-free pasta recipe using buckwheat, teff, and chickpea flours. In Journal of Food Processing and Preservation (Vol. 44, Issue 9). Hindawi Limited. https://doi.org/10.1111/jfpp.14701

91. de Lima, B. R., Botelho, R. B. A., & Zandonadi, R. P. (2017). Gluten-Free Pasta: Replacing Wheat with Chickpea. In Journal of Culinary Science & Technology (Vol. 17, Issue 1, pp. 1–8). Informa UK Limited. https://doi.org/10.1080/15428052.2017.1394952

92. Flores-Silva, P. C., Berrios, J. D. J., Pan, J., Osorio-Díaz, P., & Bello-Pérez, L. A. (2014). Gluten-free spaghetti made with chickpea, unripe plantain and maize flours: functional and chemical properties and starch digestibility. In International Journal of Food Science & Technology (Vol. 49, Issue 9, pp. 1985–1991). Wiley. https://doi.org/10.1111/ijfs.12529

93. Singh, G., Singh, B., Singh, A., Kumar, V., & Surasani, V. K. R. (2023). Development and characterisation of barley-based non-conventional pasta supplemented with chickpea flour. In International Journal of Food Science & Technology (Vol. 59, Issue 2, pp. 1104–1112). Wiley. https://doi.org/10.1111/ijfs.16553

94. Costantini, M., Summo, C., Faccia, M., Caponio, F., & Pasqualone, A. (2021). Kabuli and Apulian black Chickpea Milling By-Products as Innovative Ingredients to Provide High Levels of Dietary Fibre and Bioactive Compounds in Gluten-Free Fresh Pasta. In Molecules (Vol. 26, Issue 15, p. 4442). MDPI AG. https://doi.org/10.3390/molecules26154442

95. El-Sohaimy, S. A., Brennan, M., Darwish, A. M. G., & Brennan, C. (2020). Physicochemical, texture and sensorial evaluation of pasta enriched with chickpea flour and protein isolate. In Annals of Agricultural Sciences (Vol. 65, Issue 1, pp. 28–34). Elsevier BV. https://doi.org/10.1016/j.aoas.2020.05.005

96. Kulczyński, B., Kobus-Cisowska, J., Taczanowski, M., Kmiecik, D., & Gramza-Michałowska, A. (2019). The Chemical Composition and Nutritional Value of Chia Seeds—Current State of Knowledge. In Nutrients (Vol. 11, Issue 6, p. 1242). MDPI AG. https://doi.org/10.3390/nu11061242

97. Aranibar, C., Pigni, N. B., Martinez, M., Aguirre, A., Ribotta, P., Wunderlin, D., & Borneo, R. (2018). Utilization of a partially-deoiled chia flour to improve the nutritional and antioxidant properties of wheat pasta. In LWT (Vol. 89, pp. 381–387). Elsevier BV. https://doi.org/10.1016/j.lwt.2017.11.003

98. Menga, V., Amato, M., Phillips, T. D., Angelino, D., Morreale, F., & Fares, C. (2017). Gluten-free pasta incorporating chia (Salvia hispanica L.) as thickening agent: An approach to naturally improve the nutritional profile and the in vitro carbohydrate digestibility. In Food Chemistry (Vol. 221, pp. 1954–1961). Elsevier BV. https://doi.org/10.1016/j.foodchem.2016.11.151

99. Levent, H. (2017). Effect of partial substitution of gluten-free flour mixtures with chia (Salvia hispanica L.) flour on quality of gluten-free noodles. In Journal of Food Science and Technology (Vol. 54, Issue 7, pp. 1971–1978). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-017-2633-5

100. Khatri, M., Singh, A., Singh, R., Kamble, D. B., Dar, A. H., & Sharma, A. (2023). Optimization and evaluation of quinoa and chia based gluten free pasta formulation. In Food and Humanity (Vol. 1, pp. 174–179). Elsevier BV. https://doi.org/10.1016/j.foohum.2023.05.009

101. Kumar, D., Jhariya, N. A. (2013). Nutritional, medicinal and economical importance of corn: A mini review. Journal of Pharmaceutical Sciences, (Vol. 2, pp. 1-6).

102. Mohammed, A. A. B. A., Hasan, Z., Omran, A. A. B., Kumar, V. V., Elfaghi, A. M., Ilyas, R. A., & Sapuan, S. M. (2022). Corn: Its Structure, Polymer, Fiber, Composite, Properties, and Applications. In Polymers (Vol. 14, Issue 20, p. 4396). MDPI AG. https://doi.org/10.3390/polym14204396

103. Bresciani, A., Pagani, M. A., & Marti, A. (2022). Pasta-Making Process: A Narrative Review on the Relation between Process Variables and Pasta Quality. In Foods (Vol. 11, Issue 3, p. 256). MDPI AG. https://doi.org/10.3390/foods11030256

104. Milde Laura, B., Chigal Paola, S., Chiola Zayas María, O. (2018). Nutritional characterization of gluten free non-traditional pasta. International Journal Food Science & Nutrition, (Vol. 3, pp. 19-24).

105. Giménez, M. A., Drago, S. R., Bassett, M. N., Lobo, M. O., Sammán, N. C. (2016). Nutritional improvement of corn pasta-like product with broad bean (Vicia faba) and quinoa (Chenopodium quinoa). Food Chemistry, (Vol. 199, pp. 150-6). https://doi.org/10.1016/j.foodchem.2015.11.065

106. Ungureanu-Iuga, M., Dimian, M., Mironeasa, S. (2020). Development and quality evaluation of gluten-free pasta with grape peels and whey powders. LWT Food Science & Technology, (Vol. 130, p. 109714). https://doi.org/10.1016/j.lwt.2020.109714

107. Mirhosseini, H., Abdul Rashid, N. F., Tabatabaee Amid, B., Cheong, K. W., Kazemi, M., & Zulkurnain, M. (2015). Effect of partial replacement of corn flour with durian seed flour and pumpkin flour on cooking yield, texture properties, and sensory attributes of gluten free pasta. In LWT - Food Science and Technology (Vol. 63, Issue 1, pp. 184–190). Elsevier BV. https://doi.org/10.1016/j.lwt.2015.03.078

108. Larrosa, V.J., Lorenzo, G., Zaritzky, N.E., Califano, A.N. (2015.) Dynamic rheological analysis of gluten-free pasta as affected by composition and cooking time. Journal of Food Engineering, (Vol. 160, pp. 11-18). https://doi.org/10.1016/j.lwt.2020.109714

109. Yulianti, L. E., Sholichah, E., & Indrianti, N. (2019). Addition of Tempeh Flour as a Protein Source in Mixed Flour (Mocaf, Rice, and Corn) for Pasta Product. In IOP Conference Series: Earth and Environmental Science (Vol. 251, p. 012037). IOP Publishing. https://doi.org/10.1088/1755-1315/251/1/012037

110. El-Bialee, N., Saad, A. M., El-Didamony, M. I., & Zahran, G. (2017). Influence of Extrusion-Cooking Conditions on Corn Pasta Quality. In European Journal of Engineering Research and Science (Vol. 2, Issue 3, p. 24). European Open Access Publishing (Europa Publishing). https://doi.org/10.24018/ejers.2017.2.3.30

111. Dib, A., Wójtowicz, A., Benatallah, L., Bouasla, A., & Zidoune, M. N. (2018). Effect of hydrothermal treated corn flour addition on the quality of corn-field bean gluten-free pasta. In A. Szeląg-Sikora (Ed.), BIO Web of Conferences (Vol. 10, p. 02003). EDP Sciences. https://doi.org/10.1051/bioconf/20181002003

112. Camelo-Méndez, G. A., Tovar, J., & Bello-Pérez, L. A. (2018). Influence of blue maize flour on gluten-free pasta quality and antioxidant retention characteristics. In Journal of Food Science and Technology (Vol. 55, Issue 7, pp. 2739–2748). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-018-3196-9

113. Padalino, L., Mastromatteo, M., Sepielli, G., Nobile, M. A. D. (2011). Formulation Optimization of Gluten-Free Functional Spaghetti Based on Maize Flour and Oat Bran Enriched in b-Glucans. Materials, (Vol. 4, pp. 2119-2135). https://doi.org/10.3390/ma4122119

114. Bongianino, N. F., Steffolani, M. E., Morales, C. D., Biasutti, C. A., & León, A. E. (2023). Technological and Sensory Quality of Gluten-Free Pasta Made from Flint Maize Cultivars. In Foods (Vol. 12, Issue 14, p. 2780). MDPI AG. https://doi.org/10.3390/foods12142780

115. Bresciani, A., Pagani, M. A., & Marti, A. (2022). Pasta-Making Process: A Narrative Review on the Relation between Process Variables and Pasta Quality. In Foods (Vol. 11, Issue 3, p. 256). MDPI AG. https://doi.org/10.3390/foods11030256

116. Bolarinwa, I. F., & Oyesiji, O. O. (2021). Gluten free rice-soy pasta: proximate composition, textural properties and sensory attributes. In Heliyon (Vol. 7, Issue 1, p. e06052). Elsevier BV. https://doi.org/10.1016/j.heliyon.2021.e06052

117. Phongthai, S., D'Amico, S., Schoenlechner, R., Homthawornchoo, W., & Rawdkuen, S. (2017). Effects of protein enrichaent on the properties of rice flour based gluten-free pasta. In LWT (Vol. 80, pp. 378–385). Elsevier BV. https://doi.org/10.1016/j.lwt.2017.02.044

118. Sereewat, P., Suthipinittham, C., Sumathaluk, S., Puttanlek, C., Uttapap, D., & Rungsardthong, V. (2015). Cooking properties and sensory acceptability of spaghetti made from rice flour and defatted soy flour. In LWT - Food Science and Technology (Vol. 60, Issue 2, pp. 1061–1067). Elsevier BV. https://doi.org/10.1016/j.lwt.2014.10.001

119. Fradinho, P., Sousa, I., & Raymundo, A. (2018). Functional and thermorheological properties of rice flour gels for gluten-free pasta applications. In International Journal of Food Science & Technology (Vol. 54, Issue 4, pp. 1109–1120). Wiley. https://doi.org/10.1111/ijfs.14001

120. Marana, A. I. S., Morris, A., Prinyawiwatkul, W., Xu, Z., & King, J. M. (2023). High-protein rice flour in the development of gluten-free pasta. In Journal of Food Science (Vol. 88, Issue 4, pp. 1268–1279). Wiley. https://doi.org/10.1111/1750-3841.16522

121. Ribeiro, T. H. S., Bolanho, B. C., Montanuci, F. D., & Ruiz, S. P. (2018). Physicochemical and sensory characterization of gluten-free fresh pasta with addition of passion fruit peel flour. In Ciência Rural (Vol. 48, Issue 12). FapUNIFESP (SciELO). https://doi.org/10.1590/0103-8478cr20180508

122. Ertas, N. M., Aslan, M., Çevik, A. (2022). Improvement of Structural and Nutritional Quality of Gluten Free Pasta. Journal of Culinary Science & Technology, (Vol. 21, pp. 867-885). https://doi.org/10.1080/15428052.2021.2020198

123. Gulzar, B., Hussain, S. Z., Naseer, B., & Naik, H. R. (2021). Enhancement of resistant starch content in modified rice flour using extrusion technology. In Cereal Chemistry (Vol. 98, Issue 3, pp. 634–641). Wiley. https://doi.org/10.1002/cche.10407

124. Hagenimana, A., Ding, X., & Fang, T. (2006). Evaluation of rice flour modified by extrusion cooking. In Journal of Cereal Science (Vol. 43, Issue 1, pp. 38–46). Elsevier BV. https://doi.org/10.1016/j.jcs.2005.09.003

125. Guha, M., & Ali, S. Z. (2011). Changes in rheological properties of rice flour during extrusion cooking. In Journal of Texture Studies (Vol. 42, Issue 6, pp. 451–458). Wiley. https://doi.org/10.1111/j.1745-4603.2011.00306.x

126. Craine, E. B., & Murphy, K. M. (2020). Seed Composition and Amino Acid Profiles for Quinoa Grown in Washington State. In Frontiers in Nutrition (Vol. 7). Frontiers Media SA. https://doi.org/10.3389/fnut.2020.00126

127. Dakhili, S., Abdolalizadeh, L., Hosseini, S. M., Shojaee-Aliabadi, S., & Mirmoghtadaie, L. (2019). Quinoa protein: Composition, structure and functional properties. In Food Chemistry (Vol. 299, p. 125161). Elsevier BV. https://doi.org/10.1016/j.foodchem.2019.125161

128. Demir, B., & Bilgiçli, N. (2020). Changes in chemical and anti-nutritional properties of pasta enriched with raw and germinated quinoa (Chenopodium quinoa Willd.) flours. In Journal of Food Science and Technology (Vol. 57, Issue 10, pp. 3884–3892). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-020-04420-7

129. Demir, B., & Bilgiçli, N. (2020). Utilization of quinoa flour (Chenopodium quinoaWilld.) in gluten-free pasta formulation: Effects on nutritional and sensory properties. In Food Science and Technology International (Vol. 27, Issue 3, pp. 242–250). SAGE Publications. https://doi.org/10.1177/1082013220940092

130. Torres, O. L., Lema, M., & Galeano, Y. V. (2021). Effect of Using Quinoa Flour (Chenopodium quinoa Willd.) on the Physicochemical Characteristics of an Extruded Pasta. In D. Suhandy (Ed.), International Journal of Food Science (Vol. 2021, pp. 1–8). Hindawi Limited. https://doi.org/10.1155/2021/8813354

131. Torres Vargas, O. L., Lema González, M., & Galeano Loaiza, Y. V. (2021). Optimization study of pasta extruded with quinoa flour (Chenopodium quinoa willd). In CyTA - Journal of Food (Vol. 19, Issue 1, pp. 220–227). Informa UK Limited. https://doi.org/10.1080/19476337.2021.1883116

132. Itusaca-Maldonado, Y. M., Apaza-Humerez, C. R., Pumacahua-Ramos, A., & Mayta Pinto, E. (2024). Technological and textural properties of gluten-free quinoa-based pasta (Chenopodium quinoa Wild). In Heliyon (Vol. 10, Issue 7, p. e28363). Elsevier BV. https://doi.org/10.1016/j.heliyon.2024.e28363

133. Córdoba-Cerón, D. M., Bravo-Gómez, J. E., Agudelo-Laverde, L. M., Roa-Acosta, D. F., & Nieto-Calvache, J. E. (2023). Techno-functional properties of gluten-free pasta from hyperprotein quinoa flour. In Heliyon (Vol. 9, Issue 8, p. e18539). Elsevier BV. https://doi.org/10.1016/j.heliyon.2023.e18539

134. Sosa, M., Califano, A., & Lorenzo, G. (2018). Influence of quinoa and zein content on the structural, rheological, and textural properties of gluten-free pasta. In European Food Research and Technology (Vol. 245, Issue 2, pp. 343–353). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-018-3166-5

135. Córdoba-Cerón, D. M., Carranza-Saavedra, D., Roa-Acosta, D. F., Hoyos-Concha, J. L., & Solanilla-Duque, J. F. (2022). Physical and culinary analysis of long gluten-free extruded pasta based on high protein quinoa flour. In Frontiers in Sustainable Food Systems (Vol. 6). Frontiers Media SA. https://doi.org/10.3389/fsufs.2022.1017324

136. Khan, A., Khan, N. A., Bean, S. R., Chen, J., Xin, Z., & Jiao, Y. (2023). Variations in Total Protein and Amino Acids in the Sequenced Sorghum Mutant Library. In Plants (Vol. 12, Issue 8, p. 1662). MDPI AG. https://doi.org/10.3390/plants12081662

137. S., I. M., Bean, S. R., loerger, B. P., Hayes, C., Emendack, Y., & Jagadish S. V., K. (2023). Comparative assessment of grain quality in tannin versus non-tannin sorghums in the sorghum association panel. In Cereal Chemistry (Vol. 100, Issue 3, pp. 663–674). Wiley. https://doi.org/10.1002/cche.10643

138. Palavecino, P. M., Ribotta, P. D., León, A. E., & Bustos, M. C. (2018). Gluten-free sorghum pasta: starch digestibility and antioxidant capacity compared with commercial products. In Journal of the Science of Food and Agriculture (Vol. 99, Issue 3, pp. 1351–1357). Wiley. https://doi.org/10.1002/jsfa.9310

139. de Oliveira, L. de L., de Orlandin, L. C., de Aguiar, L. A., Queiroz, V. A. V., Zandonadi, R. P., Botelho, R. B. A., & de Alencar Figueiredo, L. F. (2022). Gluten-Free Sorghum Pasta: Composition and Sensory Evaluation with Different Sorghum Hybrids. In Foods (Vol. 11, Issue 19, p. 3124). MDPI AG. https://doi.org/10.3390/foods11193124

140. Palavecino, P. M., Bustos, M. C., Heinzmann Alabí, M. B., Nicolazzi, M. S., Penci, M. C., & Ribotta, P. D. (2017). Effect of Ingredients on the Quality of Gluten-Free Sorghum Pasta. In Journal of Food Science (Vol. 82, Issue 9, pp. 2085–2093). Wiley. https://doi.org/10.1111/1750-3841.13821

141. Susanna, S., & Prabhasankar, P. (2013). A study on development of Gluten free pasta and its biochemical and immunological validation. In LWT - Food Science and Technology (Vol. 50, Issue 2, pp. 613–621). Elsevier BV. https://doi.org/10.1016/j.lwt.2012.07.040

142. Omaraliyeva, A., Botbayeva, Z., Agedilova, M., Abilova, M., Nurtayeva, A., & Baishugulova, S. (2022). Development of the recipe composition of gluten-free flour confectionery products based on chickpea flour. In Eastern-European Journal of Enterprise Technologies (Vol. 6, Issue 11 (120), pp. 109–125). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2022.269397

143. Guennouni, M., Admou, B., El khoudri, N., Bourrhouat, A., Zogaam, L. G., Elmoumou, L., & Hilali, A. (2021). Gluten contamination in labelled gluten-free, naturally gluten-free and meals in food services in low-, middle- and high-income countries: a systematic review and meta-analysis. In British Journal of Nutrition (Vol. 127, Issue 10, pp. 1528–1542). Cambridge University Press (CUP). https://doi.org/10.1017/s0007114521002488

Funds:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Fariza Sagyntay, Almaty Technological University, Faculty of Food Technology, Tole bi 100, 050012, Almaty, Kazakhstan
Tel.: +7 707 271 97 00
E-mail: farizasagintaeva@gmail.com
ORCID: https://orcid.org/0000-0002-6805-8888

***Baltash Tarabayev**, S. Seifullin Kazakh Agro-Technical Research University, Department of Technology of Food and Processing Production, Zhenis Ave. 62, Astana 010011, Kazakhstan
Tel.: +7 771 255 70 96
E-mail: tarabaev5009@gmail.com
ORCID: https://orcid.org/0000-0002-5783-3973

Bayan Muldabekova, Almaty Technological University, Department of Bakery products and Processing Industries, Tole bi 100, 050012, Almaty, Kazakhstan
Tel.: +7 777 704 45 65
E-mail: bayan_1004@mail.ru
ORCID: https://orcid.org/0000-0003-1848-4288

Auelbek Iztaev, Almaty Technological University, Faculty of Food Technology, Tole bi 100, 050012, Almaty, Kazakhstan
Tel.: +7 700 216 225
E-mail: auelbekking@mail.ru
ORCID: https://orcid.org/0000-0002-7385--482X

Gaukhar Kossaliyeva, Northwest A&F University, Yangling, Xianyang 712100, China; Asfendiyarov Kazakh National Medical University, Tole bi 94, 050000, Almaty, Kazakhstan
Tel.: +7-777-097-4674
E-mail: kosalieva.g@kaznmu.kz
ORCID: https://orcid.org/0009-0008-0591-0229

Fatima Dikhanbayeva, Almaty Technological University, Faculty of Food Technology, Tole bi 100, 050012, Almaty, Kazakhstan
Tel.: +7 747 755 5985
E-mail: fatima6363@mail.ru
ORCID: https://orcid.org/0000-0003-4257-3774

Ramza Berzhanova, Kazakh National University, Al-Farabi Avenue, 71, Almaty, 050038, Kazakhstan
Tel.: +7 7705 448 986
E-mail: ramza05@mail.ru
ORCID: https://orcid.org/0000-0001-9251-4452

Nazym Alzhaxina, AF LPP "Kazakh Research Institute of Processing and Food Industry", Al-Farabi 47, 010000, Astana, Kazakhstan
Tel.: +7 777 704 45 65
E-mail: nazjimka@mail.ru
ORCID: https://orcid.org/0000-0001-7855-0940

Anar Kabylda, AF LPP "Kazakh Research Institute of Processing and Food Industry", Al-Farabi 47, 010000, Astana, Kazakhstan
Tel.: +7 775 900 15 07
E-mail: anara121579@gmail.com
ORCID: https://orcid.org/0000-0002-7876-9368

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 10.6.2024
Revised: 31.7.2024
Accepted: 10.8.2024
Published: 20.8.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 741-754
https://doi.org/10.5219/1992
ISSN: 1337-0960 online
www.potravinarstvo.com

Development and validation of a gas chromatography method for analysing polychlorinated biphenyls in fish roe

Valentyna Korniyenko, Svitlana Midyk, Serhii Senin, Oleksandr Machuskyy, Olena Ladohubets, Ivan Harkusha, Yuriy Balym, Alina Menchynska

ABSTRACT

A method for determining polychlorinated biphenyls (PCBs) in fish roe by gas chromatography (GC) has been developed. The suitability (validation) of the process for the determination of 14 PCBs (PCB 18, PCB 31, PCB 28, PCB 52, PCB 44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209) in fish roe by GC using an electron capture detector (ECD) was evaluated according to the following criteria: selectivity, linearity, limits of detection (LOD), limits of quantification (LOQ), accuracy and precision. Automated Soxhlet extraction and sample clean-up by solid phase extraction (SPE) were proposed for extracting PCBs from fish roe. The results of the method selectivity study showed that the determination of PCBs in fish roe is not affected by other components in the sample. The correlation coefficients for fourteen PCBs ranged from 0.9962 to 0.9999 (R^2 ≥0.995). The limits of detection (LOD) and limits of quantification (LOQ) of PCBs are below the maximum permissible levels set by the European Union (EU). The recovery percentage ranged from 81.5% to 107%, indicating the PCB extraction procedure's acceptability (R, 80 – 120%). The relative standard deviation (RSD, %) of the measurement results under convergence conditions ranged from 1.02% to 9.43% (RSD ≥15%). The obtained method suitability (validation) data meets the Commission Regulation (EU) No 589/2014 criteria.

Keywords: fish roe, polychlorinated biphenyls, PCB, gas chromatography, GC, method validation

INTRODUCTION

The world produces record quantities of fisheries and aquaculture products **[1]**. According to the European Commission, the average consumption of fish and fish products in the European Union (EU) has increased to 24.4 kg per person per year **[2]**. Global production of fish and fish products is forecast to increase by 14% by 2030. The largest importers of salmon roe in the EU are France, Germany, and Sweden, which account for 57% of total imports. In Asia are Japan, South Korea and Thailand, with a combined share of 77% of total imports. The leading suppliers of roe to the US are Taiwan, Japan and Iceland, with a combined share of 68% of total imports **[3]**.

Due to objective circumstances, Ukraine cannot catch or grow a significant amount of fish and seafood, so more than 85% of all fish products, including roe, are imported by Ukrainians. The largest quantities of fish and fish products have been imported into Ukraine from Norway, Iceland, the USA, Canada, Estonia and other countries **[4]**.

Fish roe is a particularly valuable food product, containing many nutrients **[5]**. For example, consuming 25 grams of fish roe can provide half of the body's daily protein requirement. Fish roe is also beneficial due to its micro- and macronutrients, including vitamins A, B12, B6, and D **[6]**, **[7]**.

Compared to other foods such as meat **[8]**, **[9]** and eggs **[10]**, marine fish roe contains a high amount of omega-3 fatty acids. The main fatty acids in roe are palmitic (C16:0), oleic (C18:1ω-9), linoleic (C18:2ω-6), cis-4,7,10,13,16,19-docosahexaenoic (C22:6ω-3), cis-5,8,11,14,17-eicosapentaenoic (20:5ω-3) and cis-11,14,17-eicosatrienoic (C20:3n3) acids **[11]**, **[12]**, **[13]**.

Considering the increase in the volume of fish products sold to the public **[14]**, it is necessary to strictly control the maximum permissible levels of contaminants by the current regulations in Ukraine and the world **[15]**. Among the indicators of fish roe safety, an important place is occupied by the determination of such toxic xenobiotics as polychlorinated biphenyls (PCBs). To date, 209 individual PCB congeners are known, which can accumulate in fats due to their lipophilic properties **[16]**, **[17]**. The high toxicity of these substances and their harmfulness to living organisms has been demonstrated by many scientists **[18]**, **[19]**, **[20]**, **[21]**.

According to the State Sanitary Rules and Regulations **[22]** and the requirements of the EU Directive **[23]**, the total amount of six PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180) in fish products should not exceed 75 ng/g (0.075 mg/kg). These regulations do not consider the contamination of eggs by other PCBs that may be present in fish products. Studies have also shown that the accumulation of PCBs in fish varies depending on the part of the fish analysed and that the sampling method may affect the result **[24]**.

Gas chromatography with mass spectrometric detection (GC/MS) **[25]** and gas chromatography with electron capture detection (GC/ECD) **[26]** are used to quantify PCBs.

Sample preparation by modern GC/ECD methods for determining PCBs in fat-containing objects is based on solvent extraction of the samples, purification with 90% sulfuric acid, centrifugation, and evaporation to dryness in a stream of nitrogen, followed by the addition of a solvent and purification on SPE columns **[27]**. The relevant normative documents specify the methods for determining PCBs in high-fat food products **[28]**, **[29]**, **[30]**, including fish roe, require complex and time-consuming sample preparation, i.e. they are rather labour-intensive and multi-step **[31]**, **[32]**, **[33]**. Therefore, it is important to improve the existing methods and develop new ones for the quantitative determination of PCBs in fish roe to eliminate the shortcomings as much as possible.

Scientific Hypothesis

It was anticipated that using iso-octane solvent in combination with floral and silica-based solid phase extraction cartridges for sample preparation would increase the method's selectivity. This approach will also simplify and reduce the cost of the GC/ECD method for determining selected polychlorinated biphenyls in fish roe and provide satisfactory metrological performance for evaluating its suitability.

MATERIAL AND METHODOLOGY

Samples

For the method's development and testing, we selected samples of salted black roe purchased at Kyiv (Ukraine) retail outlets. The authenticity of the roe samples was confirmed by determining their fatty acid composition.

Chemicals

PCB Test solution 14 for ECD 10 µg/mL in heptane, analytical standard. Product Number 33891. It contains the following components: PCB-No 18, PCB-No 28, PCB-No 31, PCB-No 44, PCB-No 52, PCB-No 101, PCB-No 118, PCB-No 138, PCB-No 149, PCB-No 153, PCB-No 170, PCB-No 180, PCB-No 194, PCB-No 208.

PCB No 209 solution in Isooctane 10 µg/mL is an analytical standard. Its product Number is 41612, and it is manufactured by Sigma Aldrich (Switzerland).

PCB No 30 solution in Isooctane 10 µg/mL, analytical standard. Product Number 72793, Sigma Aldrich (Switzerland).

Nitrogen gas of special purity, 99.999% (supplied by KRIOHENSERVIS LLC).

Isooctane for liquid chromatography LiChrosolv, Merck (Germany).

Sodium methoxide Natriummethoxid purum >97.0%, Fluka (Switzerland).

n-Hexan (purity GC 98%), Merck (Germany).

Methanol (HPLC grade), Lot: 1419984, Fisher Scientific (UK).

Chloroform for chromatography, Merck (Germany).

Diethyl ether (purity GC 98%), Merck (Germany).

Copure® Silica SPE Cartridge. Cat. No: Cosil61000, Biocomma (China).

Copure® Florisil SPE Cartridge. Cat. No: Cofl61000, Biocomma (China).

Biological Materials

To develop and test the methodology, we used black roe “Royal Caviar Classic”, 50 g, purchased from a retailer in one of the shopping centres in Kyiv.

Instruments

Solvent Extractor SER 148 (Soxclet apparatus); Velp Scientifica (Italy); Gas-liquid chromatography Trace GC Ultra, Thermo Fisher Scientific (United States); electron capture detector (ECD); capillary chromatography column SGE HT8-PCB (60 m× 0.25 mm, Part number 054236), Trajan Scientific and Medical; Vacuum manifold for solid-phase extraction with stand and vacuum pump N86 KT18, Laboport (France); Electronic laboratory scales Kern&Sohn GmbH ABJ 220-4M, KERN&SOHN (Germany); Shaking machine VortexMS 3 digital, IKA (Germany).

Laboratory Methods

The validation was performed at the Ukrainian Laboratory of Quality and Safety of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine (accredited according to DSTU ISO/IEC 17025:2019). The suitability of our developed method for determining PCBs in fish roe was evaluated according to the established general and specific requirements for PCB determination, which are applied during validation. This evaluation considered requirements for sample collection and the formation of a composite sample, sample storage conditions, personnel competence, and the requirements for laboratory equipment, reagents, and the laboratory's microclimate. Additionally, certificates from an authorised institution confirm the calibration of the instruments. The requirements for linearity testing and constructing a calibration curve (which includes 5 calibration points, each in 3 repetitions) were met. During the validation, the following method requirements were ensured: Low working range and limits of quantification (For most PCB congeners limit of quantification in the nanogram (10 – 9 g) range is already sufficient); High selectivity (specificity); High accuracy; Limit of quantification (for a confirmatory method shall be about one-fifth of the maximum level); the relative standard deviation (RSD, %) of the measurement results under convergence conditions within (RSD ≥15%); the recovery percentage (R, 80 – 120%); the correlation coefficients (R^2 ≥0.995) **[34]**, **[35]**.

Following the quality system requirements of DSTU ISO/IEC 17025:2019 and following the Commission Decision and Commission Regulation **[34]**, **[35]**, the following microclimate conditions were ensured in the laboratory premises during testing: temperature: 20±5°C; humidity: <80%; atmospheric pressure range: 84.0 – 106.7 kPa. All reagents and analytical standards were within their valid storage period at the testing time, meaning they were not expired. The fish roe samples were stored in a refrigerator at 2 – 8°C temperature before analysis. The prepared test samples were stored for 7 days in hermetically sealed vials. PCB analytical standards were stored at a temperature of minus 18°C. Adhering to the microclimate conditions of the laboratory premises and the storage conditions of reagents and test samples is a key requirement in the method's suitability assessment. Therefore, the method's reliability was verified only under the conditions specified by the DSTU ISO/IEC 17025:2019 quality system and the Commission Decision and Commission Regulation **[34]**, **[35]**. No deviations from the specified microclimate and storage conditions were allowed during the method suitability assessment.

Description of the Experiment

Sample preparation: Fish roe samples were homogenised to a uniform mass. 5±0.1 g of fish roe was weighed and transferred to a cellulose cartridge for extraction. A layer of cotton wool was placed over the sample, and PCBs were extracted using a Soxhlet apparatus. The extraction (fat extraction) was performed at 80 °C – 30 min in diethyl ether, 60 min over ether and 30 min diethyl ether evaporation. 2 ml of isooctane was added to the dry residue of the extract, shaken vigorously for 1 min and transferred to 5 ml test tubes. A second portion of isooctane (2 ml) was added, shaken vigorously for 1 min, and the extract was combined in a 5 ml test tube. The extract was then purified by solid phase extraction using two cartridges: the upper one - Florisil SPE cartridge, the lower one - Silica SPE cartridge. The cartridges were conditioned with 2 ml of isooctane, and the extract was applied to the upper cartridge (Florisil SPE). Elution was performed with isooctane (3 times with 2 ml). The purified extracts obtained were made up to a volume of 10 ml and analysed by gas-liquid chromatography with an electron capture detector (ECD) using the chromatographic conditions recommended by the manufacturer of the SGE HT8-PCB capillary chromatography column on which the separation of PCBs was carried out.

Number of samples analyzed: 20 samples of fish roe.

Number of repeated analyses: 2.

Number of experiment replications: the number of replications of each experiment to determine a value was two.

Design of the experiment: In the first stage, we developed a method that included optimising sample preparation (optimal solvents, sample cleaning consumables) and selecting chromatographic conditions.

To determine the PCB content, a gas-liquid chromatograph Trace GC Ultra, Thermo Fisher Scientific (United States), and electron capture detector (ECD) were used. PCBs were separated on an SGE HT8-PCB capillary chromatography column (60 m × 0.25 mm, part number 054236).

Description of instrument settings and chromatographic conditions:

Injection volume – 2 µl, injection type – splitless; carrier gas type – nitrogen, flow rate – 2 ml/min, flow mode: constant flow. Temperature program for the GC oven:

Initial temperature – 70 °C, Hold time (minutes) – 1.00;

Ramp 1: Rate (°C/min) – 40.0, temperature (°C) – 130, Hold Time (minutes) – 0.00;

Ramp 2: Rate (°C/min) – 2.5, temperature (°C) – 290, Hold Time (minutes) – 5.00.

Oven Run-Time (min) – 71.50. Prep Run Timeout (min): 10.00. Equilibration Time (min): 0.50.

An Electron-Capture Detector (ECD) from Thermo Fisher Scientific was used. Description of the detector settings: Base Temperature (°C) – 300, ECD Temperature (°C) – 300, Reference Current (nA): 1.0, Pulse Amplitude (V) – 50, Pulse Width (µsec): 1.0. Makeup Flow (ml/min): 40.

Results were evaluated using the specialised instrument software Xcalibur. The mass fraction of each PCB was calculated using the external standard method and expressed in mg/kg. The second step was to assess the suitability of this method, i.e. its validation according to the following criteria: selectivity, linearity, limits of detection (LOD), limits of quantification (LOQ), accuracy and precision, and recovery. The suitability assessment of the method was carried out by Commission Regulation (EU) No 589/2014 **[34]** and Commission Decision 2002/657/EC **[35]**, which ensures the implementation of EU Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results **[36]**.

The limits of detection (LOD) for each PCB were calculated as the mass concentration of the component that produces a signal (peak area) that is 3 times higher than the signal of the blank sample (matrix). The limits of quantification (LOQ) for each PCB were calculated as the mass fraction of the component that produces a signal (peak area) that is 10 times higher than the signal of the control sample of the analyzed matrix **[34]**, **[35]**.

Statistical Analysis

The results were evaluated using the dedicated gas chromatography software Xcalibur, version 2.0.7 (Thermo Scientific Software). This programme consists of several separate blocks (Processing Setup, Quan Browser-Calibration) and allows automatic identification of component peaks by their release time and determination of linearity (R^2 calculation) during calibration. One of the functions of Xcalibur is also the export of primary data (retention times (Rt) PCBs, peak areas (S) and noise/signal (S/N)) to Microsoft Excel. To perform statistical analysis of the validation criteria and to determine the Relative Standard Deviation (RSD), Mean, Standard Square Deviation and Expanded Uncertainty, the appropriate formulae were written in Microsoft Excel. That is, statistical data analysis was performed using Microsoft Excel and Xcalibur.

The measurement results were statistically processed according to the Sante 11312/2021 guideline, calculating only the RSD (Relative Standard Deviation) in percentages. Calculating p-values is not a mandatory criterion when validating chromatographic methods **[37]**.

RESULTS AND DISCUSSION

The GC/MS method **[38]** with the recommended QuEChERS sample preparation **[39]** should be considered for determining PCBs in high-fat foods. It allows fast and efficient extraction of residual amounts of target compounds and purification of the extract from matrix components **[40]**, **[41]**.

Researchers have optimised a method for determining polychlorinated biphenyls in fish based on accelerated extraction with acetone/n-hexane (1:1) solvents **[42]**.

The GC-ECD method is also used to determine PCBs with recoveries of 95.7 – 101% **[43]**.

Several variants of PCB determination methods are described in the scientific literature **[44]** and official regulatory documents **[45]**. One method is for determining 19 PCBs in solid matrices using a capillary chromatography column (30 m) with different diameters. The sample preparation steps for determining the 19 PCB congeners can be used to analyse and identify other congeners. Still, not all 209 PCB congeners can be identified using the chromatography columns and analytical procedures described in this method **[46]**.

There are also several methods for extracting solid samples to determine PCBs, which are listed in the official methods. Solid samples can be extracted with hexane-acetone (1:1) or methylene chloride-acetone (1:1) using Method 3540C (Soxhlet) **[47]**, Method 3541 (automated Soxhlet) **[48]**, Method 3545 (pressurised fluid extraction) **[49]**, Method 3546 (microwave extraction) **[50]**, Method 3550 B (ultrasonic extraction) **[51]**, Method 3562 (supercritical fluid extraction) **[52]**.

Capillary columns with an internal diameter of 0.25 mm, 0.32 mm or 0.53 mm are used for GC/ECD chromatography. Columns with smaller inner diameters have a higher chromatographic resolution than those with larger inner diameters. Chromatographic columns with a smaller inner diameter (0.25 mm) and long length (>60 m) can generally be more efficient in separating a larger number of PCB congeners **[53]**.

An analytical method has been developed based on the extraction of organochlorine pesticides (p,p'DDT, p,p'DDE, p,p'DDD, α-HCCG, β-HCCG, γ-HCCG, heptachlor) and PCBs with organic solvents, purification of the extracts obtained and subsequent quantitative determination of these compounds by GC/ECD **[45]**. A method for determining 6 PCBs in chicken eggs is also described. It involves extraction for 3 hours on a magnetic stirrer using n-hexane/acetone 85:15 (v:v), ultrasonication, evaporation under a nitrogen stream, addition of sulphuric acid and ENVI-carb®, overnight soaking, centrifugation, repeated evaporation under a nitrogen stream, addition of n-hexane solvent, purification with Bond Elut-PCB, repeated evaporation under a nitrogen stream and dissolution of the residue in isooctane **[54]**.

In the scientific literature **[26]**, a multi-step preparation of roe samples for GC/ECD analysis is described, involving 24-hour sample extraction using a Soxhlet apparatus, evaporation on a rotary evaporator, centrifugation and concentration of the sample under a nitrogen stream using reagents such as sodium sulphate, sulphuric acid, hexane and dichloromethane. Other methods for determining PCBs are also known and have advantages and disadvantages **[21]**.

At present, the scientific community working in the field of analytical methods development aims to find new methods, to develop simplified methods of sample preparation for the determination of PCBs in foods with high-fat content, which would be characterised by the shortest possible execution time, minimal use of human resources, require the use of small volumes of solvents, i.e. have a low cost, but at the same time provide effective extraction of the components of interest and acceptable metrological characteristics of the method **[40]**.

To overcome the disadvantages of traditional sample preparation (long and multi-step) **[28]**, **[29]**, **[30]**, **[31]**, **[55]** in the determination of PCBs in fish roe, we proposed a method for the determination of 14 PCBs in fish roe using automated Soxhlet extraction followed by purification of the extracts by solid phase extraction (SPE).

The essence of the method modification is the extraction of fat from fish roe with diethyl ether (using a Soxhlet apparatus), followed by SPE purification of the samples using 2 types of cartridges: Florisil SPE cartridge (pore volume 0.70 – 0.90 mL/g) and Silica SPE cartridge containing silicon dioxide (SiO_2), magnesium dioxide (MgO) and sodium dioxide (Na_2SO_4), using is ethane as eluent.

The sample preparation procedure for our modified method is described in this article's 'Sample preparation' section.

Assessment of method suitability. The method was validated according to the criteria of the European Commission Regulation (EU) No 589/2014 **[34]** and Commission Decision 2002/657/EC **[35]**: selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy.

Optimisation of the chromatographic conditions allowed the determination of retention times (Rt) for all the PCBs analysed. Chromatogram of a 14-component analytical standard for PCBs (Rt for PCB 18 – 24.94; for PCB 31 – 28.79; for PCB 28 – 29.08; for PCB 52 – 31.32; for PCB 44 – 33.08; for PCB 101 – 38.36; for PCB 149 – 42.55; for PCB 118 – 43.90; for PCB 153 – 45.22; for PCB 138 – 47.25; for PCB 180 – 52.49; for PCB 170 – 54.49; for PCB 194 – 59.39; for PCB209 – 62.02) at the level of 0.01 mg/kg is shown in Figure 1.

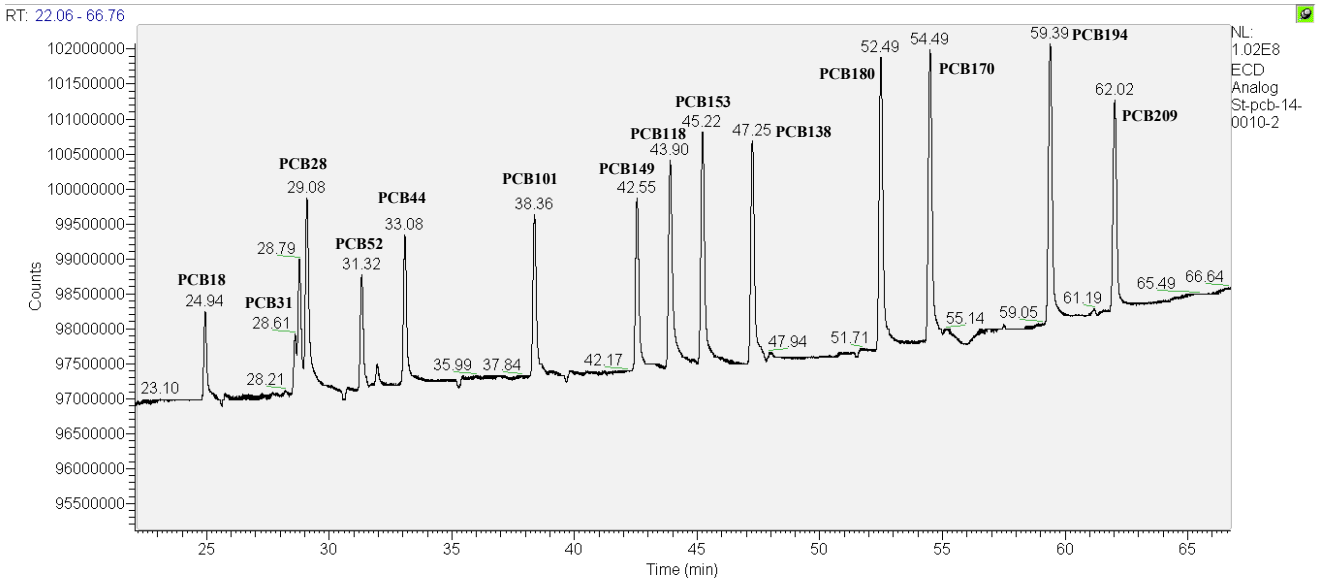


Figure 1 Chromatogram of analytical standard PCBs Test solution 14 for ECD (PCB 18, PCB 31, PCB 28, PCB 52, PCB44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209) at 0.01 mg/kg.

Selectivity. To determine the selectivity of the method, samples of black salted fish roe free of PCBs (blank samples) and simulated samples containing 14 PCBs (PCB 18, PCB 31, PCB 28, PCB 52, PCB 44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209) at concentrations of 0.005 mg/kg, 0.01 mg/kg, 0.02 mg/kg; 0.04 mg/kg were analysed. A comparison of the chromatograms obtained showed that the determination of PCBs in fish roe was not affected by other components in the sample (Figur 2). It should also be noted that gas-liquid chromatography with an electron capture detector (GC/ECD) is a highly selective

method, allowing the determination of an analyte in the presence of matrix components due to the operation of the detector **[26]**.

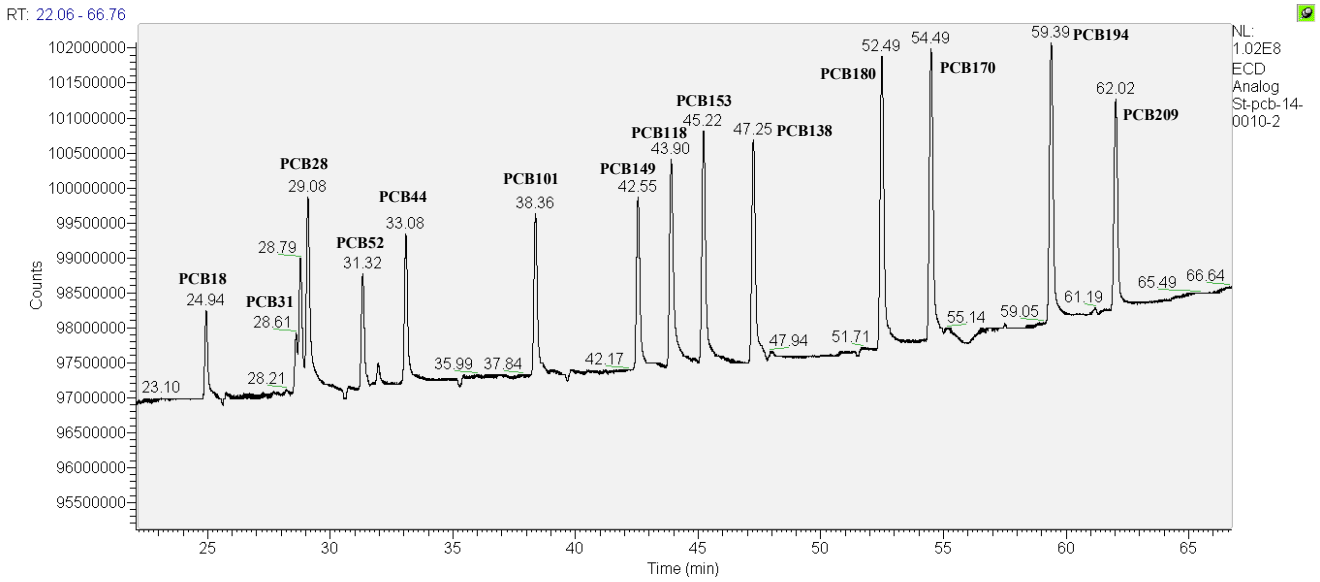


Figure 2 Chromatogram of added 14 PCBs in a model fish roe sample at 0.01 mg/kg.

Table 1 Results of the evaluation of linearity, limit of detection and limit of quantification of the developed method.

Name according to IUPAC[1] nomenclature	Linear range, mg/kg	R^2	LOQ, mg/kg	LOD, mg/kg
2,2',5-Trichlorobiphenyl (PCB18)	0.002 – 0.050	0.9988	0.003	0.0009
2,4',5-Trichlorobiphenyl (PCB31)	0.002 – 0.050	0.9990	0.001	0.0003
2,4,4'-Trichlorobiphenyl (PCB 28)	0.002 – 0.050	0.9966	0.001	0.0003
2,2',5,5'-Tetrachlorobiphenyl (PCB 52)	0.002 – 0.050	0.9991	0.001	0.0003
2,2',3,5'-Tetrachlorobiphenyl (PCB44)	0.002 – 0.050	0.9984	0.001	0.0003
2,2',4,5,5'-Pentachlorobiphenyl (PCB101)	0.002 – 0.050	0.9999	0.001	0.0003
2,2',3,4',5',6-Hexachlorobiphenyl (PCB149)	0.002 – 0.050	0.9993	0.001	0.0003
2,3',4,4',5-Pentachlorobiphenyl (PCB118)	0.002 – 0.050	0.9989	0.001	0.0003
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB153)	0.002 – 0.050	0.9979	0.001	0.0003
2,2',3,4,4',5'-Hexachlorobiphenyl (PCB138)	0.002 – 0.050	0.9972	0.001	0.0003
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB180)	0.002 – 0.050	0.9978	0.001	0.0003
2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB170)	0.002 – 0.050	0.9998	0.001	0.0003
2,2',3,3',4,4',5,5'-Octachlorobiphenyl(PCB194)	0.002 – 0.050	0.9962	0.001	0.0003
2,2′,3,3′,4,4′,5,5′,6,6′-Decachlorobiphenyl(PCB209)	0.002 – 0.050	0.9974	0.001	0.0003

Note: IUPAC[1] – International Union of Pure and Applied Chemistry; R^2 – correlation coefficients.

Linearity. To establish the calibration curve, 5 solutions of the analytical standard 14 PCBs with the specified PCB concentrations (0.05 mg/kg, 0.02 mg/kg, 0.01 mg/kg, 0.005 mg/kg, 0.002 mg/kg) were used. For each standard solution, two values of the analytical signal (chromatographic peak area) were obtained using one batch of reagents, equipment and consumables. Regression analysis of the data was performed by the least squares method using a linear model.

The calibration curves for all PCBs showed R^2 values ranging from 0.9962 to 0.9999, which meets the required level of ≥0.995 (Table 1, Figure 3 and Figure 4).

Therefore, the standard curves for polychlorinated biphenyls (PCB 18, PCB 31, PCB 28, PCB 52, PCB 44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209) are linear in the range 0.002 to 0.05 mg/kg.

Limits of detection (LOD). The limit of detection for each of the 14 PCBs was determined based on the signal-to-noise ratio (S/N) of the chromatographic peak, which is three times higher than the S/N of the blank sample (S/N = 3).

The results showed that the limit of detection for PCBs in fish roe was 0.0009 mg/kg for PCB 18 and 0.0003 mg/kg for PCB 31, PCB 28, PCB 52, PCB 44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209 (Table 1).

Limits of quantification (LOQ). The limit of quantification for each PCB was determined based on the signal-to-noise ratio (S/N) of the chromatographic peak, which is 10 times higher than the S/N of the blank (S/N=10).

It was found that the limit of quantification (LOQ) of PCBs in fish roe was 0.003 mg/kg for PCB 18 and 0.001 mg/kg for PCB 31, PCB 28, PCB 52, PCB 44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209 (Table 1).

According to the current regulatory document in Ukraine **[22]** and EU Commission Regulation (EU) No 1259/2011 **[23]**, the total amount of six PCBs (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180) in fish products (including roe) should not exceed 75 ng/g (0.075 mg/kg).

Therefore, the limit of detection and limit of quantification of polychlorinated biphenyls according to the developed method is acceptable for their determination in fish roe by the regulatory documents of Ukraine and EU Commission Regulation No. 589/2014 **[34]**.

Accuracy. The accuracy of PCB determination in fish roe was evaluated using the injected-found method. The analytical standard of 14 PCBs (PCB 18, PCB 31, PCB 28, PCB 52, PCB 44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209) at levels of 0.005, 0.01, 0.02 and 0.04 mg/kg was added to the fish roe samples which did not contain residual PCBs. After all the steps of sample preparation and GC/ECD analysis of the extracts obtained, the concentration of PCBs was determined and the degree of recovery (R, %) was calculated for 5 model matrices of artificially enriched PCBs.

The results of the study showed that the lowest recovery (R, %) of PCBs at the level of 0.01 mg/kg was obtained for PCB 31, which was 81.50%; the highest recovery was obtained for PCB 118, which was 107% (at an added concentration of 0.02 mg/kg), and for PCB170, which was 106.75% (at an added concentration of this PCB of 0.04 mg/kg) (Table 2).

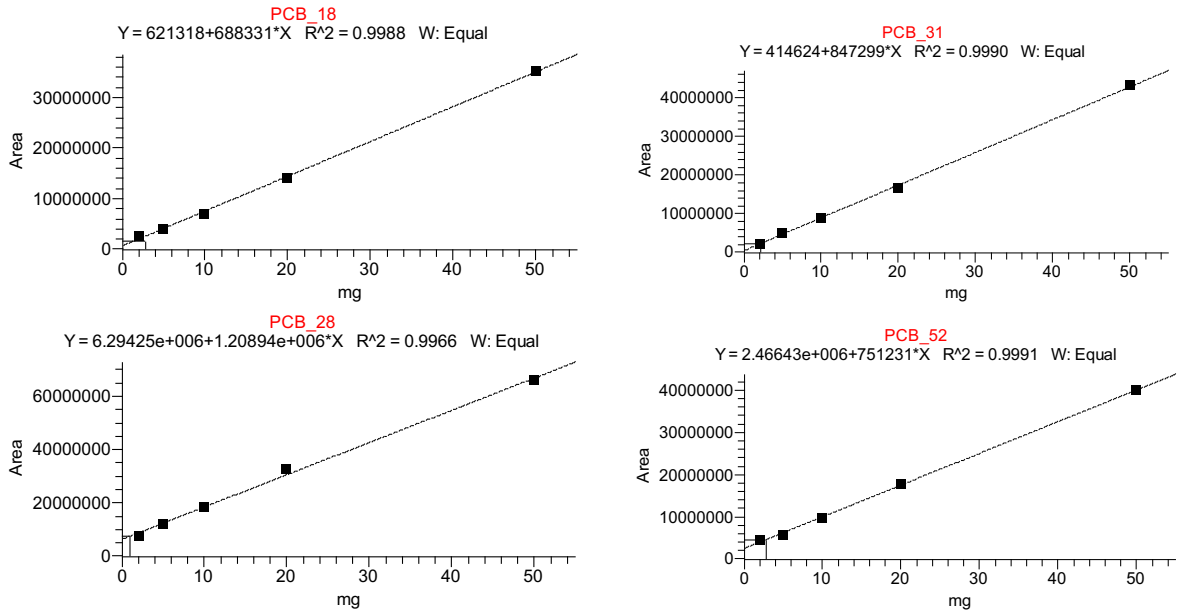


Figure 3 Linearity of solutions of 5 concentrations of the PCBs analytical standard (PCB 18, 28, 31 and 52).

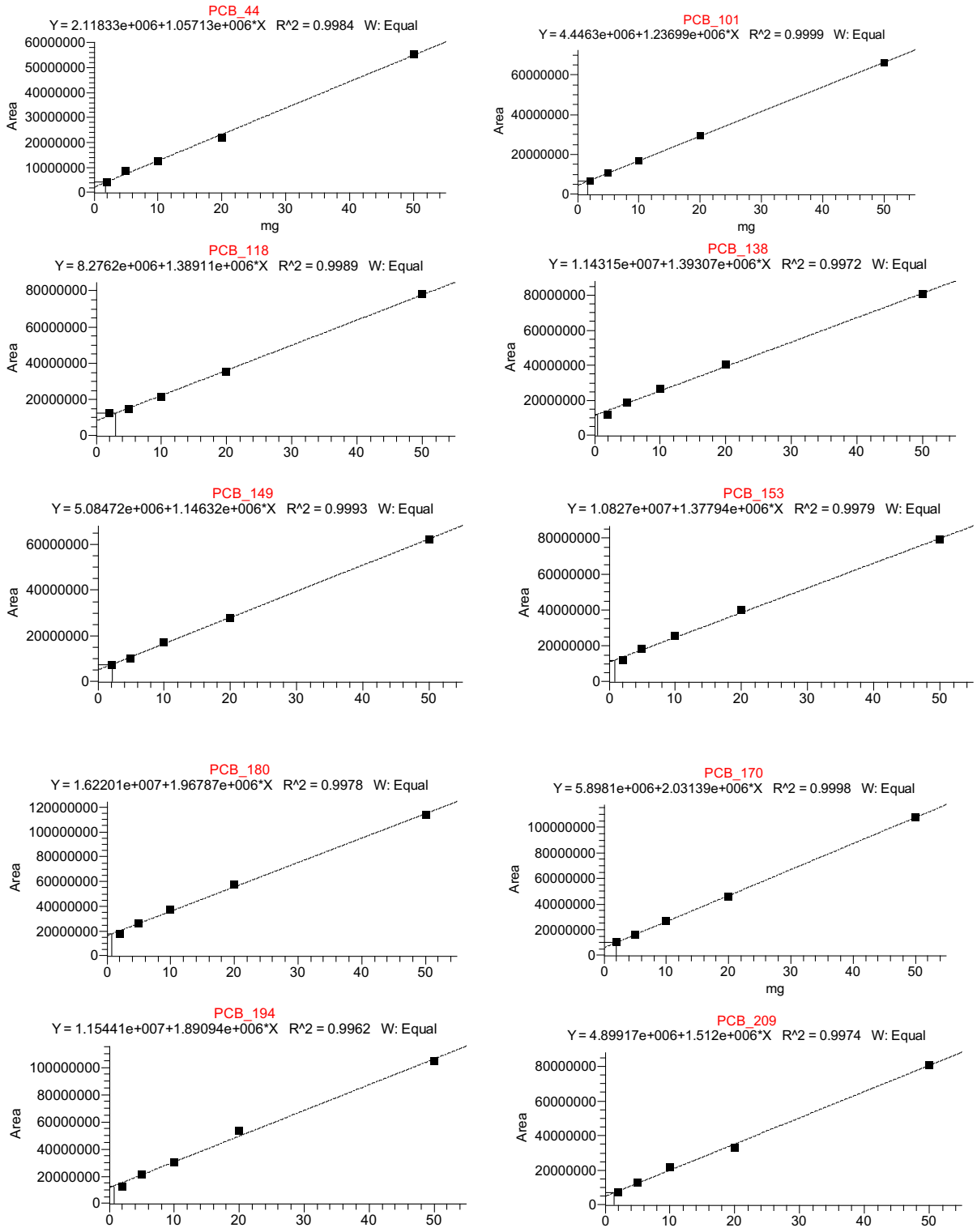


Figure 4 Linearity of solutions of 5 concentrations of the PCBs analytical standard (PCB: 44, 101, 118, 138, 149, 153, 170, 180, 194, 209).

Table 2 Results of the evaluation of the accuracy and precision of the determination of 14 PCBs (n=5).

PCBs	The concentration of PCBs added to the sample							
	0.005 mg/kg		0.01 mg/kg		0.02 mg/kg		0.04 mg/kg	
	[1]R, %	[2]RSD, %	[1]R, %	[2]RSD, %	[1]R, %	[2]RSD, %	[1]R, %	[2]RSD, %
PCB18	99.40	4.29	92.00	4.84	95.00	7.07	91.23	2.66
PCB31	100.00	2.83	81.50	5.24	93.50	4.54	99.06	1.02
PCB28	102.00	2.77	99.65	1.92	94.25	4.88	88.75	5.98
PCB52	88.60	9.43	89.50	5.53	96.75	2.56	99.75	1.42
PCB44	94.00	6.02	99.50	7.82	99.00	1.43	97.50	2.90
PCB101	90.00	3.14	93.00	6.08	97.50	2.15	96.63	1.79
PCB149	95.00	4.47	99.90	6.21	104.00	1.36	100.88	1.60
PCB118	104.00	2.72	107.00	1.32	106.75	2.09	102.13	1.56
PCB153	101.00	1.40	101.00	1.40	103.00	3.47	103.25	1.37
PCB138	97.00	1.46	98.00	1.44	96.80	5.29	99.00	2.96
PCB180	99.80	4.10	101.50	2.21	102.00	1.69	103.00	2.40
PCB170	100.00	6.84	99.50	5.42	101.75	1.04	106.75	1.85
PCB194	100.50	7.44	101.50	4.56	101.25	1.05	93.38	1.33
PCB209	100.70	1.83	98.50	2.15	102.05	3.78	96.63	1.49

[1]R – degree of recovery; [2]RSD – relative mean square deviation.

Thus, the obtained results of the degree of PCB extraction from fish roe correspond to the range of permissible values (80 – 120%) given in the EU Commission Regulation No. 589/2014 **[34]**.

Precision. The precision of the GC/ECD method for the analysis of PCBs in fish roe was determined by the convergence of the measurement results. Convergence was estimated according to the following scheme:

- model (artificially spiked with PCBs at levels of 0.005, 0.01, 0.02 and 0.04 mg/kg) samples of black fish roe were analysed in 10 replicates;
- this experiment was performed under conditions of convergence (under the same conditions, with the same equipment, by the same researcher, within a short period);
- the relative standard deviation (RSD, %) values were calculated for the concentration values obtained.

It was found that the relative standard deviation (RSD, %) of the results of measurement of model fish roe samples under conditions of convergence for PCB at concentrations of 0.005 mg/kg, 0.01 mg/kg, 0.02 and 0.04 mg/kg for PCB 18 (2.66 – 7.07 %); for PCB 31 (1.02 – 5.24 %); for PCB 28 (1.92 – 5. 98 %); for PCB 52 (1.42 – 9.43 %); for PCB 44 (1.43 – 7.82 %); for PCB 101 (1.79 – 6.08 %); for PCB 149 (1.36 – 6.21 %); for PCB 118 (1.32 – 2. 72 %); for PCB 153 (1.37 – 3.47%); for PCB 138 (1.44 – 2.96%); for PCB 180 (1.69 – 4.10%); for PCB 170 (1.04 – 6.84%); for PCB 194 (1.05 – 7.44%); for PCB 209 (1.49 – 3.78%) (Table 2).

Thus, the relative standard deviation of the measurement results under convergence conditions ranged from 1.02% to 9.43%, which does not exceed the established values (RSD ≥15%) given in the EU Commission Regulation No. 589/2014 **[34]**. The results of the convergence evaluation indicated the absence of systemic errors in the developed methodology.

The evaluation of the suitability (validation) of the method for determining 14 PCBs (PCB 18, PCB 31, PCB 28, PCB 52, PCB 44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209) in fish roe by GC/ECD using automated Soxhlet extraction followed by purification of the extracts by SPE (Florisil, Silica) showed acceptable metrological characteristics **[34]**.

Thus, the results obtained provide the basis for further studies on method modification using solvents and solid phase purification methods for the determination not only of PCBs in fatty products but also of other fat-soluble persistent organic pollutants, in particular polyaromatic hydrocarbons and organochlorine pesticides.

CONCLUSION

The method for the determination of polychlorinated biphenyls (PCB 18, PCB 31, PCB 28, PCB 52, PCB 44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209) in fish roe by gas-liquid chromatography with electron capture detector (GC/ECD) using automated Soxhlet extraction followed by purification of the extracts by SPE (Silica, Florisil SPE cartridges) showed acceptable metrological characteristics. The correlation coefficients (R^2) for PCBs were ≥0.995. The limit of detection (LOD) and limit of quantification (LOQ) of PCBs were below the European Union (EU) limits. The recovery of PCBs from fish roe ranged from 81.5% to 107%, indicating the proposed method's acceptability (R, 80 – 120%). The relative standard deviation of the recovery results ranged from 1.02% to 9.43%. These values do not exceed the established standards, as specified in the EU Regulation No. 589/2014. The results of the studies give grounds to believe that the method developed by GC/ECD for the determination of 14 polychlorinated biphenyls (PCB 18, PCB 31, PCB 28, PCB 52, PCB 44, PCB 101, PCB 149, PCB 118, PCB 153, PCB 138, PCB 180, PCB 170, PCB 194, PCB 209) in fish roe meets the requirements of Commission Regulation (EU) No 589/2014 and is suitable for use in specialised chemical analytical laboratories for food safety control.

REFERENCES

1. Holembovska, N., Tyshchenko, L., Slobodyanyuk, N., Israelian, V., Kryzhova, Y., Ivaniuta, A., Pylypchuk, O., Menchynska, A., Shtonda, O., & Nosevych, D. (2021). Use of aromatic root vegetables in the technology of freshwater fish preserves. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 296–305). HACCP Consulting. https://doi.org/10.5219/1581
2. Makarenko, A., Mushtruk, M., Rudyk-Leuska, N., Kononenko, R., Shevchenko, P., Khyzhniak, M., Martseniuk, V., Kotovska, G., Klymkovetskyi, A., & Glebova, J. (2022). Investigation of internal organs and additive tissue of hybrid hypophthalmichthys (Hypophthalmichthys spp.) as a promising raw material for the production of dietary nutritional products. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 411–430). HACCP Consulting. https://doi.org/10.5219/1760
3. Bondarchuk, M., Khartii, M. (2022). Global trends in the development of the caviar products market. In Efektyvna ekonomika (Vol. 2, pp. 1–9). Dnipro State Agrarian and Economic University. https://doi.org/10.32702/2307-2105-2022.2.77
4. Overview of the fish market of Ukraine for 2022 and 2023. (2024, January, 4). In UIFSA. https://uifsa.ua/news/news-of-ukraine/overview-of-the-fish-market-of-ukraine-for-2022-and-2023
5. Makarenko, A., Mushtruk, M., Rudyk-Leuska, N., Kononenko, I., Shevchenko, P., Khyzhniak, M., Martseniuk, N., Glebova, J., Bazaeva, A., & Khalturin, M. (2021). The study of the variability of morphobiological indicators of different size and weight groups of hybrid silver carp (Hypophthalmichthys spp.) is a promising direction of development of the fish processing industry. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 181–191). HACCP Consulting. https://doi.org/10.5219/1537
6. Bunga, S., Carne, A., Bekhit, A. El-D. A. (2022). Chapter 3 - Composition and nutrition of fish roes. In Bekhit, A. El-D. A. (Ed.), Fish roe: Biochemistry, Products, and Safety (pp. 41–92). Academic Press. https://doi.org/10.1016/B978-0-12-819893-3.00011-4
7. Wirth, M., Kirschbaum, F., Gessner, J., Krüger, A., Patriche, N., Billard, R. (2000). Chemical and biochemical composition of caviar from different sturgeon species and origins. In Nahrung (Vol. 44, Issue 4, pp. 233–237). Akademie-Verlag.
8. Bal'-Prylypko, L. V., Derevyanko, L. P., Slobodyanyuk, N. M., Starkova, E. R., & Androshchiuk, O. S. (2018). Using of the Ampullaria glauca snails' caviar for correction of the effects of the ionizing radiation exposure in small dose. In Nuclear Physics and Atomic Energy (Vol. 19, Issue 2, pp. 159–165). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/jnpae2018.02.159
9. Ugnivenko, A., Nosevych, D., Antoniuk, T., Chumachenko, I., Ivaniuta, A., Slobodyanyuk, N., Kryzhova, Y., Rozbytska, T., Gruntovskyi, M., & Marchyshyna, Y. (2022). Manifestation of living and post-slaughter traits of productivity in inbred and outbred bull calves of Ukrainian meat cattle breed. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 356–366). HACCP Consulting. https://doi.org/10.5219/1769
10. Honchar, V., Iakubchak, O., Shevchenko, L., Midyk, S., Korniyenko, V., Kondratiuk, V., Rozbytska, T., Melnik, V., & Kryzhova, Y. (2022). The effect of astaxanthin and lycopene on the content of fatty acids in the yolks of chicken eggs under different storage regimes. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 473–489). HACCP Consulting. https://doi.org/10.5219/1774
11. Gong, Ya., Huang, Ya., Gao, L., Lu, J., Hu, Yu., Xia, L., Huang, H. (2013). Nutritional Composition of Caviar from Three Commercially Farmed Sturgeon Species in China. In Journal of Food and Nutrition Research (Vol. 1, Issue 5, pp. 108–112). Graduate School Foundation Inc.

12. Ma, S., Li, L., Hao, S., Yang, X., Huang, H., Cen, J., Wang Yu. (2020). Journal of Oleo Science (Vol. 69, Issue 10, pp.1199–1208). Japan Oil Chemists' Society. https://doi.org/10.5650/jos.ess20061
13. Abbas, E., Hrachya, G. (2015). Fatty acid composition of caviar and liver from cultured great sturgeon (Huso huso). In International Food Research Journal (Vol. 22, Issue 3, pp.1083–1086). Elsevier BV.
14. Ivaniuta, A., Menchynska, A., Nesterenko, N., Holembovska, N., Yemtcev, V., Marchyshyna, Y., Kryzhova, Y., Ochkolyas, E., Pylypchuk, O., & Israelian, V. (2021). The use of secondary fish raw materials from silver carp in the technology of structuring agents. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 546–554). HACCP Consulting. https://doi.org/10.5219/1626
15. DSTU 8096, 2015. Salted fish roe. Specifications.
16. Khyzhnyak, S., Voitsitskiy, V., Korniyenko, V. (2022). Polychlorinated biphenyls in the environment and methods of their determination. In Topical issues of the development of veterinary medicine and breeding technologies: scientific monograph (pp. 546–570). Baltija Publishing. https://doi.org/10.30525/978-9934-26-258-6-17
17. Shang, X., Dong, G., Zhang, H., Zhang, L., Yu, X., Li, J., Wang, X., Yue, B., Zhao, Y., Wu, Y. (2016). Polybrominated diphenyl ethers (PBDEs) and indicator polychlorinated biphenyls (PCBs) in various marine fish from Zhoushan fishery, China. In Food Control (Vol. 67, pp. 240–246). EFFoST. https://doi.org/10.1016/j.foodcont.2016.03.008.
18. Panesar, H. K., Kennedy, C. L., Keil, Stietz, K. P., Lein, P. J. (2020). Polychlorinated Biphenyls (PCBs): Risk Factors for Autism Spectrum Disorder? In Toxics (Vol. 8, pp. 1–32). MDPI AG. https://doi.org/10.3390/toxics8030070
19. Abolghasemi, M., Esmaeilzadeh, S., Mirabi, P., Golsorkhtabaramiri, M. (2021). Human Exposure to Polychlorinated Biphenyls (PCBs) and The Risk of Endometriosis: A Systematic Review and Meta-Analysis Protocol. In International Journal of preventive medicine (Vol. 12, p. 108). Isfahan University of Medical Sciences. https://doi.org/10.4103/ijpvm.IJPVM_178_19
20. Bober, A., Liashenko, M., Protsenko, L., Slobodyanyuk, N., Matseiko, L., Yashchuk, N., Gunko, S., & Mushtruk, M. (2020). Biochemical composition of the hops and quality of the finished beer. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 307–317). HACCP Consulting. https://doi.org/10.5219/1311
21. Liu, J., Li, G., Liu, J., Wang, P., Wu, D., Zhang, X., & Wu, Y. (2023). Recent Progress on Toxicity and Detection Methods of Polychlorinated Biphenyls in Environment and Foodstuffs. In Critical Reviews in Analytical Chemistry (Vol. 53, Issue 4, pp. 928–953). Taylor & Francis. https://doi.org/10.1080/10408347.2021.1997570
22. State sanitary rules and standards. (2020). Maximum permissible levels of certain pollutants in food products. Order No. 1238 dated 05.22.2020. Retrieved from: https://zakon.rada.gov.ua/laws/show/z0684-20#Text
23. Commission Regulation (EU) No 1259. (2011). Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. In Official Journal of the European Union (Vol. 3, pp. 18–23). Publications Office of the European Union.
24. Lüth, A., Lahrssen-Wiederholt, M., Karl, H. (2018). Studies on the influence of sampling on the levels of dioxins and PCB in fish. In Chemosphere (Vol. 212, pp. 1133–1141). Elsevier BV. https://doi.org/10.1016/j.chemosphere.2018.09.011
25. Eghbaljoo, H., Ghalhari, M. R., Shariatifar, N., Khaniki, G. J., Aghaee, E. M., Sani, M. A., Mansouri, E., Majid Arabameri (2023). Analysis of polychlorinated biphenyls (PCBs) in edible oils using the QuEChERS/GC-MS method: A health risk assessment study. In Heliyon (Vol. 9, p. 21317). Elsevier BV. https://doi.org/10.1016/j.heliyon.2023.e21317
26. Grafton, A., Lee, D., Libero, D., Miller, J., Rapko, K. (2006). Polychlorinated Biphenyls (PCBs) in Fish Roe. In Journal of Young Investigators (Vol. 1). Philadelphia University. Retrieved from: https://www.jyi.org/2006-january/2017/10/8/polychlorinated-biphenyls-pcbs-in-fish-roe
27. Colangelo, R., Diletti, G., Scortichini, G., Calitri, A., Casamassima, F., Iammarino, M., Nardelli, V., Zianni, R., Ingegno, M. (2023). Determination of non-dioxin like polychlorinated biphenyls by GC-ECD and GC-HRMS analysis in seafood. Conference: Chimica sotto l'Albero 2023 - Innovazione e Resilienza: Giovani Ricercatori per un Futuro Sostenibile.
28. DSTU EN 1528-1, 2002. Fatty food products. Determination of pesticides and polychlorinated biphenyls (PCB). Part 1. General provisions (EN 1528-1:1996, IDT).
29. EN 1528-2, 1998. Fatty food. Dnetermination of pesticides and polychlorinated biphenyls (PCBs). Part 2: Extraction of fat, pesticides and PCBs, and determination of fat content
30. EN 1528-3, 1996. Fatty food. Determination of pesticides and polychlorinated biphenyls (PCBs). Part 3. Clean-up methods.

31. EN 1528-4, 1996. Fatty food. Determination of pesticides and polychlorinated biphenyls (PCBs). Part 4: Determination, confirmatory tests, miscellaneous.

32. BS EN 1528-3, 1997. Fatty food. Determination of pesticides and polychlorinated biphenyls (PCBs) Clean-up methods Retrieved from: https://www.epa.gov/sites/default/files/2015-12/documents/8082a.pdf

33. Commission Regulation (EU) No 589. (2014). Commission Regulation (EU) No 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 252/2012. In Official Journal of the European Union (Vol. 3, pp.18–40). Publications Office of the European Union.

34. Commission Decision of 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (notified under document number C (2002) 3044), text with EEA relevance 2002/657/ EC. In Official journal of the European Union (L 221, pp. 8–36).

35. Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues there of in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC.

36. SANTE 11312/2021 (2021). Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. Retrieved from: https://www.eurl-pesticides.eu/userfiles/file/EurlALL/SANTE_11312_2021.pdf

37. Levchuk, I. V. (2014). Determination of polychlorinated biphenyls (PCBs) in oils and fats. In Intehrovani Tekhnolohii ta Enerhozberezhennya (Vol. 1, pp. 113–120). National Technical University "Kharkiv Polytechnic Institute".

38. Ostadgholami, M., Zeeb, M., Amirahmadi, M., Daraei, B. (2024). Multivariate Optimization and Validation of a Modified QuEChERS Method for Determination of PAHs and PCBs in Grilled Meat by GC-MS. In Foods (Vol. 13, p. 143). Multidisciplinary Digital Publishing Institute (MDPI). https://doi.org/10.3390/foods13010143

39. Chmil, V. D., Golohova, O. V., Vydrin, D. J., Kalashnikov, A. A. (2016). Prospects of using the QuEChERS method to assess the toxicological safety of food products. In Problemy harchuvannja (Vol. 2, pp. 69–78). Scientific center of preventive toxicology, food and chemical safety named after Academician LI Medved.

40. Senin, S. A., Midyk, S. V., Kornienko, V. I., Konovalova, O.Yu., Berezovskiy, O. V., Ladohubets, E. V., Harkusha, I. V. (2023). Determination of Mycotoxins in Wheat Grain by LC-MS/MS using Modified QuEChERS Sample Preparation. In Methods and Objects of Chemical Analysis (Vol. 18, Issue 1, p. 22–28). Taras Shevchenko National University of Kyiv. https://doi.org/10.17721/moca.2023.22-28

41. Ottonello, G., Ferrari, A., Magi, E. (2014). Determination of polychlorinated biphenyls in fish: Optimisation and validation of a method based on accelerated solvent extraction and gas chromatography–mass spectrometry. In Food Chemistry (Vol. 142, pp. 327–333). Elsevier BV. https://doi.org/10.1016/j.foodchem.2013.07.048

42. Afful, S., Awudza, J. A. M., Twumasi, S. K., Osae, S. (2013). Determination of indicator polychlorinated biphenyls (PCBs) by gas chromatography–electron capture detector. In Chemosphere (Vol. 93, Issue 8, pp. 1556–1560). Elsevier BV. https://doi.org/10.1016/j.chemosphere.2013.08.001

43. Skrynnyk M.M. (2017). Determination of organochlorine pesticides and polychlorinated biphenyls in aquatic organisms by chromato-mass-spectrometry method. [Candidate dissertation, Uzhgorod National University]. Uzhgorod National University. Retrieved from: https://www.uzhnu.edu.ua/uk/infocentre/get/11325

44. DSTU 4514, 2006. Fish, other water fresh resources and food stuffs from them. Detection of chlorine-organik pesticids and polychlorinated bifenils by method of gas-liquid chromatography.

45. Method 8082A. (2000). Polychlorinated biphenyls (PCBs) by gas chromatography. https://www.accustandard.com/media/assets/8082a.pdf

46. Method 3540C. (1996). Soxhlet extraction. Retrieved from: https://www.epa.gov/sites/default/files/2015-12/documents/3540c.pdf

47. Method 3541. (1994). Automated Soxhlet extraction. Retrieved from: https://www.epa.gov/sites/default/files/2015-06/documents/epa-3541.pdf

48. Method 3545. (1996). Pressurized fluid extraction (PFE). Retrieved from: http://www.cromlab.es/Articulos/Metodos/EPA/3000/3545.PDF

49. Method 3546. (2007). Microwave extraction. Retrieved from: https://www.epa.gov/sites/default/files/2015-12/documents/3546.pdf

50. Method 3550 B. (1996). Ultrasonic extraction. http://www.cromlab.es/Articulos/Metodos/EPA/3000/3550B.PDF

51. Method 3562. (2007). Supercritical fluid extraction of polychlorinated biphenyls (PCBs) and organochlorine pesticides. Retrieved from: https://www.epa.gov/sites/default/files/2015-12/documents/3562.pdf

52. Zheplinska, M., Mushtruk, M., Vasyliv, V., Sarana, V., Gudzenko, M., Slobodyanyuk, N., Kuts, A., Tkachenko, S., & Mukoid, R. (2021). The influence of cavitation effects on the purification processes of beet sugar production juices. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 18–25). HACCP Consulting. https://doi.org/10.5219/1494

53. Nardelli, V., D'Amico, V., Casamassima, F., Gesualdo, G., Li, D., Marchesiello, W. M. V., Nardiello, D., Quinto, M. (2019). Development of a screening analytical method for the determination of non-dioxin-like polychlorinated biphenyls in chicken eggs by gaschromatography and electron capture detection. In Food Additives & Contaminants: Part A (Vol. 36, Issue 9, pp. 1393–1403). Taylor & Francis. https://doi.org/10.1080/19440049.2019.1627002

54. DSTU IEC 61619, 2019. Insulation liquids. Contamination by polychlorinated biphenyls (PCBs). Method of determination by capillary column gas chromatography (IEC 61619:1997, IDT).

Funds: This research received no external funding.

Acknowledgments: We would like to thank you to Dr. For Valentyna Korniyenko.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Statement: This article does not contain any studies that would require an ethical statement.

Contact Address:

Valentyna Korniyenko, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, Ukrainian Laboratory of Quality and Safety of Agricultural Products, Heroiv Oborony Str., 15, 03041 Kyiv, Ukraine,
E-mail: kornienko-valentina1966@ukr.net
ORCID: https://orcid.org/0000-0003-0403-7727

***Svitlana Midyk**, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, Ukrainian Laboratory of Quality and Safety of Agricultural Products, Heroiv Oborony Str., 15, 03041 Kyiv, Ukraine,
E-mail: svit.mid@gmail.com
ORCID: https://orcid.org/0000-0002-2682-2884

Serhii Senin, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, Ukrainian Laboratory of Quality and Safety of Agricultural Products, Heroiv Oborony Str., 15, 03041 Kyiv, Ukraine,
E-mail: sergejsen@gmail.com
ORCID https://orcid.org/0000-0001-7972-5714

Oleksandr Machuskyy, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, Heroiv Oborony Str., 15, 03041 Kyiv, Ukraine,
E-mail: dr.machuskyy@yahoo.com
ORCID: https://orcid.org/0000-0002-3154-1972

Olena Ladogubets, State Biotechnological University, St. Alchevsky, 44, Kharkiv, 61002, Ukraine,
E-mail: ladohubets@gmail.com
ORCID: https://orcid.org/0000-0002-0977-5940

Ivan Harkusha, State Biotechnological University, St. Alchevsky, 44, Kharkiv, 61002, Ukraine,
E-mail: 0957785723@btu.kharkov.ua
ORCID: https://orcid.org/0000-0002-6249-2878

Yuriy Balym, State Biotechnological University, St. Alchevsky, 44, Kharkiv, 61002, Ukraine,
E-mail: yuriy.balim@gmail.com
ORCID: https://orcid.org/0000-0002-2494-1329

Alina Menchynska, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products Department of Technology of meat, fish and Marine Products, Polkovnyka Potekhina, str. 16, Kyiv, 03040, Ukraine,
E-mail: menchynska@ukr.net
ORCID: https://orcid.org/0000-0001-8593-3325

Corresponding author: *

potravinárstvo®

OPEN ACCESS

Received: 26.6.2024
Revised: 23.7.2024
Accepted: 24.7.2024
Published: 21.8.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 755-775
https://doi.org/10.5219/1997
ISSN: 1337-0960 online
www.potravinarstvo.com

The influence of natural antioxidants on the quality and storage capacity of semi-finished horse meat products

Nazym Abilmazhinova, Aigul Tayeva, Bagila Jetpisbayeva, Madina Kozhakhieva, Gaukhar Kuzembayeva, Karlygash Abdiyeva

ABSTRACT

The article is devoted to the influence of natural antioxidants on the quality parameters of meat semi-finished products from horse meat and the microbiological stability of the product during its storage. In solving the set tasks, the standard, generally accepted methods of research were used. Statistical processing of the obtained results and assessment of data reliability were carried out by statistical methods using Microsoft Excel and Statistica (version 12.0). A new antioxidant of natural origin, dihydroquercetin, has been proposed and tested. Effective doses of its application have been established: 0.05 - 0.075% for raw horse fat and 0.075% for semi-finished meat products from horse meat produced with its addition. It has been shown that the administration of dihydroquercetin (Dx) together with ascorbic acid (AA) and tocopherol (Tp) is 10-30% more effective. The effect of dihydroquercetin on the indicators of oxidative spoilage in the technological process, from the production of horse meat to finished products using the latter, has been studied. The organoleptic parameters (appearance, odour, colour) of the control sample and the sample with 0.025% Dx by fat weight corresponded in their characteristics to the experimental samples with 0.05%, 0.075% and 0.1% by 45 days of storage. The introduction of dihydroquercetin, ascorbic acid, and tocopherol also makes it possible to slow down the microbiological spoilage of the semi-finished products that have been obtained during storage.

Keywords: semi-finished meat product, horse meat, raw horse fat, dihydroquercetin, ascorbic acid, tocopherol, peroxide value, acid value, thiobarbituric value

INTRODUCTION

Horse meat is used as a source of meat in Asia and some European countries. Horse meat is especially popular in Kazakhstan, Mongolia, and Kyrgyzstan, but it is also consumed in European countries, such as Sweden, Belgium, and France. Horsemeat has a high content of substances important for the human body, particularly amino acids, vitamins (A, group B, E and PP) and minerals (phosphorus, sodium, copper, iron, potassium, magnesium). Compared to the meat of other animals, horse meat contains more complete protein, organic acids and water. Horse meat is distinguished by a high content of complete, high-quality protein balanced in amino acid composition. Absorption of horse meat in the body occurs approximately eight times faster than beef absorption. According to nutritionists, horse meat fats differ from fats from other meat types in their high polyunsaturated fatty acids content. Due to its ability to lower cholesterol in the blood, horse meat helps regulate metabolic processes in the body, and its low-fat content allows it to be used in weight loss diets **[1]**.

The Republic of Kazakhstan has great opportunities to increase the industrial processing of horse meat into various meat products, which have recently become increasingly popular worldwide, especially in Western Europe. After epidemics of foot-and-mouth disease and “mad cow disease”, the popularity of horse meat in European countries is growing. In France, where residents previously consumed horse meat as a delicacy, consumption of this meat has increased by more than 60 %. They plan to replace beef with horse meat in the menu

of school canteens in Italy. In European countries, horse meat costs are high **[2]**. In this regard, producing high-quality export-oriented meat products from horse meat is promising for the Republic of Kazakhstan **[3]**.

The leaders in the number of horses worldwide are the USA, Mexico, Brazil, Mongolia, and China. Kazakhstan has more than 3 million horses, and the population growth in recent years has been about 10 % **[4]**.

Nevertheless, the shortage of raw materials and the predominance of imported meat, which is not always of acceptable quality, determine the tasks for scientists' research in creating technologies for new meat products using domestic raw materials of animal and plant origin.

The increasing demand for chopped semi-finished meat products from the modern consumer has determined the direction of current research based on the use of raw domestic meat materials and components of plant origin in the technology of products with high nutritional value and low cost **[5]**.

Widely popular among the Republic of Kazakhstan's population, cutlet meat is formed after isolation, consisting of pieces of meat pulp of various sizes and weights obtained from trimmings from different parts of the carcass. Scientific literature analysis and patent searches showed that horse meat cutlets are limited due to the need for more technology based on scientific research **[6]**.

At the same time, horse meat products are produced in small quantities at meat processing plants, which is explained by the specifics of the raw material and the limited scientifically based recommendations for its integrated use in producing meat products. Important The production and social task is stabilising oxidative processes in horsemeat lipids through additives to increase the shelf life of minced semi-finished meat products **[7]**, **[8]**.

Oxidative changes in fats are inevitable during storage of any food product. Scientists have researched lipid oxidation and its mechanism, and much attention has been given to the problem of fat oxidation in meat products because oxidative processes influence the formation of the quality and safety of meat and meat products. Oxidative processes cause a decrease in the content of essential fatty acids and vitamins, deterioration in colour, taste, consistency and the appearance of foreign odours, and also reduce the shelf life of products **[9]**. Preventing fat oxidation is paramount to the meat industry, helping to increase the production of quality, flavorful products and extend their shelf life **[10]**.

In this regard, it is of interest to search for innovative methods of processing horse meat, such as the use of natural stabilisers, which allow you to regulate the properties of raw materials, increase shelf life, and expand the range while simultaneously improving the quality of the finished product.

The purpose of these studies is to study the effect of antioxidants of natural origin on the quality indicators and shelf life of semi-finished horse meat products.

Scientific Hypothesis

The use of antioxidants of natural origin during storage inhibits lipid oxidation processes without reducing the quality and safety indicators in meat semi-finished products.

MATERIAL AND METHODOLOGY

Samples

The following raw materials were used to produce experimental samples of meat semi-finished products from horse meat: horse meat of 1 grade, raw horse fat, dihydroquercetin, ascorbic acid, and tocopherol.

Chemicals

Hexane (for HPLC ≥ 95%), chloroform (anhydrous ≥ 99%, contains 0.5–1.0% ethanol as a stabiliser – $CHCl_3$), methanol (anhydrous 99.8% – CH_3OH), ethyl alcohol (C_2H_5OH), potassium hydroxide (KOH), phenolphthalein, sodium chloride (BioXtra ≥ 99.5% (AT) – NaCl), nitrogen ≥ 99.998% (N_2), barium chloride ($BaCl_2$), ferrous sulfate heptahydrate/iron (II) sulfate heptahydrate/($FeSO_4$), hydrochloric acid reagent grade, 37% (HCl), ammonium thiocyanate (NH_4SCN), iron (II) chloride – anhydrous, beads −10 mesh, 99.99% trace metals basis ($FeCl_2$), trichloroacetic acid, 25% alcohol solution (AS), butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA), malondialdehyde – $CH_2(CHO)_2$(MDA) and ascorbic acid (AA), tocopherol (Tp) were purchased from Sigma-Aldrich, Inc. (Merck KGaA, Darmstadt, Germany). Dihydroquercetin (Dx) with purity ≥ 96% was purchased from Flavitlife Bio JSCo (Sofia, Bulgaria) **[10]**.

Instruments

A Camspec model M550 dual-beam UV-VIS spectrophotometer (Camspec Ltd, Kembridge, UK) was used to determine peroxide number (POV) and 2-thiobarbituric acid reactive substances (TBARS).

Laboratory Methods

Laboratory studies of raw materials were conducted at JSC "Almaty Technological University" (Almaty, Kazakhstan) and in the meat processing training center at the "University of Food Technologies" (Plovdiv, Bulgaria).

The POV was determined spectrophotometrically based on the oxidation of Fe2+ to Fe3+ in hydroperoxides. The formation of a colour compression between the Fe3+ and SCN was obtained by the method of Stine et al. **[11]** and refined by Schmedes and Holmer **[12]**. The absorption was measured at 507 nm.

The extracted lipids' acid value (AV) was determined according to EVSEN ISO 660:2009 procedure **[13]**. The extracted lipids were dissolved in ethyl alcohol (99%) and heated for about 2 min before being titrated while still hot against 0.1 M KOH using phenolphthalein as an indicator.

TBARS were determined by Botsoglou et al. **[12]**.

Organoleptic evaluation establishes whether products comply with the standard's requirements regarding the main qualitative indicators (appearance, colour, odour, aroma, taste, and consistency) **[14]**.

Determination of microbiological parameters according to SST 9958-81 **[15]**. Studies were conducted to determine the number of mesophilic aerobic and facultative-anaerobic microorganisms, *E. coli* bacteria (coliforms), sulfite-reducing clostridia, and *S. aureus*. Meat-peptone agar was used for all microbiological analyses. (BioMedia, Russia).

Description of the Experiment

Sample preparation: A chilled to 1 °C horse shoulder (first category of fatness) was used. The horse meat was in the shape of semi-circular pieces, not heavier than 0.2 kg, and had a thickness of about 10 cm. The meat pieces were ground in a meat mincer (wolf machine) with holes that were 3 mm in diameter and separated into 9 samples of 100 g each. Then add raw horse fat and mix. Weigh dihydroquercetin, ascorbic acid and tocopherol on a bench scale. In one part of the test samples we add dihydroquercetin, ascorbic acid and tocopherol in dry form in different dosages. In the other part of the experimental samples, we add dihydroquercetin in 25% alcohol solution at room temperature until complete dissolution after adding ascorbic acid and tocopherol. The ready minced meat is mixed in a mincer.

Number of samples analyzed: A total of 13 samples were analysed.

Number of repeated analyses: All instrument readings were measured three times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was three times.

Design of the experiment: Standard methods were used to determine the effect of storage time on lipid oxidation, organoleptic studies, and microflora content during the storage of semi-finished meat products from horse meat.

Statistical Analysis

To analyse the test parameters of meat semi-finished products from horse meat, statistical analysis of the obtained data was carried out, and the reliability of the obtained data was assessed by statistical methods using Microsoft Excel and Statistica program (version 12.0). Statistical functions of mean value and standard deviation were used to describe continuous variables. Graphical interpretation of the results was performed using Microsoft Excel. The statistical analysis results are presented in Figures 10-19, with each value being the mean of at least 9 determinations. We then calculated each measurement's error and the squares of the errors to calculate the absolute error of the measurement. We chose a reliability value of p = 0.95 **[16]**.

RESULTS AND DISCUSSION

Research has been carried out on studying the influence of dihydroquercetin, ascorbic acid, and tocopherol on quality and safety indicators of meat semi-finished products from horse meat during storage at their joint use.

In several scientific research works, scientists have experienced the introduction of dihydroquercetin in poultry meat **[17]**, ground beef **[18]**, semi-finished broiler meat **[19]**, semi-finished products from moose meat **[20]**, and pork fat **[21]**.

Samples were developed from raw horse fat to determine application methods and select an effective dosage of dihydroquercetin. Dx was added in dry form to one part of the experimental samples in dosages of 0.01%, 0.025%, 0.05%, 0.075, 0.1%, and 0.2%. The minimum study dosage of 0.01% was chosen according to the recommendations of the Dx manufacturer.

From an analysis of literary sources **[22]**, it is known that Dx is highly soluble in a water-spirit solution; therefore, samples of raw horse fat with similar dosages of Dx but previously dissolved in a 25% alcohol solution (AS) were also produced.

The control sample without adding Dx. After production, the prototypes were stored at t =4 ±2°C.

The results obtained on the accumulation of peroxides (Figure 1) clearly show that in the control sample, from the fourth day of storage, there was an active accumulation of primary fat oxidation products.

In samples with a Dx content of 0.01%, the peroxide value by 12 days of storage was only 1.5% and 4.5% lower than that of the control, indicating an insufficient Dx dosage. Dosages of Dx starting from 0.05% and up to

0.075% were more effective in reducing peroxides. As seen in Figure 1, an alcohol solution of Dx is much more effective in reducing the accumulation of peroxides.

Dynamics of changes in acid value also showed (Figure 2) that a dosage of 0.01% is not enough. The acid value for the control sample and samples with a dosage of 0.01 - 0.025% remained almost the same throughout the entire storage period. At the same time, the acid value of the remaining samples was 1 mg/kg less than that of the first three samples. The acid value values for samples containing an alcohol solution of Dx were significantly lower than samples with the same amount of Dx but added in dry form.

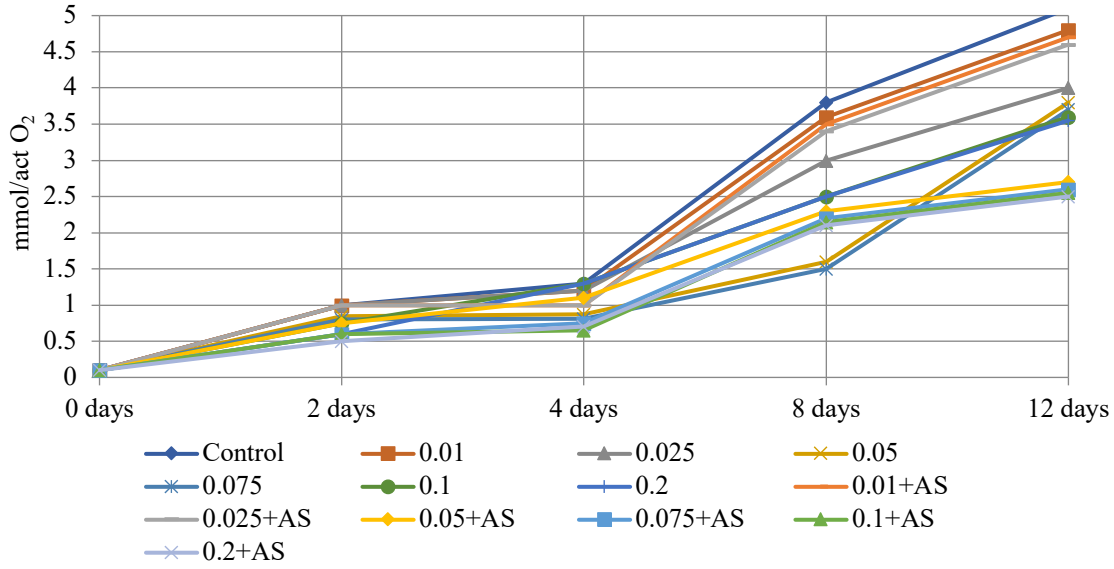


Figure 1 Dynamics of changes in the peroxide value of horse fat during storage (mmol/act O_2).

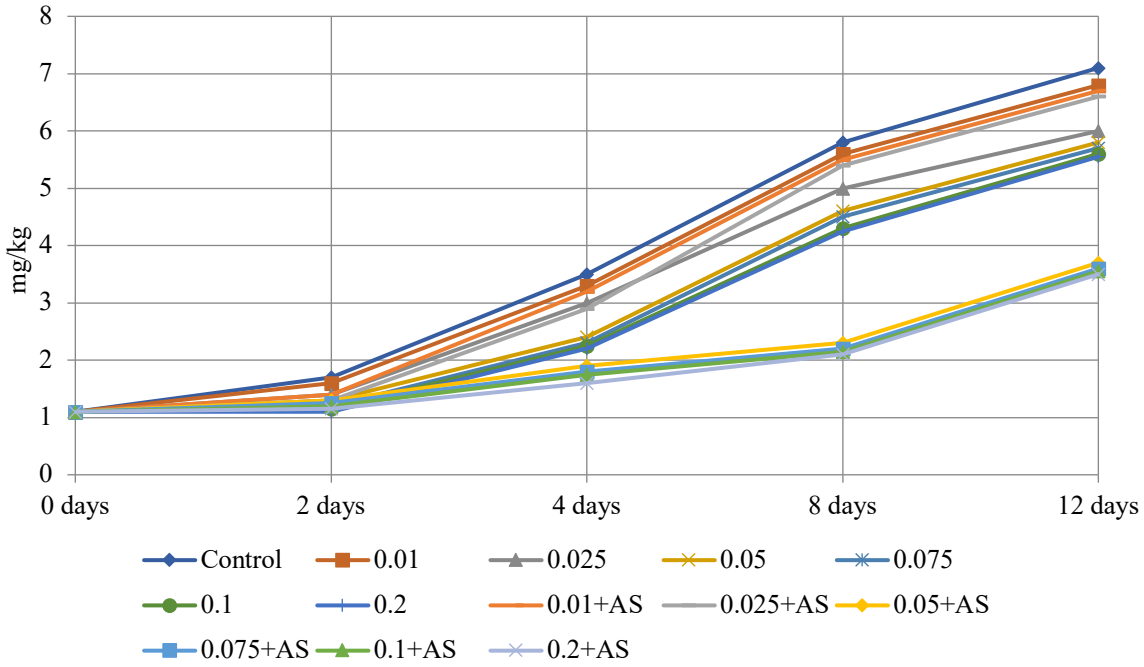


Figure 2 Dynamics of changes in the acid value of horse fat during storage (mg/kg).

The accumulation of products of secondary breakdown of fats occurred very intensively in the control sample (Figure 3). It exceeded the established norm for thiobarbituric value of 0.5 mg/kg already on the 8th day of storage. In contrast, not a single test sample with Dx content had the normalised value for thiobarbituric value was exceeded. However, in samples with a Dx content of more than 0.05%, the accumulation of secondary fat oxidation products occurred two times slower.

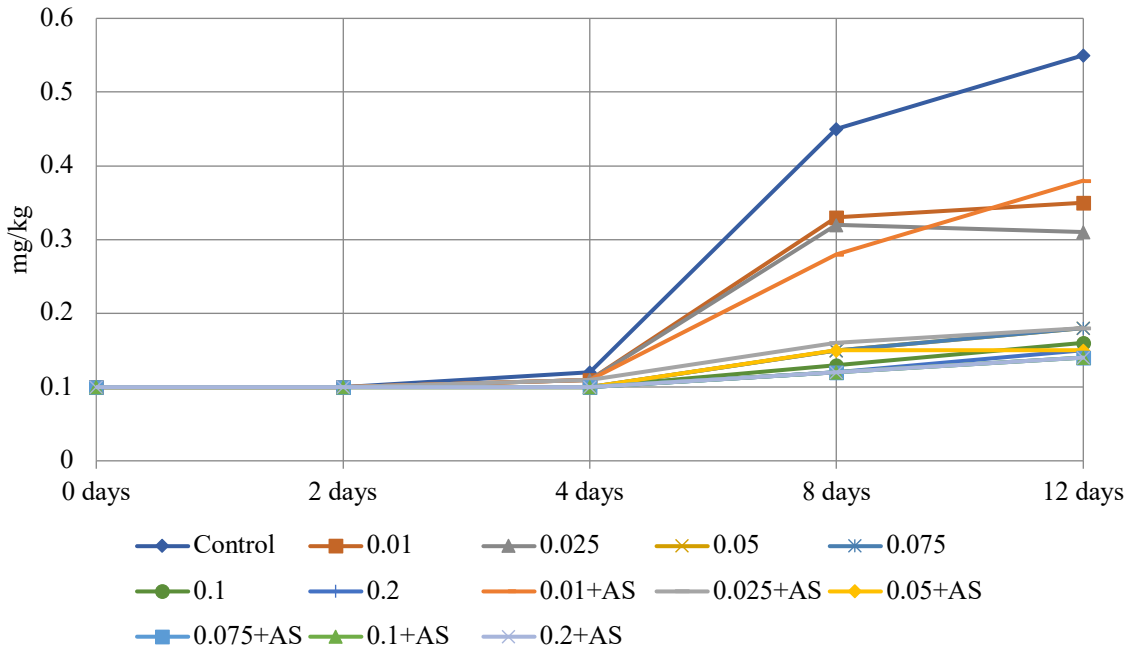


Figure 3 Dynamics of changes in the thiobarbituric value of horse fat during storage (mg/kg).

Studies conducted on raw horse fat have shown that a dosage of 0.01% Dx is ineffective in reducing fats' oxidative spoilage. Dosages of Dx from 0.05% to 0.2% suppressed the development of oxidative spoilage in test samples, and increasing the dosage fourfold (up to 0.2%) slightly reduced the values of peroxide, acid, and thiobarbituric.

The results obtained also clearly show that introducing Dx in the form of an alcohol solution was more effective, obviously due to its more uniform distribution over the fat mass.

Thus, a dosage of Dx from 0.05% to 0.075% by weight of fat in an alcohol solution is most effective for maintaining the quality and safety of raw horse fat.

In meat products technology, ascorbic acid is added to the recipes of semi-finished meat products in the recommended dosage of 0.05% and tocopherol—0.02%. Thus, it is advisable to study the effect of the above components on the antioxidant capacity of dihydroquercetin and, if necessary, adjust the dosage in meat systems.

To select an effective dosage of dihydroquercetin, experimental samples were developed from raw horse fat, into which 0.01%, 0.025%, 0.05%, and 0.75% by weight of the dihydroquercetin raw material were added in the form of an aqueous-alcohol solution together with the addition of ascorbic acid and tocopherol in dosages of 0.05% and 0.02%, respectively. The control was a sample without the addition of ascorbic acid and tocopherol.

Changing the amount of tocopherol and ascorbic acid added is undesirable since their purpose in meat-containing products is to prevent oxidative spoilage of fats and stabilise colour characteristics.

The results obtained on the accumulation of peroxides (Figure 4) clearly show that the combined addition of tocopherol and ascorbic acid with Dx slows the accumulation of primary fat oxidation products.

In all samples, the peroxide value was 15-30% lower after 12 days of storage when adding tocopherol and ascorbic acid.

The dynamics of changes in acid value (Figure 5) also showed that adding tocopherol and ascorbic acid helps prolong shelf life without losing organoleptic characteristics.

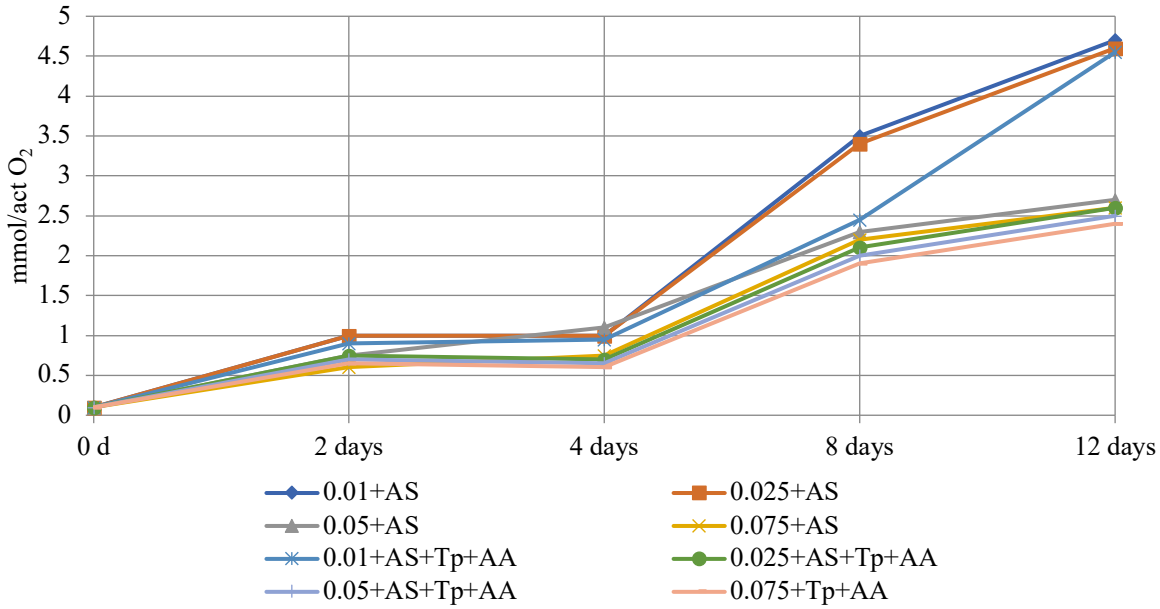


Figure 4 Dynamics of changes in the peroxide value of horse fat during storage (mmol/act O_2).

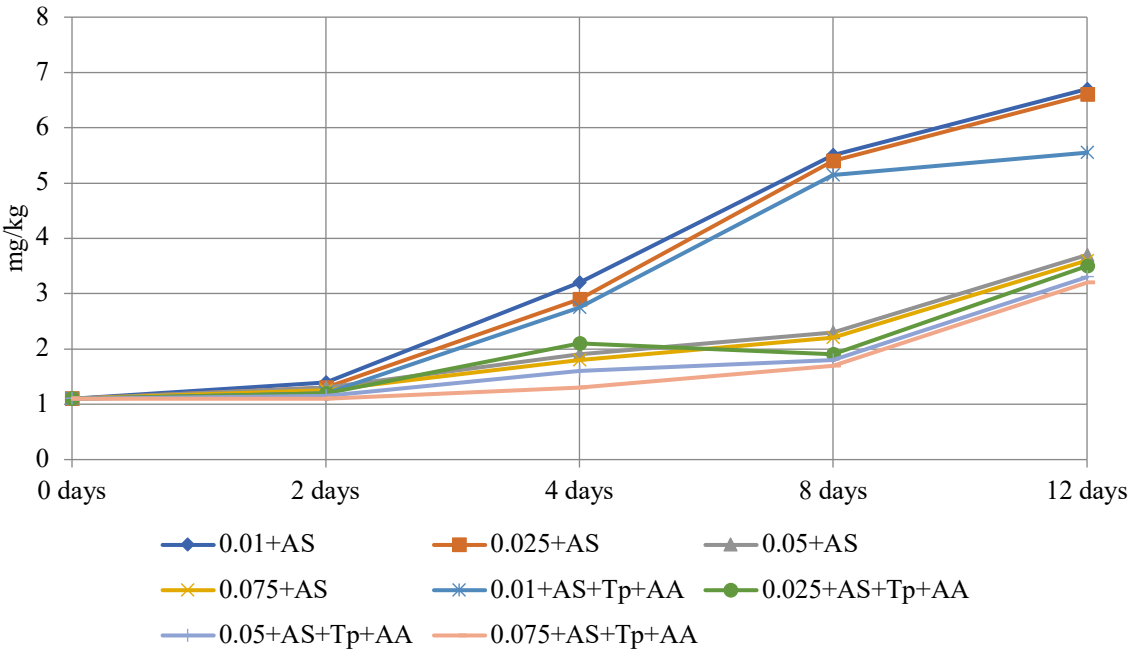


Figure 5 Dynamics of changes in the acid value of horse fat during storage (mg/kg).

The accumulation of products of secondary fat breakdown did not occur intensively in all samples; in control samples (Figure 6), it exceeded the established norm for thiobarbituric value of 0.5 mg/kg 24 days of storage, while in test samples with dosages of 0.025%—0.075%, the normalised value for thiobarbituric value was exceeded only on the 28th day of storage.

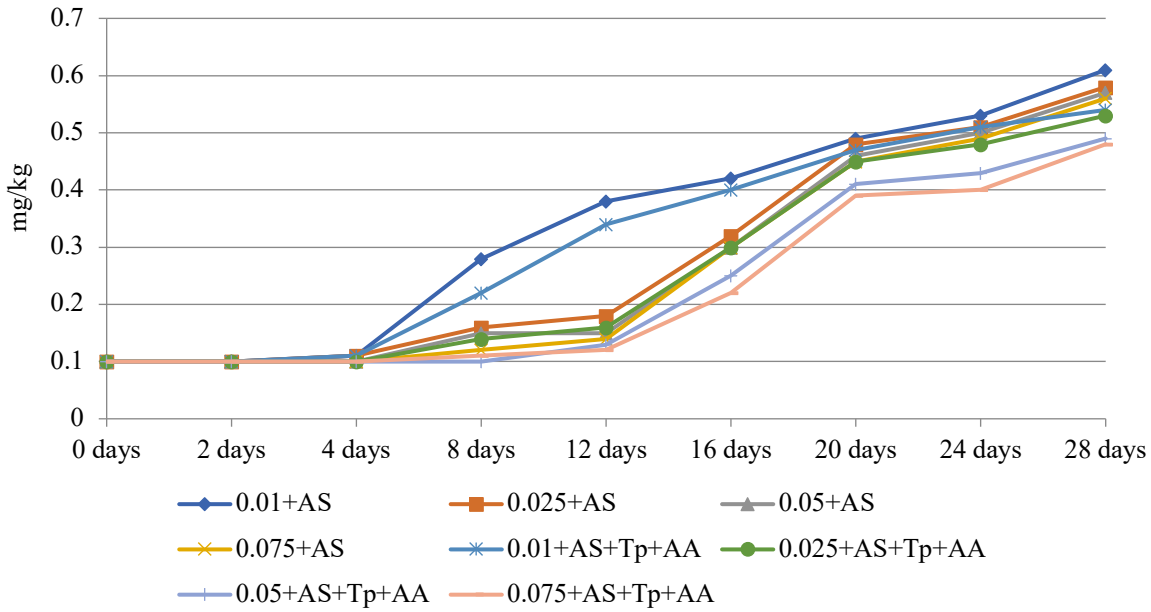


Figure 6 Dynamics of changes in the thiobarbituric value of horse fat during storage (mg/kg).

Fats deteriorate as volatile fatty acids and carbon dioxide accumulate, resulting in a change in aromatic characteristics. In addition, compounds with strong oxidising properties are formed: peroxides, hydroperoxides, free radicals, and atomic oxygen.

To confirm the selected dosage of Dx in raw horse fat, experimental studies were conducted on the storage of horse meat. To solve this problem, test samples were developed: control - without adding Dx, and test samples into which an alcohol solution was added containing Dx to the fat mass of 0.025%, 0.05%, 0.075%, 0.1%, respectively. Ascorbic acid and tocopherol were added to each test sample in the recommended dosages - 0.05% and 0.02%, respectively.

Horse meat with a fat content of 15±1% was used for the research. Horse meat was chosen as a research object for testing the selected dosage of Dx because it contains a large amount of moisture—about 70%—and Dx is poorly soluble in water.

After processing, all obtained samples were separated and stored at -18 ±2°C.

The results of studying the dynamics of accumulation of oxidative spoilage products (Figures 7, 8 and 9) showed that in the control sample, there was an intensive accumulation of peroxides from 3.1 to 22.0 mmol of active 02, i.e. increased over 60 days of storage, by more than 7.1 times the original value. At the same time, an increase in acid value was also observed in the control sample, and by 30 days of storage, the established norm was exceeded by 2 g KOH/kg. The thiobarbituric value value increased 14 times compared to the initial level.

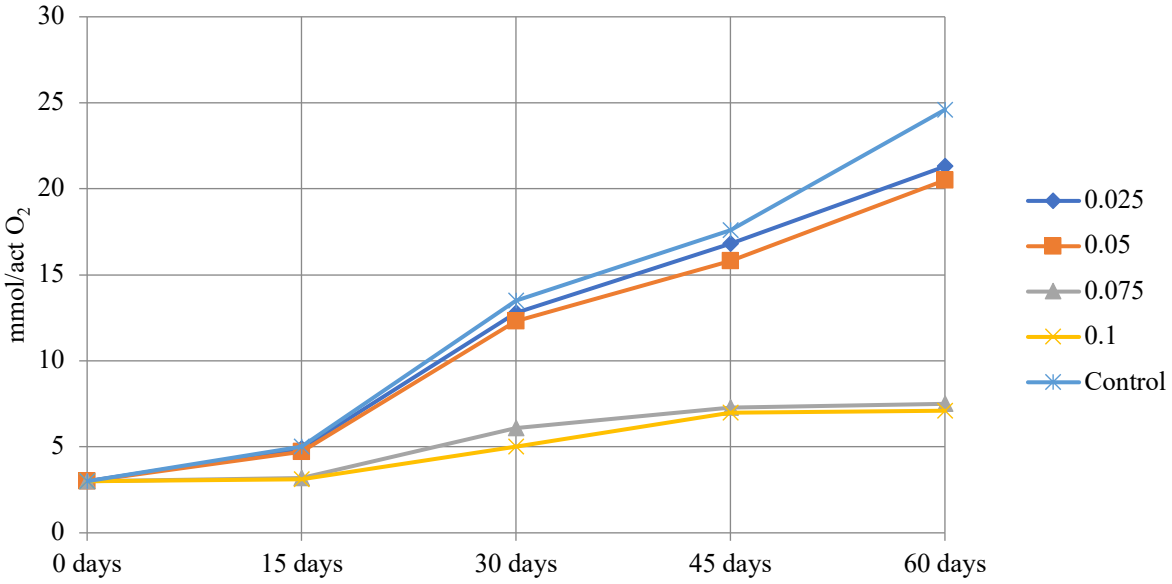


Figure 7 Dynamics of changes in the peroxide value of horse meat during storage (mmol/act O_2).

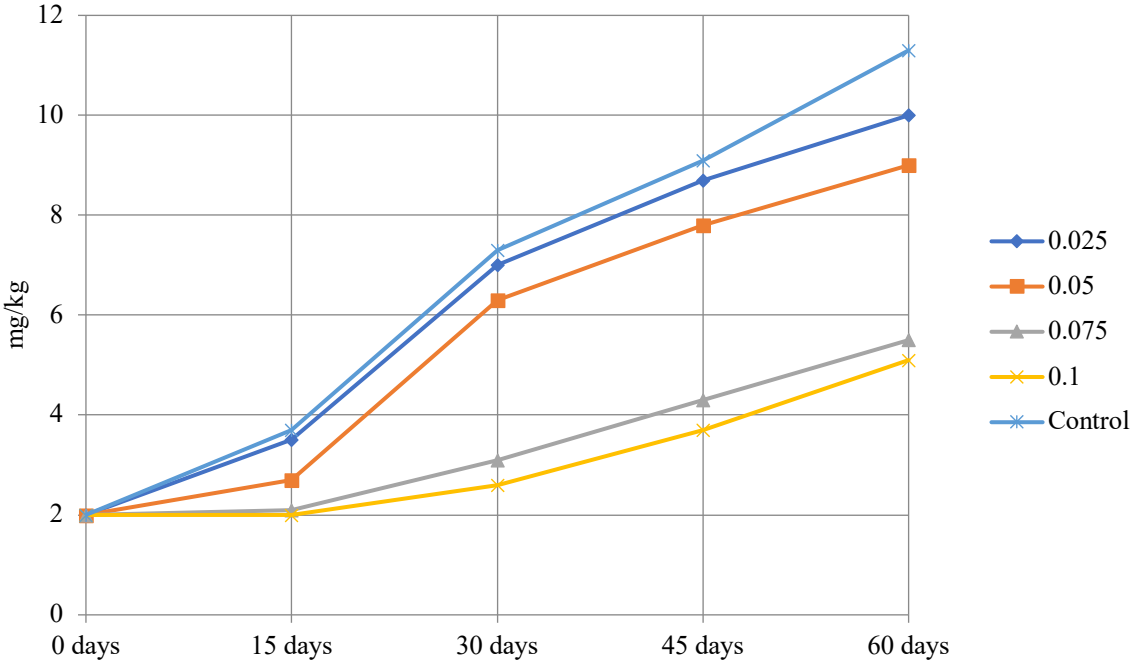


Figure 8 Dynamics of changes in the acid value of horse meat during storage (mg/kg).

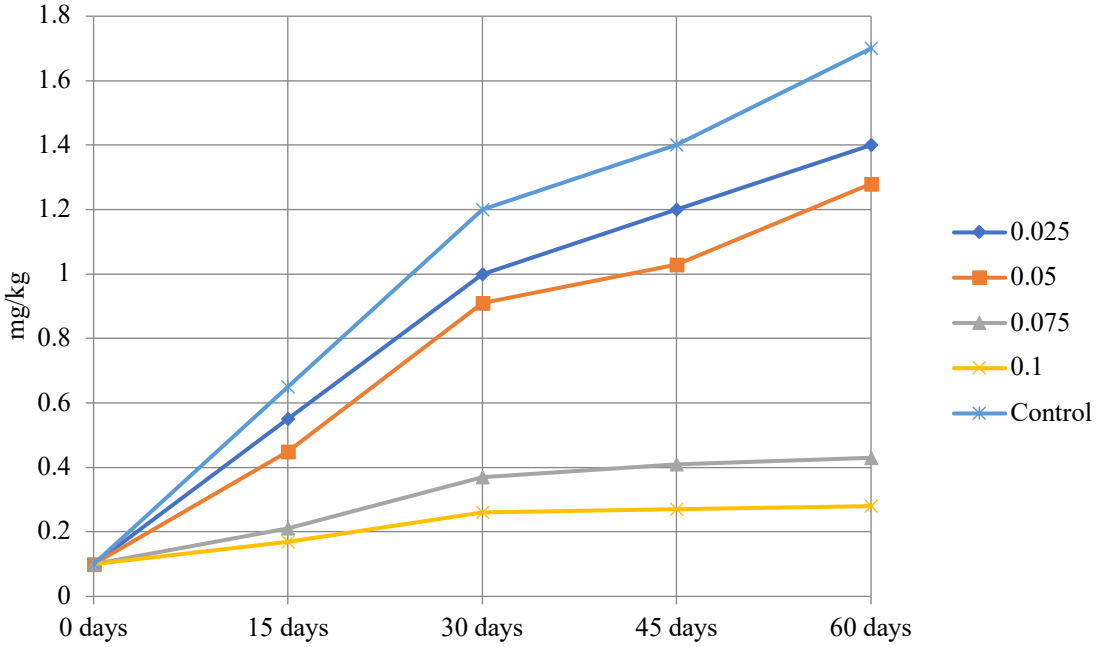


Figure 9 Dynamics of changes in the thiobarbituric value of horse meat during storage (mg/kg).

As can be seen from the results obtained, dosages of 0.025% and 0.05% Dx by fat weight are not effective enough to inhibit the accumulation of oxidation products. By 60 days of storage, samples with a dosage from 0.025% to 0.05% practically did not differ from the control for all studied indicators.

In test samples with a Dx dosage of 0.075% to 0.1%, the accumulation of peroxides proceeded much slower than in the first two samples and at approximately the same rate.

Hydrolytic breakdown of fat in model samples of horse meat during storage led to the accumulation of free fatty acids, which increased the acid value. Samples containing 0.075% and 0.01% Dx by fat weight were resistant to the accumulation of free fatty acids and did not significantly exceed the permissible limit on the 60th day of storage.

According to the organoleptic indicators (appearance, smell, colour) presented in Table 1, on the thirtieth day of storage, the control sample and the sample with 0.025% Dx by weight of fat corresponded in their characteristics to the experimental samples with 0.05%, 0.075% and 0.1 % to 45 days of storage. Already on the 30th day of storage, a pronounced foreign odour was noted in the first two samples, which was associated with the accumulation of fat oxidation products and microbiological spoilage.

The effects of dihydroquercetin, tocopherol, and ascorbic acid on the preservation of hydrolytic and oxidative spoilage rates during the storage of fats and meat products have been documented in several studies. Dihydroquercetin has been shown to improve the stability of ghee fats, with higher concentrations correlating with lower oxidative spoilage rates, thereby extending shelf life **[23]**. Tocopherol, especially α-tocopherol and its acetate form, has been found to protect against lipid oxidation in various meat products by preserving the colour and reducing volatile aldehyde formation **[24]**, **[25]**, **[26]**, **[27]**. Ascorbic acid has demonstrated efficacy in preventing a colour change in irradiated beef and reducing lipid oxidation. However, its effectiveness may vary depending on the age of the meat and storage time after irradiation **[24]**, and ascorbic acid used in combination with rosemary extract effectively retards lipid oxidation in pork meat without affecting other quality parameters **[28]**. Similarly, tocopherol and ascorbic acid have been demonstrated to reduce oxidative reactions in frozen fish **[29]**.

Interestingly, although ascorbic acid generally acts as an antioxidant, it has been observed to stimulate lipid oxidation in certain contexts, such as in cooked pork cutlets **[26]**. Moreover, combining ascorbic acid with tocopherol may have a synergistic effect, as shown in the protection against stress-induced lipid oxidation in chicken meat **[30]**. However, in some cases, ascorbic acid alone did not protect against oxidation, and no synergism was observed between α-tocopherol acetate and ascorbic acid in chicken meat **[25]**.

Table 1 Organoleptic studies of minced horse meat.

Name semi-finished product	Type of packaging	Reaction with copper sulfate	Appearance, color, smell	Broth clarity and aroma
		0 days storage		
Minced horse meat	Control 0.025% 0.05% 0.075% 0.1%	Broth transparent fresh meat	Pink colour. The smell is specific, characteristic of fresh meat.	Transparent, fragrant
		15 days of storage		
Horse meat	Control 0.025% 0.05% 0.075% 0.1%	Broth transparent fresh meat	Pink colour. The smell is specific, characteristic of each type of fresh meat.	Transparent, fragrant
		30 days storage		
Minced horse meat	Control 0.025%	Broth with flakes of questionable freshness	Pink colour. Smell with a hint of mustiness.	Cloudy with a slight unpleasant odor
	0.05% 0.075% 0.1%	Clear broth fresh meat	Pink colour. The smell is specific, characteristic of each type of fresh meat.	Transparent, fragrant
		45 days storage		
Minced horse meat	Control 0.025%	A small amount of flaked meat of questionable freshness	Pink colour.. The smell is slightly musty.	Cloudy with a slight unpleasant odor
	0.05% 0.075% 0.1%	Broth transparent fresh meat	Pink colour. The smell is specific, characteristic of each type of fresh meat.	Transparent, fragrant
		60 days storage		
Minced horse meat	Control	The broth is cloudy, the meat is stale	–	–
	0.025% 0.05%	A small amount of flaked meat of questionable freshness	Pink colour. The smell is slightly musty.	Cloudy with a slight unpleasant odor
	0.075% 0.1%	Broth transparent fresh meat	Pink colour. The smell is specific, characteristic of each type of fresh meat.	Transparent, fragrant

Thus, the optimal dosage for adding an antioxidant component is the addition of an alcohol solution containing Dx to the fat mass from 0.075% to 0.1%, respectively, with the additional addition of ascorbic acid and tocopherol in the recommended dosages - 0.05% and 0.02%, respectively.

As emphasised by **[31]**, adding dihydroquercetin to semi-finished products from meat of broilers enhances their biological activity. It positively affects the quality and yield of finished products without affecting the organoleptic characteristics.

Dihydroquercetin has antioxidant properties that can inhibit the oxidation process in various meat products. Studies have shown that the addition of dihydroquercetin to ground meat can slow down lipid oxidation, thereby increasing oxidative stability and potentially extending the shelf life of the product. These findings are consistent with researchers Bozhko et al. **[32]** and by Bee Cheah et al. **[33]**.

Studies show that dihydroquercetin maximises the consumer properties of chopped semi-finished products in developing oxidative spoilage processes **[34]**. Previous studies of antioxidants in fat storage **[21]** also confirm that it slows the decomposition rate of fats resistant to oxidative deterioration.

Experiments were conducted to determine the optimal ratio of ascorbic acid and dehydroquercetin to be added to horse meat products. The experiment was carried out as follows: dehydroquercetin and ascorbic acid were added to the horse meat product in different proportions, the samples were encrypted, and nothing was added to the control sample. The samples were examined for acid, peroxide and thiabarbituric values.

Below are the results and data processed in the Statistica 12.0 program—determination of acid value. The acid value indicates the degree of hydrolytic breakdown of lipids, in this case, a horse meat product.

The results obtained are presented in the form of response surface graphs. Figure 10 shows the response surface of the acid value depending on the dose of Dx and AA.

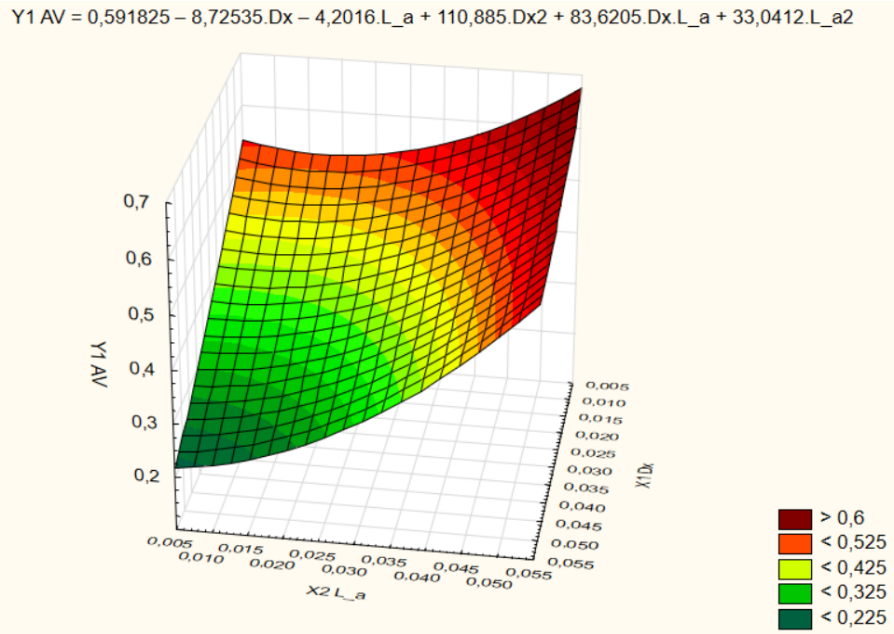
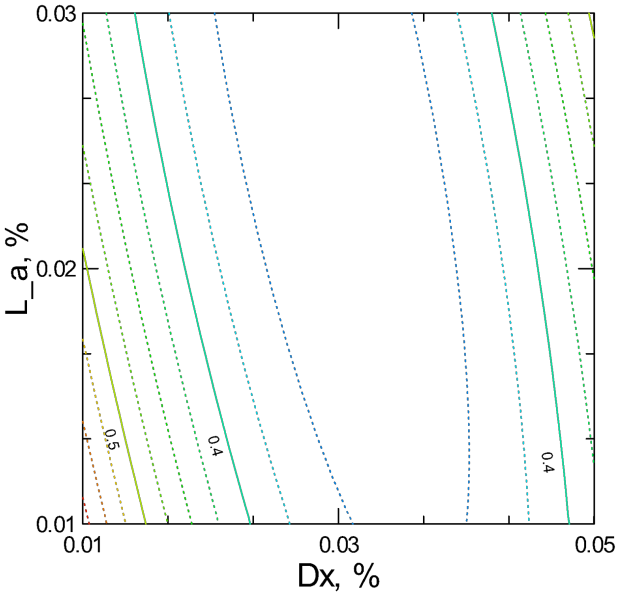


Figure 10 Response surface of the acid value depending on the dose of Dx and AA.

The following mathematical and statistical indicators were obtained when processing experimental data: R-squared = 96.9135%, R - squared (adjusted ford. f.) = 94.7089%, Standard Error of Est. = 0.006, Mean absolute error = 0.0026, Durbin-Watson statistic = 1.84348 (P=0.3264), Lag 1 residual autocorrelation = 0.0780817.

Based on the results of mathematical and statistical analysis with a reliability of 94.7%, it can be said that the acid value is optimal for horse meat with a concentration of ascorbic acid (AA = 0.020%) and concentration of dehydroquercetin (Dx = 0.035%). Figure 11 shows the data processing results, which shows the optimum values.



Where: concentration of AA, %
concentration of Dx, %

Figure 11 Minimum value of AV at concentration AA (AA=0.020%) and concentration Dx (Dx=0.035%).

When processing data in the Statistica 12.0 program, a second-degree equation was obtained indicating the dependence of the AV on the concentration of two antioxidants:

$AV = 0.591825 - 8.72535.Dx - 4.2016.AA + 110.885.Dx^{2} + 83.6205.Dx.AA + 33.0412.AA^{2,}$ cm ^{3} KOH/g

The reliability of the model was checked statistically, and the following data were obtained:

r^2CoefDet	DF Adj r^2	Fit Std Err	F-val
0.97	0.94	0.006158807	43.96

Figure 12 shows the critical limit of the AV, based on the Fisher criterion (blue line) and the degree of significance of various factors:

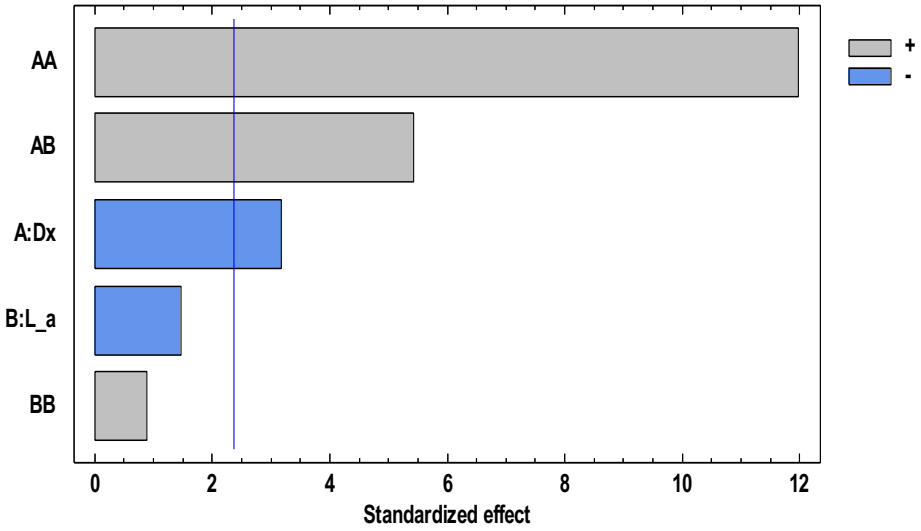


Figure 12 Critical limit of acid value.

The influence of the selected factors on the peroxide value (POV) is shown in Figure 13, as a peroxide value response surface.

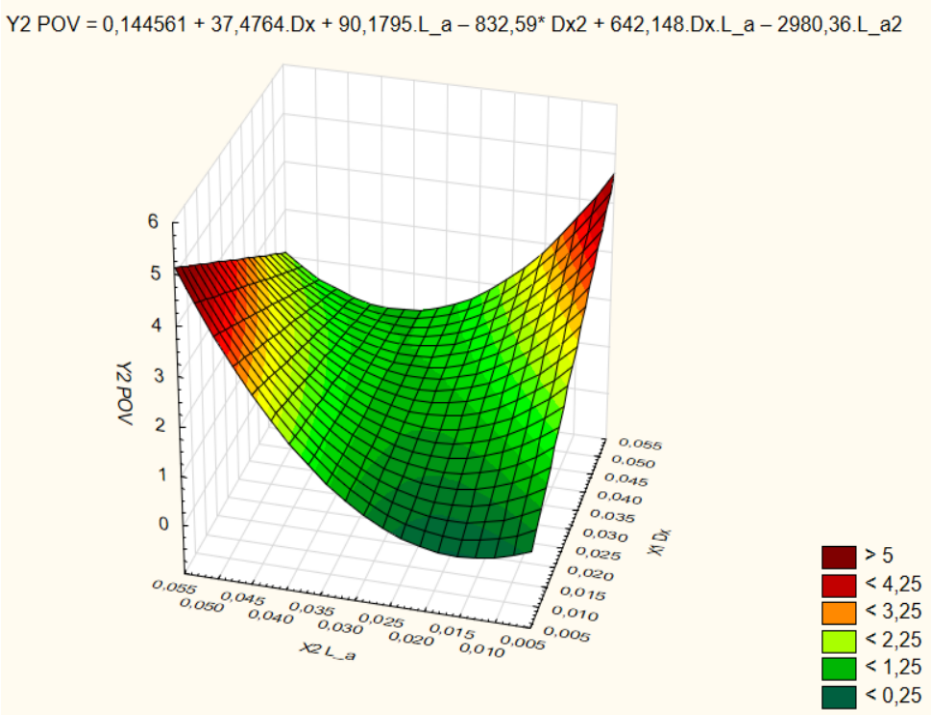


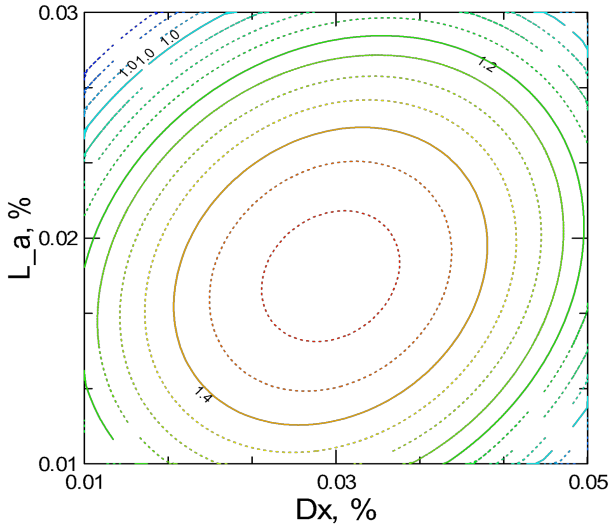
Figure 13 Surface response of peroxide value depending on the dose of Dx and AA.

When determining the peroxide value, which indicates the degree of primary oxidation of lipids and the production of primary oxidation products - hydroperoxides, data were obtained with the following mathematical and statistical indicators:

R-squared = 99.5%, R-squared (adjusted for df) = 99.13%, Standard Error of Est. = 0.0272, Mean absolute error = 0.0166366, Durbin-Watson statistic = 2.53 (P = 0.7746).

Lag 1 residual autocorrelation = -0.321102.

The mathematical and statistical analysis results show that the model is described with an accuracy of 99.5%.



Where : concentration of AA, %
concentration of Dx, %

Figure 14 Minimum values of POV.

As can be seen from Figure 14, the minimum values of the peroxide value are achieved at the concentration of ascorbic acid (AA = 0.030%) and the concentration of dehydroquercetin (Dx = 0.010%) or at the concentration of ascorbic acid (AA = 0.010%) and the concentration of dehydroquercetin (Dx = 0.050%)

Figure 15 shows the critical limit of the peroxide value based on the Fisher criterion (blue line) and the degree of significance of various factors:

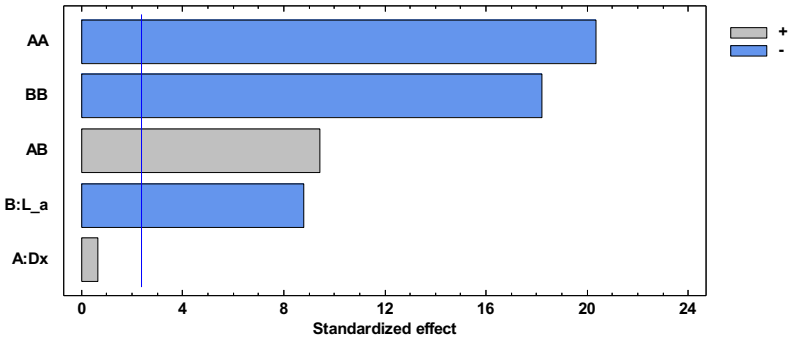


Figure 15 Critical limit of peroxide value.

A second-degree equation is derived, indicating the dependence of the peroxide value on the concentration of two antioxidants:

$$POV = 0.144561 + 37.4764.Dx + 90.1795.AA - 832.59^{*} Dx^{2} + 642.148.Dx.AA - 2980.36.AA^{2,}\ meqvO_{2}/kg$$

Reliability has been statistically proven by the following results:

r^2CoefDet	DF Adj r^2	Fit Std Err	F-val
0.994915	0.991283	0.00166366	273.9187

Thiobarbituric value was also determined, which indicates the degree of secondary oxidation of lipids and the production of secondary oxidation products—malonaldehyde.

Figure 16 shows a thiobarbituric value response surface showing the effect of selected factors on the thiobarbituric value.

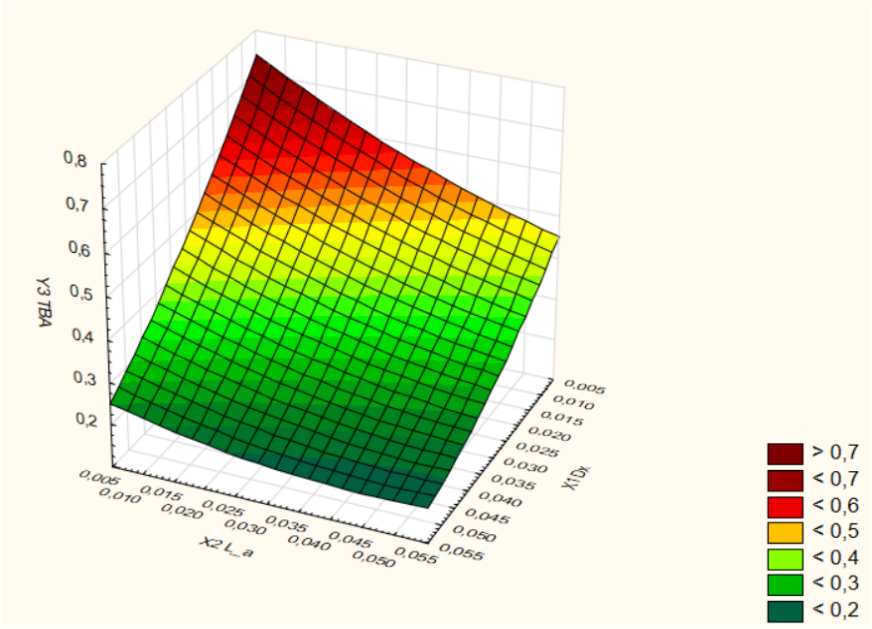
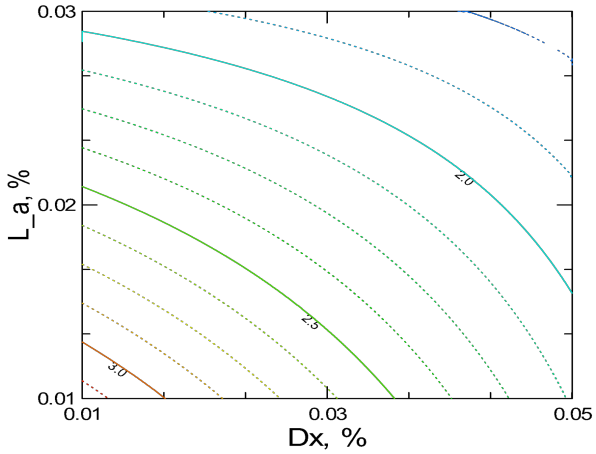


Figure 16 Response surface of the thiobarbituric value depending on the dose of Dx and AA.

When processing the experimental results, the following mathematical and statistical indicators were obtained:

R-squared = 97.0878%, R-squared (adjusted for df) = 95.3404%, Standard Error of Est. = 0.088, Mean absolute error = 0.0509082.

According to mathematical and statistical analysis, the resulting model is 97% reliable, and the standard error is 0.08.



Where: concentration of AA, %
concentration of Dx, %

Figure 17 Thiabarbituric number values.

The minimum values of thiabarbituric value are shown at the concentration of ascorbic acid (AA = 0.030%) and the concentration of dehydroquercetin (Dx = 0.050%)

Figure 18 shows the critical limit of the thiabarbituric value, based on the Fisher criterion (blue line) and the degree of significance of various factors:

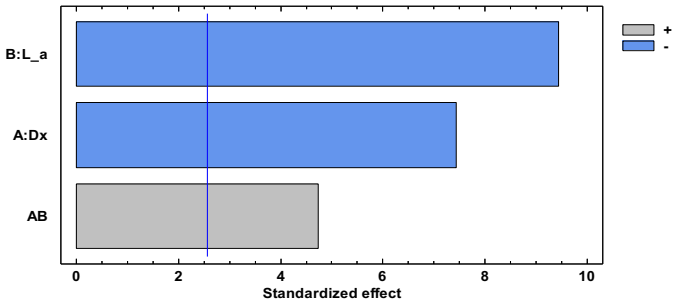


Figure 18 Critical boundary thiabarbituric value

As a result of the research and the results obtained, the Triple Optimum of the problem was obtained (yellow area) which is located at concentration of ascorbic acid (AA = 0.027-0.030%) and dehydroquercetin concentrations (Dx = 0.024 – 0.035%) is shown in Figure 19.

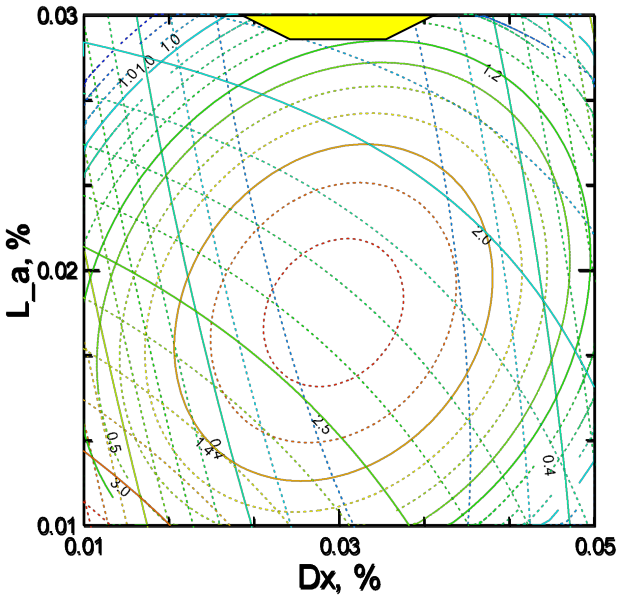


Figure 19 Triple optimum for finding the concentration of two antioxidants.

Plant-based derivatives not only improve some of meat's technological characteristics but can also help reduce the perception of meat products as unhealthy by allowing consumers to include functional compounds beneficial to human health in their daily diet.

In practice, the optimal solution is to add two antioxidants to the semi-finished horse meat product in the following concentration: 0.05-0.02 % ascorbic acid (0.05-0.02 g/kg) and 0.075-0.01 % dehydroquercetin (0.075-0.01 g/kg) **[35]**.

Manufacturers add horse meat to semi-finished meat products to reduce costs and rationally use raw materials.

Horse meat is subject to rapid spoilage, so it is important to study the influence of the proportion of horse meat added on the storage capacity of semi-finished meat products (minced meat).

4 prototypes were compiled:

– control (horse meat without horse fat) with the addition of ascorbic acid, tocopherol and an alcohol solution of Dx at a dosage of 0.075%;

– experiment 1 (horse fat with the addition of 15%) with the addition of ascorbic acid, tocopherol and an alcohol solution of Dx at a dosage of 0.075%;

– experiment 2 (horse fat with the addition of 25%) with the addition of ascorbic acid, tocopherol and an alcohol solution of Dx at a dosage of 0.075%;

– experiment 3 (horse fat with the addition of 35%) with ascorbic acid, tocopherol and an alcohol solution of Dx at a dosage of 0.075%.

The produced semi-finished products were frozen and stored at -18°C for 60 days.

The results of a study of the dynamics of the accumulation of oxidative spoilage products (Figures 20, 21, 22) showed that in the control sample, the accumulation of peroxides occurred somewhat more slowly, which is explained by the lower moisture content. However, all processed samples remained of good quality throughout the entire storage period, and the accumulation of breakdown products of fatty acids and peroxides was within normal limits.

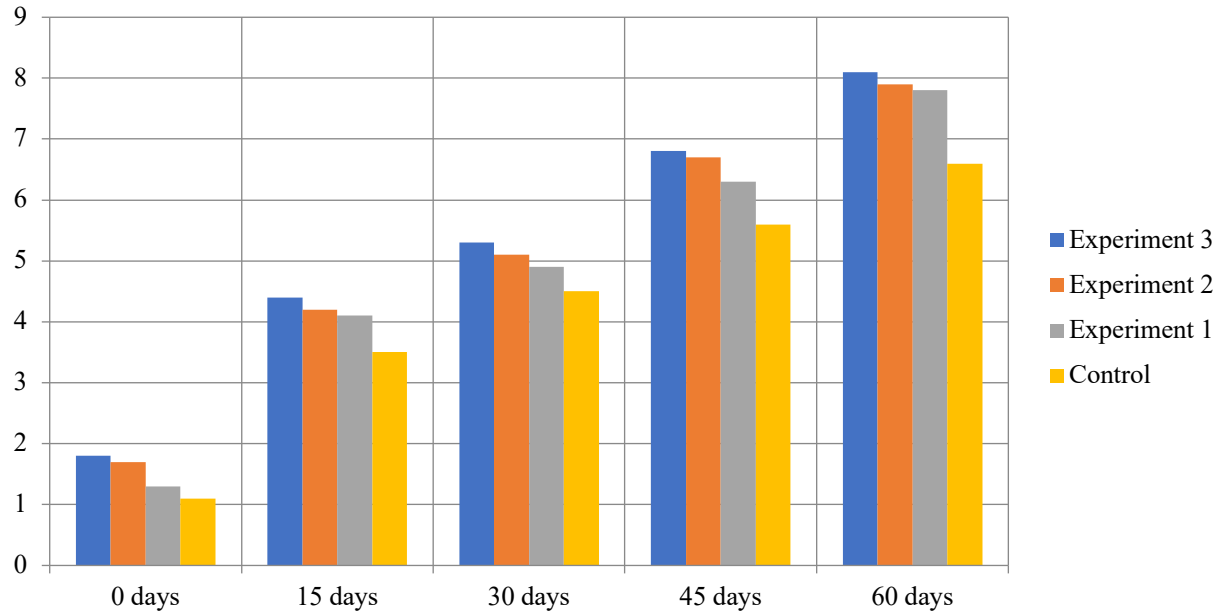


Figure 20 Dynamics of changes in the peroxide value of semi-finished meat products during storage (mmol/act O_2).

The beneficial effect of natural antioxidants on the inhibition of oxidative reactions during storage of both meat semi-finished products and finished products was noted in **[36]**.

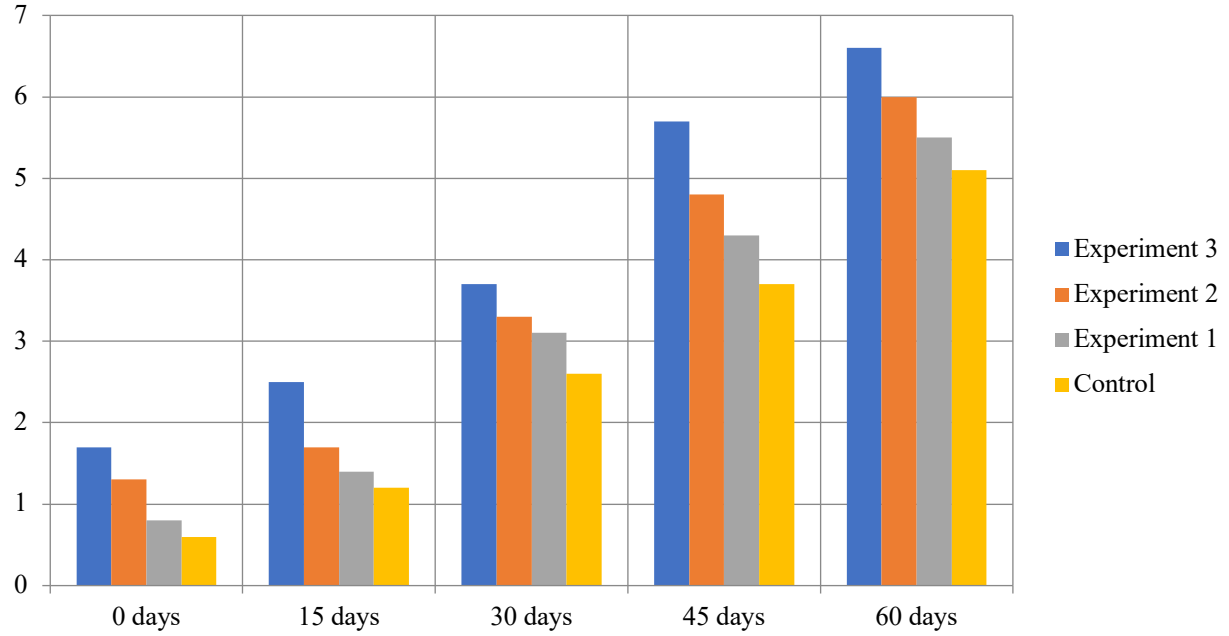


Figure 21 Dynamics of changes in the acid value of semi-finished meat products during storage (mg/kg).

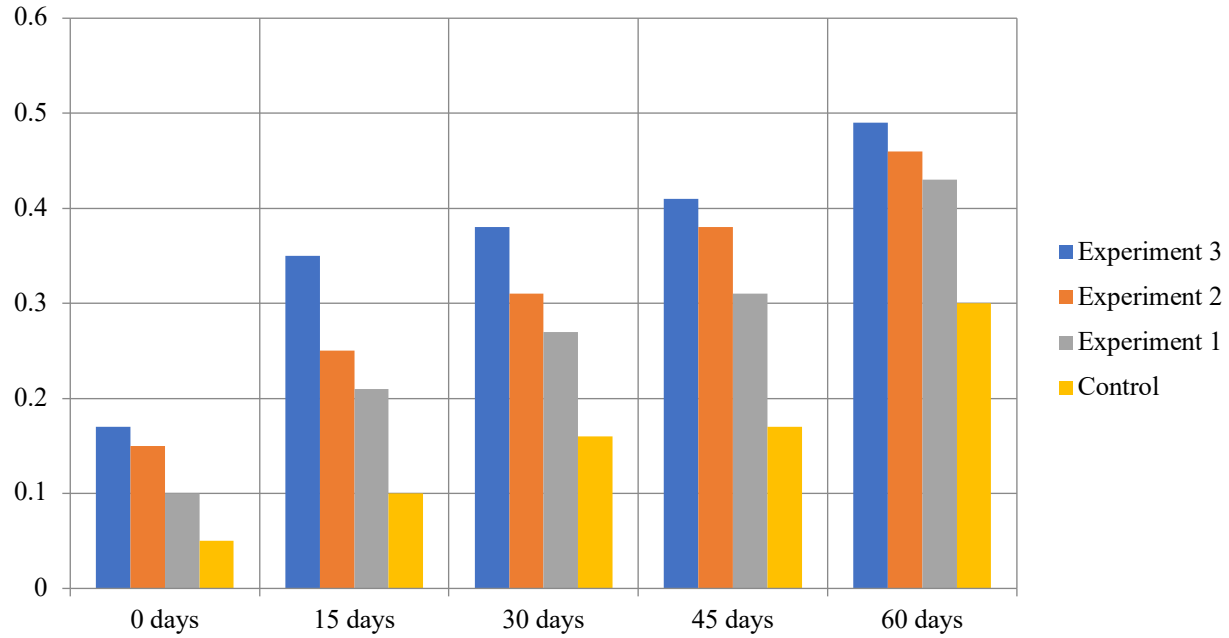


Figure 22 Dynamics of changes in the thiobarbituric value of semi-finished meat products during storage (mg/kg).

The conducted research **[34]** testifies about the achievement of positive technological effects from the application of the natural antioxidant - dihydroquercetin due to the retardation of the development of oxidative spoilage processes. Also, it shows that its use allows keeping consumer properties of minced meat semi-finished products within 6 months and more.

Cold-smoked pork sausage improved the hygienic quality of the sausage with no significant effect on the growth of lactic acid bacteria **[37]**. According to Kuz'mina et al. **[38]** objectively established that dihydroquercetin exhibits strong antioxidant activity, thereby reducing oxidative spoilage of semi-finished products and creates an opportunity to manage its qualitative characteristics during storage. It is noted that using food additives allows the preservation of organoleptic and microbiological properties of semi-finished products for a long period of storage due to the inhibition of oxidation product formation **[39]**.

The results obtained confirm similar data available in the literature. For example, the addition of grape seed powder and green tea extract to meat semi-finished products **[40]**, dihydroquercetin **[41]**, **[42]**, **[43]**, tiger nuts and quinoa **[44]**, milk thistle meal **[45]**, grape seed powder, green tea extract and amaranth/flaxseed flour **[46]**, as well

as rosemary **[47]** prevents the accumulation of oxidation products and protects semi-finished products from oxidative damage.

The introduction of Dx, ascorbic acid, and tocopherol also makes it possible to inhibit microbiological spoilage of the resulting semi-finished meat products during storage (Table 2). The research object was minced meat produced from horse meat with the addition of 35% since the presence of a large amount of raw fat accelerates the development of not only oxidative processes but also microbiological spoilage.

Table 2 Dynamics of microflora content during storage of semi-finished meat products at a temperature of -18°C.

Index	Unit measurements	Limit	Changes in microflora									
			control sample					experimental sample				
			0	15	30	45	60	0	15	30	45	60
NMAFAM	CFU	n/m than1.0 10^4 / g	1.8 10^2	9.2 10^2	3.7 10^3	7.9 10^3	1.9 10^4	1.6 10^2	6.9 10^2	1.1 10^3	1.5 10^3	3.8 10^3
Escherichia coli	CFU	n/a in 2 g	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
L. monocytogenes	CFU	n/a in 25 g	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d
Pathogenic, including *Salmonella*	CFU	n/a in 25 g	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

Note: NMAFAM (Number of mesophilic aerobic and facultative-anaerobic microorganisms), n/m – not more, n/a – not allowed, n/d – not detected.

Based on the results of microbiological studies, it was established that the use of Dx together with tocopherol and ascorbic acid leads to inhibition of microflora growth and has a bacteriostatic effect, which helps to increase the shelf life of semi-finished meat products compared to control samples.

Figure 23 Semi-finished meat products from horse meat.

CONCLUSION

The use of Dx in the composition of fat mixtures and horse meat allows you to slow down the development of oxidative processes. The dosage of Dx from 0.05% to 0.075% by weight of fat in an alcoholic solution is most effective for maintaining the quality and safety of raw horse fat. When applying AA and Tp in dosages of 0.05% and 0.02%, respectively, it allows reducing the dosage of Dx to 0.025% without losing the qualitative characteristics of raw fat in for 24 days of storage. Organoleptic studies of minced horse meat the optimal dosage for the introduction of an antioxidant component is the introduction of an alcoholic solution with a Dx content by fat weight from 0.075% to 0.1%, respectively, with additional addition of AA and Tp in recommended dosages – 0.05% and 0.02%, respectively. The introduction of Dx from 0.075% to 0.01% by weight of fat in an alcoholic solution together with AA and Tp allows you to extend the shelf life of horse meat and meat semi-finished products (at a temperature of -18°C) up to 60 days.

REFERENCES

1. Lorenzo, J. M., Munekata, P. E. S., Campagnol, P. C. B., Zhu, Z., Alpas, H., Barba, F. J., & Tomasevic, I. (2017). Technological aspects of horse meat products – A review. In Food Research International (Vol. 102, pp. 176–183). Elsevier BV. https://doi.org/10.1016/j.foodres.2017.09.094
2. Belaunzaran, X., Lavín, P., Barron, L. J. R., Mantecón, A. R., Kramer, J. K. G., & Aldai, N. (2017). An assessment of the fatty acid composition of horse-meat available at the retail level in northern Spain. In Meat Science (Vol. 124, pp. 39–47). Elsevier BV. https://doi.org/10.1016/j.meatsci.2016.10.014
3. Belaunzaran, X., Bessa, R. J. B., Lavín, P., Mantecón, A. R., Kramer, J. K. G., & Aldai, N. (2015). Horse-meat for human consumption — Current research and future opportunities. In Meat Science (Vol. 108, pp. 74–81). Elsevier BV. https://doi.org/10.1016/j.meatsci.2015.05.006
4. Gill, C. O. (2005). Safety and storage stability of horse meat for human consumption. In Meat Science (Vol. 71, Issue 3, pp. 506–513). Elsevier BV. https://doi.org/10.1016/j.meatsci.2005.04.030
5. He, M. L., Ishikawa, S., & Hidari, H. (2005). Fatty Acid Profiles of Various Muscles and Adipose Tissues from Fattening Horses in Comparison with Beef Cattle and Pigs. In Asian-Australasian Journal of Animal Sciences (Vol. 18, Issue 11, pp. 1655–1661). Asian Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.2005.1655
6. Osoro, K., Ferreira, L. M. M., García, U., García, R. R., Martínez, A., & Celaya, R. (2012). Grazing systems and the role of horses in heathland areas. In Forages and grazing in horse nutrition (pp. 137–146). Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-755-4_15
7. Lorenzo, J. M., Sarriés, M. V., Tateo, A., Polidori, P., Franco, D., & Lanza, M. (2014). Carcass characteristics, meat quality and nutritional value of horsemeat: A review. In Meat Science (Vol. 96, Issue 4, pp. 1478–1488). Elsevier BV. https://doi.org/10.1016/j.meatsci.2013.12.006
8. De Palo, P., Tateo, A., Maggiolino, A., & Centoducati, P. (2014). Effect of nutritive level on carcass traits and meat quality of IHDH foals. In Animal Science Journal (Vol. 85, Issue 7, pp. 780–786). Wiley. https://doi.org/10.1111/asj.12203
9. Medeubayeva, Z., Tayeva, A., Shambulova, G., Syzdykova, L., & Astakhov, M. (2022). Revealing the influence of plant-based additives on qualitative indicators of a semi-finished product made from camel meat. In Eastern-European Journal of Enterprise Technologies (Vol. 2, Issue 11 (116), pp. 29–35). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2022.255676
10. Abilmazhinova, N., Vlahova-Vangelova, D., Dragoev, S., Abzhanova, S., & Balev, D. (2020). Optimization of the Oxidative Stability of Horse Minced Meat Enriched with Dihydroquercetin and Vitamin C as a New Functional Food. 'Prof. Marin Drinov' Publishing House of Bulgarian Academy of Sciences. https://doi.org/10.7546/crabs.2020.07.18
11. Stine, C. M., Harland, H. A., Coulter, S. T., & Jenness, R. (1954). A Modified Peroxide Test for Detection of Lipid Oxidation in Dairy Products. In Journal of Dairy Science (Vol. 37, Issue 2, pp. 202–208). American Dairy Science Association. https://doi.org/10.3168/jds.s0022-0302(54)70245-x
12. Schmedes, A., & Hølmer, G. (1989). A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. In Journal of the American Oil Chemists' Society (Vol. 66, Issue 6, pp. 813–817). Wiley. https://doi.org/10.1007/bf02653674
13. Kardash, E. i Tur'yan, Y.I. (2005). Acid Value Determination in Vegetable Oils by Indirect Titration in Aqueous-alcohol Media. Croatica Chemica Acta, 78 (1), 99-103. Preuzeto https://hrcak.srce.hr/2797
14. SST 9959-2015 Meat and meat products. General conditions for conducting an organoleptic assessment.
15. SST 9958-81 Sausages and meat products. Methods of bacteriological analysis.
16. Yermukanova, A., Leonid, P., Georgii, S., Zhiyenbayeva, S., & Mrkvicová, E. (2024). Mathematical modelling and optimization of the granulation process of loose compound feed for broilers. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 18, pp. 20–35). HACCP Consulting. https://doi.org/10.5219/1925
17. Voitsekhovskaya, L. I., Franko, Ye. V., Verbytskyi, S. B., & Okhrimenko, Yu. I. (2021). Effects of bioactive compounds upon the deterioration of the chilled mechanically deboned poultry meat during storage. In Vestnik MGTU (Vol. 24, Issue 4, pp. 396–407). FSEI HPE Murmansk State Technical University. https://doi.org/10.21443/1560-9278-2021-24-4-396-407
18. Stoyanov Staykov, A., Georgiev Dragoev, S., Kostadinov Balev, D., Borislavova Vlahova-Vangelova, D., & Rumenova Pavlova, M. (2016). Preserving the quality and prolongation the shelf-life of beef packed under vacuum or modified atmosphere using ternary antioxidant blend. In Journal of microbiology, biotechnology and food sciences (Vol. 5, Issue 6, pp. 617–622). Slovak University of Agriculture in Nitra. https://doi.org/10.15414/jmbfs.2016.5.6.617-622

19. Kuzmina, N. N., Petrov, O. Yu., Semenov, V. G., Baimukanov, D. A., Dzhanabekova, G. K., Sarimbekova, S. N., Nesipbayeva, A. K., & Zhylkyshybayeva, M. M. (2019). Comparative assessment of the impact of natural antioxidants on the qualitative indicators of semi-finished products from broiler-chicken meat and oxidative processes in their storage. In THE BULLETIN (Vol. 6, Issue 382, pp. 231–240). National Academy of Sciences of the Republic of Kazakshtan. https://doi.org/10.32014/2019.2518-1467.166
20. Shagaeva, N.N., Kolobov, S.V. & Pchelkina, V.A. (2018). Study of the effect of dihydroquercetin on the microstructure of minced meat semi-finished products. In Polzunovskiy vestnik (Vol. 4, pp. 95-99). (In Russ.) Altai State Technical University. https://doi.org/10.25712/astu.2072-8921.2018.04.019
21. Nasonova, V. V., & Tunieva, E. K. (2019). Comparative studies in the effectiveness of antioxidants. In Proceedings of universities applied chemistry and biotechnology (Vol. 3, Issue 9, pp. 563–569). Irkutsk National Research Technical University. https://doi.org/10.21285/2227-2925-2019-9-3-563-569
22. Rudakov, O. B., & Rudakova, L. V. (2020). Dihydroquercetin in meat products. In Meat Technology Magazine (Vol. 15, Issue 1, pp. 44–47). Otraslevije Vedomosty Publishing House Ltd. https://doi.org/10.33465/2308-2941-2020-05-44-47
23. Shagaeva, N. N., Zachesova, I. A., Kolobov, S. V., & Evdokimova, O. V. (2021). Effect of dihydroquercetin on the stability of the properties of rendered fats. In IOP Conference Series: Earth and Environmental Science (Vol. 640, Issue 4, p. 042019). IOP Publishing. https://doi.org/10.1088/1755-1315/640/4/042019
24. Ahn, D. U., & Nam, K. C. (2004). Effects of ascorbic acid and antioxidants on color, lipid oxidation and volatiles of irradiated ground beef. In Radiation Physics and Chemistry (Vol. 71, Issues 1–2, pp. 151–156). Elsevier BV. https://doi.org/10.1016/j.radphyschem.2004.04.012
25. Grau, A., Guardiola, F., Grimpa, S., Barroeta, A. C., & Codony, R. (2001). Oxidative Stability of Dark Chicken Meat Through Frozen Storage: Influence of Dietary Fat and α-Tocopherol and Ascorbic Acid Supplementation. In Poultry Science (Vol. 80, Issue 11, pp. 1630–1642). Elsevier BV. https://doi.org/10.1093/ps/80.11.1630
26. Haak, L., Raes, K., & De Smet, S. (2009). Effect of plant phenolics, tocopherol and ascorbic acid on oxidative stability of pork patties. In Journal of the Science of Food and Agriculture (Vol. 89, Issue 8, pp. 1360–1365). Wiley. https://doi.org/10.1002/jsfa.3595
27. King, A. J., Uijttenboogaart, T. G., & de Vries, A. W. (1995). α-Tocopherol, β-Carotene and Ascorbic Acid as Antioxidants in Stored Poultry Muscle. In Journal of Food Science (Vol. 60, Issue 5, pp. 1009–1012). Wiley. https://doi.org/10.1111/j.1365-2621.1995.tb06281.x
28. Perlo, F., Fabre, R., Bonato, P., Jenko, C., Tisocco, O., & Teira, G. (2018). Refrigerated storage of pork meat sprayed with rosemary extract and ascorbic acid. In Ciência Rural (Vol. 48, Issue 4). FapUNIFESP (SciELO). https://doi.org/10.1590/0103-8478cr20170238
29. Wang, T., Li, Z., Yuan, F., Lin, H., & Pavase, T. R. (2016). Effects of brown seaweed polyphenols, α-tocopherol, and ascorbic acid on protein oxidation and textural properties of fish mince (Pagrosomus major) during frozen storage. In Journal of the Science of Food and Agriculture (Vol. 97, Issue 4, pp. 1102–1107). Wiley. https://doi.org/10.1002/jsfa.7835
30. Young, J., Stagsted, J., Jensen, S., Karlsson, A., & Henckel, P. (2003). Ascorbic acid, alpha-tocopherol, and oregano supplements reduce stress-induced deterioration of chicken meat quality. In Poultry Science (Vol. 82, Issue 8, pp. 1343–1351). Elsevier BV. https://doi.org/10.1093/ps/82.8.1343
31. Kuzmina, N. N., Petrov, O. Y., & Savinkova, E. A. (2020). Influence of natural antioxidants on quality indicators of semi-finished products from meat of broilers. In IOP Conference Series: Earth and Environmental Science (Vol. 421, Issue 2, p. 022074). IOP Publishing. https://doi.org/10.1088/1755-1315/421/2/022074
32. Bozhko, N., Tischenko, V., Pasichnyi, V., Marynin, A., & Polumbryk, M. (2017). Analysis of the influence of rosemary and grape seed extracts on oxidation the lipids of peking duck meat. In Eastern-European Journal of Enterprise Technologies (Vol. 4, Issue 11 (88), pp. 4–9). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2017.108851
33. Cheah, P. B., & Gan, S. P. (2000). Antioxidative/Antimicrobial Effects of Galangal and α-Tocopherol in Minced Beef. In Journal of Food Protection (Vol. 63, Issue 3, pp. 404–407). Elsevier BV. https://doi.org/10.4315/0362-028x-63.3.404
34. Shagaeva, N. N. (2021). Chopped semi-finished meat consumer properties' changes during storage. In Bulletin of KSAU (Issue 10, pp. 189–194). Krasnoyarsk State Agrarian University. https://doi.org/10.36718/1819-4036-2021-10-189-194

35. Tayeva, A. M., Abilmazhinova, N. K., Tlevlessova, D. A., Jetpisbayeva, B. Sh., & Kuzembaeva, G. K. (2022). Revealing the effectiveness of the effect of plant extracts in meat products. In The Journal of Almaty Technological University (Issue 3, pp. 114–121). Almaty Technological University JSC. https://doi.org/10.48184/2304-568x-2022-3-114-121
36. Kumar, Y., Yadav, D. N., Ahmad, T., & Narsaiah, K. (2015). Recent Trends in the Use of Natural Antioxidants for Meat and Meat Products. In Comprehensive Reviews in Food Science and Food Safety (Vol. 14, Issue 6, pp. 796–812). Wiley. https://doi.org/10.1111/1541-4337.12156
37. Gustiene, S., Zaborskiene, G., Rokaityte, A., & Riešute, R. (2019). Effect of Biofermentation with Taxifolin on Physicochemical and Microbiological Properties of Cold-Smoked Pork Sausages. In Food technology and biotechnology (Vol. 57, Issue 4, pp. 481–489). Faculty of Food Technology and Biotechnology - University of Zagreb. https://doi.org/10.17113/ftb.57.04.19.6250
38. Kuz'mina N. N., Petrov O. Yu., Guseva I. V. (2017). Quality management convenience food poultry meat with antioxidants during storage. In Vestnik of the Mari State University. Chapter "Agriculture. Economics". (Vol. 3, Issue 3 (11), pp. 33-37.) Mari State University
39. Shagaeva, N. N., Kolobov, S. V., & Zachesova, I. A. (2021). The effect of dihydroquercetin on the stability of consumer properties of chopped semi-finished meat. In S. Belousov & S. Roshchupkin (Eds.), E3S Web of Conferences (Vol. 285, p. 05011). EDP Sciences. https://doi.org/10.1051/e3sconf/202128505011
40. Perumalla, A. V. S., & Hettiarachchy, Navam. S. (2011). Green tea and grape seed extracts — Potential applications in food safety and quality. In Food Research International (Vol. 44, Issue 4, pp. 827–839). Elsevier BV. https://doi.org/10.1016/j.foodres.2011.01.022
41. Grikshas, S.A., & Al, A.G. (2021). Development of technology for the production of chopped semi-finished products from poultry meat using natural antioxidants. Trends in the development of science and education (Vol. 79, Issue 6, pp. 157–161). Private Enterprise, Vladislav Ivanov. https://doi.org/10.18411/trnio-11-2021-279
42. Borozda A.V. (2010). Development of technology for chopped semi-finished products using roe deer meat: dis. dis. Blagoveshchensk. 139 p. Available at: https://www.dissercat.com/content/razrabotka-tekhnologii-rublenykh-polufabrikatov-s-ispolzovaniem-myasa-kosuli
43. Nasonova V.V. (2008). Comparative study of the antioxidant activity of dihydroquercetin in meat products: dis. dis. Moscow. 168 p. Available at: https://www.dissercat.com/content/sravnitelnoe-izuchenie-antiokislitelnoi-aktivnosti-digidrokvertsetina-v-myasoproduktakh
44. Bayumi Ahmed Adel Ahmed Sayed. (2021). Development of technology for low-calorie minced meat semi-finished products using vegetable raw materials: dis. dis. Moscow. 177 p. Available at: https://www.dissercat.com/content/razrabotka-tekhnologii-nizkokaloriinykh-myasnykh-rublenykh-polufabrikatov-s-ispolzovaniem
45. Boleshenko O.P. (2006). Development of technology for semi-finished products from horse meat for functional purposes: dis. dis. Moscow. 135 p. Available at: https://www.dissercat.com/content/razrabotka-rublenykh-polufabrikatov-iz-koniny-dlya-funktsionalnogo-naznacheniya
46. Tavdidishvili, D., Khutsidze, T., Pkhakadze, M., Kalandia, A., & Vanidze, M. (2020). The effect of antioxidants on the quality of semi-finished minced rabbit meat. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 429–436). HACCP Consulting. https://doi.org/10.5219/1335
47. Y.I. Sharygina. (2011). Improving the technology of minced meat semi-finished products using natural substances with antioxidant properties: dis. dis. Kaliningrad. 212 p. Available at: https://www.dissercat.com/content/sovershenstvovanie-tekhnologii-rublenykh-myasnykh-polufabrikatov-s-ispolzovaniem-prirodnykh-

Funds:

This article was not supported by grants.

Acknowledgments: -

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Nazym Abilmazhinova,** Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77074540634
E-mail: abilmazhinova85@mail.ru
ORCID: https://orcid.org/0000-0001-7398-2242

Aigul Tayeva, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77072350000
E-mail: aigul_taeva@mail.ru
ORCID: https://orcid.org/0000-0001-6663-4282

Bagila Jetpisbayeva, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77072389275
E-mail: dzhetpisbaeva.b@atu.edu.kz
ORCID: https://orcid.org/0000-0002-0792-3903

Madina Kozhakhiyeva, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77077226842
E-mail: Madinamko@mail.ru
ORCID: https://orcid.org/0000-0001-5767-5154

Kuzembayeva Gaukhar, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77012616119
E-mail: kuzembaevagk@mail.ru
ORCID: https://orcid.org/0000-0003-0558-9531

Karlygash Abdiyeva, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77750697276
E-mail: k.abdieva@mail.ru
ORCID: https://orcid.org/0000-0003-0111-6737

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 10.5.2024
Revised: 23.7.2024
Accepted: 24.7.2024
Published: 28.8.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 776-791
https://doi.org/10.5219/1985
ISSN: 1337-0960 online
www.potravinarstvo.com

The technology of cooking falafel with high biological value for vegans

Larysa Bal-Prylypko, Inna Levytska, Liubov Tkachenko, Yuliya Kryzhova, Yaroslav Rudyk, Yevheniia Marchyshyna, Maxim Ryabovol, Yuliia Slyva

ABSTRACT

The article presents the results of scientific research on the possibility of increasing the biological value of falafel dishes for vegans through the use of flax seeds and blueberry extract. Vegan nutrition analysis in Ukraine shows that dishes for this population group are mostly deficient in biologically active substances. One way to solve this problem is to develop dishes for vegans using raw plant materials with a high content of biologically active substances. The chemical composition of flax seeds and blueberry extract is analysed in terms of using these products for vegan cuisine. The feasibility of using flax seeds and blueberry extract to increase the biological value of the falafel vegan dish was proven. The feasibility of replacing vegetable oil and a fraction of wheat flour to optimise the vitamin-mineral composition of falafel was determined. Based on the organoleptic evaluation of the control and test samples, the optimal ratio of falafel ingredients was established: 2.5% dry blueberry extract and 7.5% flax seeds per 100 g of the dish. The falafel chemical composition with the addition of flax seeds and blueberry extract was studied compared to the control sample. According to the research results, the technology of cooking falafel for vegans with an increased content of biologically active substances was developed. Based on the data obtained, it can be concluded that the developed dish will enrich vegan nutrition with deficient biologically active substances. The social effectiveness of the developed technology of cooking falafel is to expand the range of vegan dishes. The developed dish can be recommended for the nutrition of fasting people. It is advisable to use the technology developed to cook falafel in restaurants.

Keywords: falafel, vegan nutrition, biological value, organoleptic properties, restaurant technologies

INTRODUCTION

Human nutrition is essential in ensuring a high level of health, increasing life expectancy, and preserving working capacity **[1]**, **[2]**.

It is known that human food products should contain about 600 substances or nutrients necessary for the normal functioning of the body **[3]**. These substances are important in biochemical processes' complex, harmonious mechanisms. For 96% of organic and inorganic compounds obtained with food products, one or another biological property is inherent. Therefore, human health depends on the amount and ratio of these substances in the diet **[4]**.

Today, various forms of vegetarianism are becoming increasingly popular in the world's developed countries **[5]**. One of them is veganism – a form of strict vegetarianism accompanied by the rejection of fish, eggs, milk, honey and, of course, meat, that is, the rejection of absolutely all products of animal origin. Studies by scientists **[6]** prove that veganism positively affects the gastrointestinal tract and cardiovascular system. Adherence to a strict form of vegetarianism is one of the ways to prevent atherosclerosis, hypertension, some forms of cancer, and diabetes. However, the complete rejection of any animal products has several disadvantages: people are deficient in protein, iron, calcium, zinc, phosphorus, and copper, as well as fat-soluble vitamins A and E and omega-3 fatty acids. According to the World Health Organization, a vegan diet without additional intake of

vitamin B_{12} can cause anemia **[7]**. Therefore, the urgent task is to develop new technologies for vegan dishes with the optimal macro- and micronutrients necessary for the human body and improved organoleptic indices.

Vegetarianism has been a part of human culture since ancient times. Adherents of plant-based diets existed in ancient Greece and Asia **[8]**. Pythagoras, Zarathustra, Aristotle, and Plato did not eat meat. In the 1700s, Christians in Europe and North America promoted the concept of vegetarianism as part of a religious lifestyle that also included pacifism, abolition of slavery, and abstinence from alcohol. Until the 19th century, moral and metaphysical arguments justified the refusal to eat animals. However, in the early 1800s, an increased pursuit of better health in combination with increased authority of science helped to shape the physiological arguments for vegetarianism. As food science has developed since the mid-twentieth century, vegetarianism has gained general acceptance as a healthy dietary alternative. But since this alternative is still often chosen for moral or religious reasons, familiarisation of future vegetarians with possible negative health consequences remains an important activity. Today, vegetarianism is the most widespread system of non-traditional nutrition. Eating food is a significant part of social culture and identity, as is the appearance of vegetarianism and veganism, which are associated with a new culture of nature management and humanism.

It has been determined **[9]** that vegetarianism is a diet that refuses to consume meat and meat products, including meat of cattle, poultry, fish, and flesh of any other animal. This diet is gradually gaining great popularity around the world. Thus, one of the largest vegetarian communities lives in India, and many people who adhere to this diet live in European countries, such as Italy, Great Britain, Germany, and the Netherlands. At the same time, veganism is a more stringent diet that involves a complete rejection of food products associated with animals, including milk, eggs, and other products for the manufacture of which animals are involved in one way or another. According to various estimates**[10]**, vegans today comprise 0.25 to 2.5% of the UK population and 0.2 to 1.3% of the US population. Many vegans live in Holland, Sweden, Denmark, and other European countries.

A recent study **[11]** shows a rapid tendency to increase the number of vegans in Ukraine. The reasons for adhering to vegetarianism are various, from ethical reasons, religious beliefs, and features of the closest environment to improvement in health. The results of many scientific studies **[12]**, **[13]** on the positive effects of vegetarian diets on human health are known. Thus, vegetarianism as a type of diet can be attributed to healthy nutrition.

However, new vegan cafes, restaurants, catering establishments, bakeries, and enterprises that produce food products and various vegan products exist and are constantly opening abroad. Many online stores specialise in vegan products on the Internet **[14]**. The works of many scientists are devoted to the issue of rationing the nutrient composition of health-improving food products to a person's physiological needs in nutrients: P.O. Karpenko, M.I. Peresichnyi, L.V. Bal-Prylypko, V.N. Korzuna, A.M. Dorokhovych, H.O. Simakhina, N. M. Zubar, O.I. Cherevka, M.R. Shpahat, H. Godfray, V. Bezsheiko, NSRizzo et al. **[15]**, **[16]**. Based on the analysis of literature sources, it can be concluded that developing new technologies for cooking dishes with increased biological value for vegan cuisine is a timely and promising task for scientists.

Scientific Hypothesis

Given the rapid trend of increasing the number of vegans in Ukraine, an analysis of the range and composition of vegan nutrition dishes has been conducted. It has been determined that there is a need for more biologically active substances in vegan dishes. The development of technologies for cooking dishes for vegan nutrition using plant raw materials, which have a high content of biologically active substances, is an urgent task. Given this, using flax seeds and blueberry extract in vegan cuisine will purposefully improve falafel's functional and organoleptic indices. Accordingly, the developed technology of cooking falafel dishes will make it possible to enrich the diet of vegans with deficient biologically active substances and expand the range of menus for restaurant industry facilities.

MATERIAL AND METHODOLOGY

Samples

The study was performed in a laboratory of healthy nutrition and restaurant technologies of the Faculty of Food Technology and Product Quality Management of the Agro-Industrial Complex of the NULES of Ukraine.

Chemicals

Water (chemical formula H_2O) was used to soak different flax seed varieties during extraction. Water corresponds to the national standard DSTU ISO 7887:2003 **[25]**. A salt solution (1% NaCl) extracted polysaccharides from flax seeds.

Animals, Plants and Biological Materials

The following was used for the study:

- dry blueberry extract – manufacturer: Frutta (Ukraine) (Figure 1-a);
- highest quality flax seeds (humidity 7.8%, purity – 97.9% (VIOLA brand (Ukraine) (Figure 1-b);
- chickpeas with a humidity of 10.6%, protein content – of 21%, and fibre of 17.1% (Kyshentsi LLC, Cherkasy region, Ukraine) (Figure 1-c);
- vegetable oil **[17]**: sunflower oil, refined, first pressing (Oleina™, Ukraine)
- wheat flour **[18]**: of the highest quality (Kyivmlyn LLC, Ukraine)
- spring onion, garlic, parsley leaves.

a b c

Figure 1 Main ingredients for cooking falafel: a – dry blueberry extract, b – flax seeds, c – chickpeas.

Instruments

Laboratory scales AXIS AD 510 (Poland) with a permissible weighing error of ±0.01 g.
Laboratory mill OlisLab 2100 manufactured by Olis LLC (Ukraine).
Blender BOSCH MSM2620B (Germany).
Convection oven XF 023 9 Brand: Unox (Italy).
Glass beaker with a volume of 500 cm^3 according to DSTU ISO 4787:2009.
Porcelain tableware, according to DSTU 2084-92.
Laboratory thermometer (TLS-200, manufactured by Inter-Syntez LLC, Ukraine).

Laboratory Methods

The methods of research are organoleptic, experiment planning, and mathematical processing of experimental data based on computer technologies. The organoleptic quality assessment was carried out using a five-point scale. For each organoleptic indicator, a weighting factor was determined: appearance: 0.3, consistency: 0.2, colour: 0.1, odour: 0.2, and flavour: 0.2 **[19]**.

Description of the Experiment

Sample preparation: To develop the technology of cooking the falafel dish, 300 g of chickpeas were weighed, washed with running water, and transferred to a glass beaker. Then, the chickpeas in the beaker were poured with water and left to soak for 4 hours. After soaking, the chickpeas were boiled for 2 hours, cooled, and crushed in a laboratory mill to a homogeneous consistency.

While the chickpeas were cooked, other components of the dish were prepared for each sample: one control sample and three experimental samples.

Onion, garlic, and parsley were peeled, washed, and crushed, and the estimated amount for each study option was weighed. The flour was sifted and weighed separately for the control variant, 15 g, and the experimental variant, 10 g.

Flax seeds and dry blueberry extract were used only for experimental variants. Flax seeds were crushed in a laboratory mill and weighed according to the experimental variant: Experiment No. 1 – 7.5 g; Experiment No. 2 – 5 g; Experiment No. 3 – 2.5 g. The dry blueberry extract was weighed according to the experimental variant: Experiment No. 1 – 2.5 g; Experiment No. 2 – 5 g; Experiment No. 3 – 7.5 g.

For each variant, 60 g of crushed chickpeas were weighed, and an appropriate number of prepared components were added. All elements of the control variant and the three experimental variants were separately mixed to a homogeneous consistency using a blender. The falafel balls were formed and baked in a convection oven at 180ºC for 5 min.

Number of samples analyzed: during the experimental studies, four samples were taken, namely, three experimental samples and one control sample.

Several repeated analyses: The experiments were repeated five times; thus, the methods of mathematical statistics of experimental data processing were used.

Number of experiment replications: Each experiment was conducted five times, and the number of samples – three- led to repeated analyses.

Design of the experiment: Cooking the falafel dish was carried out according to the expected technological scheme, which consists of the following operations: acceptance and mechanical culinary processing (MCP) of raw materials; soaking of chickpeas for 4 hours; boiling of chickpeas for 2 hours; grinding of flax seeds; weighing of components; dosing of components by experimental options; mixing of components; forming of falafels; baking of samples at a temperature of 180 °C; cooling and preparation for organoleptic evaluation.

The experimental design accounted for variations in sample composition and preparation processes to ensure the reliability and reproducibility of results. Using several samples and repeated experiments minimised the influence of random errors and ensured the statistical significance of the obtained data. Various options for components and their ratio allowed us to assess the influence of each element on the final quality of falafel, which contributed to the development of optimal cooking technology.

Statistical Analysis

Statistical processing was performed using Microsoft Excel 2016 in combination with XLSTAT. The accuracy of the experimental data obtained was determined according to Student's t-criterion with a confidence probability of ≤0.05 for several replicate measurements – minimum 5. Linear programming problems were solved using the settings of MS Excel "Finding a solution" (ExcelSolver) **[19]**.

RESULTS AND DISCUSSION

Vegetarian cuisine in general, and vegan cuisine in particular, combines original traditional dishes of different world peoples with their adapted versions—so-called ‘veganized’ **[20]**. Recipes can be 'veganized' by replacing animal products with plant-based ones. The development of technology for cooking food products for vegan nutrition is based on the knowledge of the norms of each nutrient content in the finished product to meet human needs optimally **[21]**.

In Ukraine, where supporters of vegetarianism and veganism represent a fairly large proportion of the population, more specialised restaurant industry facilities and a narrow range of products offered on the menus of the facilities mentioned above need to be observed **[22]**.

It has been determined **[23]** that in vegetarian and vegan nutrition with the refusal of consumption of meat and meat products, dairy products, that is, products that are sources of animal protein, protein deficiency is replaced due to the widespread use of legumes, namely soybeans, peas, beans, chickpeas.

Chickpeas (*Cicer arietinum*) – one of the most consumed legumes in the world (more than 2.3 million tons come on the world market annually) **[24]**. Studies have shown that chickpeas, one of the most consumed legumes in the world, are an essential source of proteins, carbohydrates, vitamins, minerals, and dietary fibre. Due to this, chickpea seeds are used for vegetarian nutrition **[25]**.

To increase the biological value of the falafel dish, research was conducted on various types of plant raw materials grown in Ukraine and characterised by a high content of biologically active substances **[26]**.

A vegan diet requires special attention to nutrients such as protein, iron, calcium, vitamin B12, vitamin D, and omega-3 fatty acids with long chains to prevent deficiencies and ensure overall health and well-being **[27]**.

Different raw materials from plants with a high biologically active content are used for desserts and beverages **[28]**. The functional and technological features of ice cream for vegetarians have also been researched **[29]**. A promising direction is to use raw materials from medicinal plants to create food products that improve health **[30]**.

Blueberries were selected after analysing the use of wild raw materials to provide food products with biologically active substances **[31]**. Blueberries were traditionally used in Ukrainian cuisine for making beverages, filling for pies, and dumplings **[32]**.

Today, the technology of low-temperature extraction of raw plant materials, namely blueberries and elderberries, which makes it possible to preserve all valuable components, has become widely used. For extraction, environmentally friendly raw materials are used, which undergo several stages of processing: grinding, extraction, moisture removal, and lyophilic drying. As a result, a finely dispersed, highly hygroscopic powder is obtained, with an insoluble substance content of not more than 5% **[33]**. Dry blueberry extract produces such technology, allowing it to preserve vitamins, microelements, and other biologically active substances **[34]**.

The composition of dry blueberry extract includes **[35]** anthocyanins (up to 25%); oligo-elements: potassium, calcium, magnesium, and iron; vitamins: C, B_1, B_2. The extract also contains organic fruit acids: lemon, apple,

milk, oxalic, amber, quinic, tanning substances, tannins, epimirtin, quercetin, hyperoside, and isoquercitrin. The chemical composition of the dry blueberry extract is given in Table 1.

Table 1 Chemical composition of dry blueberry extract.

Name of indicator	Value, mg % per 100g
Ascorbic acid, C	10
Thiamine, B1	1.4
Riboflavin, B2	0.8
Pantothenic acid, B3	2.1
Carotenes	0.75-1.6
Flavonoids: ***hyperin, astragalin, quercitrin, isoquercitrin, rutin***	460-600
Anthocyanins: ***delphinidine, malvidine, petunidine, idain, myrtiline***	650-780
Phenolic acids: ***coffee, quinic, chlorogenic***	28-35
Phenols and their derivatives: ***hydroquinone, monotropeoside, asperuloside***	30
Tanning substances	120
K (potassium)	870-1,200
Ca (calcium)	98-1,120
Cl (chlorine)	76-435
P (phosphorus)	125-286
S (sulfur)	123-232
Zn (zinc)	10.4-31.4
Fe (iron)	729-3,120
Cu (copper)	570 -3,230

Dry blueberry extract has astringent, rot-preventing, and antimicrobial properties **[36]**. Blueberry carotenoids improve night vision and visual apparatus function. Dry blueberry extract is of particular value for vegans due to many anthocyanins, which reduce the aggregation ability of red blood cells in vitro and have a hematopoietic function **[37]**.

Flax seed is also a traditional raw material for enriching food products **[38]**, and it is used mainly in bakery products. However, it has a unique chemical composition and pharmacological properties. The content of essential substances in mature flax seeds, protein substances 18-33%, mucus 5-12%, carbohydrates 12-26%, nitrogen-free extractive substances 22%, fatty oils 30-50%, fatty acid triglycerides: linolenic 30-45%, linoleic 25-59%, oleic 18-20%, stearic glyceride 8-9%, palmitic, arachidonic, myristic, and α-tocopherol **[39]**. The uniqueness of flax seed is due to its very high content of polyunsaturated a-linolenic fatty acid, which is essential for the human diet. Like certain hormones, it contributes to the performance of important biochemical functions in the human body – it is part of cell membranes, participates in the regeneration of the cardiovascular system, in brain growth and development, has vasodilating properties, and exhibits anti-stress and antiarrhythmic effects. Seeds contain phytosterols, enzymes, and vitamins C, A, and F. High molecular weight compounds that release lino-caffeine and linocinamarine during hydrolysis have been found in the flaxseed shell. Flaxseed contains oil consisting of glycerides of linoleic and linolenic acids; organic acids and carotene; macronutrients: potassium, calcium, magnesium, iron; micronutrients: manganese, copper, zinc, selenium, and boron. Flaxseed also has anti-inflammatory, analgesic, anti-sclerotic, and mildly laxative effects. Attractive appearance and the ability of flax shell substances (lignan compounds – antioxidants) to prevent hormone-dependent cancer, namely, prostate and mammary gland cancer, to stabilise blood sugar levels, determine the use of flax raw materials in whole grains.

The results **[40]** of experimental studies show that flaxseed is characterised by an increased content of proteins and fat, which account for 66-68% of the total weight (Table 2).

Table 2 Chemical composition of flaxseed.

Indices	Features of flaxseed, %
Moisture	9.26
Fat	36.55
Protein	30.65
Sugar	4.43
Pentosans	7.80
Cellulose	13.30
Ash	4.18

The seeds' lipids are of particular physiological and nutritional value; they can be used as a natural source of physiologically active forms (Omega-3 and Omega-6) of polyunsaturated fatty acids **[41]**. Flaxseed tocopherols are also essential functional components that positively impact human health.

The results of experimental studies **[42]** of the fractional composition of lipids in flaxseed show that neutral lipids dominate in flaxseed, which make up 98% of its total amount (Table 3).

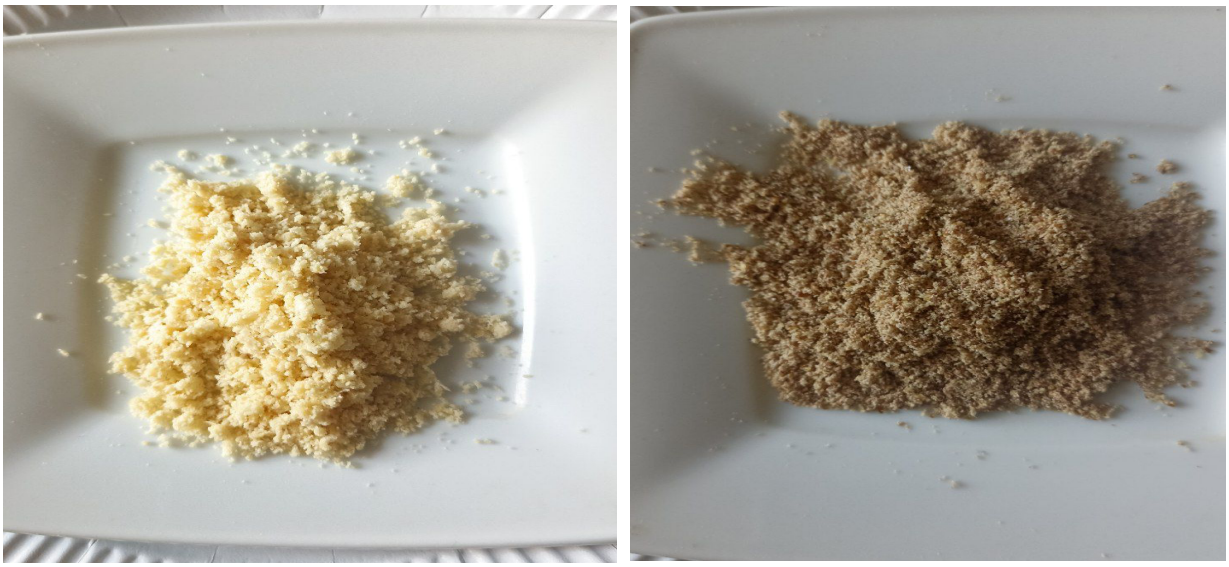
Table 3 Fractional composition of lipids in flaxseed.

Composition of lipids	Features of flaxseed
Triglycerides	97.83
Phospholipids	0.83
Free fatty acids	0.08
Sterols	0.46
Sterol esters	0.12
Mono- and diglycerides	0.11
Tocopherols, mg %	49
In particular, unsaturated fatty acids:	88.10 ±4.32
palmito-oleic	0.22 ±0.13
oleic	21.40 ±1.11
linoleic	12.40 ±1.03
linolenic	54.08 ±3.14

Having analysed the chemical composition and technological properties of blueberry extract and flaxseed, it should be noted that using these components in producing specialised food products for vegans is a promising solution. Blueberry extract and flaxseed's rich vitamin and mineral composition confirm the feasibility of using these ingredients in cooking falafel with chickpeas for vegan nutrition.

The proposed dry blueberry extract and flaxseed can be considered enrichment agents of vitamins A, E, F, and C and group B and minerals iron, calcium, zinc, phosphorus, and copper, which should additionally enter the body of a person who refuses to eat any products of animal origin.

The classic falafel recipe includes chickpeas, garlic, onion, parsley, cilantro, salt, flour, vegetable oil, and additives such as dry blueberry extract and flax seeds. Figure 2 shows smashed chickpeas and crushed flax seeds (b).



a b

Figure 2 Smashed chickpeas (a) and crushed flax seeds (b).

The development of technologies with different amounts of dry blueberry extract and flax seeds was carried out. To determine the optimal ratio of ingredients, experimental samples of falafel with model compositions based on chickpeas with the addition of dry blueberry extract and flax seeds were prepared. The model compositions of the experiments are shown in Table 4.

Table 4 Model compositions of falafel based on chickpeas with the addition of dry blueberry extract and flax seeds.

Name of ingredient	Control	Experiment 1	Experiment 2	Experiment 3
Chickpeas	60	60	60	60
Garlic	5	5	5	5
Onions	10	10	10	10
Parsley	5	5	5	5
Flour	15	10	10	10
Vegetable oil	5	-	-	-
Dry blueberry extract	-	2.5	5	7.5
Flaxseed	-	7.5	5	2.5
Output weight	100	100	100	100

The organoleptic evaluation of model compositions of falafel based on chickpeas with the addition of dry blueberry extract and flax seeds was carried out. The organoleptic parameters of falafel were identified, particularly appearance, colour, consistency, flavour, and odour. The organoleptic evaluation of experimental and control samples of falafel was carried out on a five-point system by generally accepted recommendations **[43]**. The results of the organoleptic assessment of experimental and control samples of cocktails are shown in Table 5.

Table 5 The organoleptic evaluation of falafel based on chickpeas with adding dry blueberry extract and flax seeds (points).

Quality indicators	Falafel based on chickpeas with the addition of dry blueberry extract and flax seeds			
	Control	Experiment 1	Experiment 2	Experiment 3
Appearance	4.8	4.7	4.7	4.7
Flavor	4.8	5.0	5.0	5.0
Odor	4.8	5.0	4.9	5.0
Colour	4.8	4.8	4.8	4.7
Consistency	4.9	4.9	4.7	4.7
Overall score	4.82	4.88	4.84	4.83

The model composition of falafel (Experiment 1) received the highest organoleptic evaluation, in which the complete amount of vegetable oil and a third of the total amount of flour were replaced with 2.5% dry blueberry extract and 7.5% flax seeds. A new type of falafel with blueberry extract and flax seeds was called „Falafel Active“. Figure 3 shows the experimental and control samples of falafel.

Figure 3 Samples of falafel: a - control, b - experimental 1, c - experimental 2, d - experimental 3

Figure 4 shows the serving of the ready-made dish ‘Falafel Active’.

Figure 4 'Falafel Active' dish for vegans using flax seeds and blueberry extract.

The developed technological scheme of cooking a 'Falafel Active' dish for vegan cuisine is shown in Figure 5.

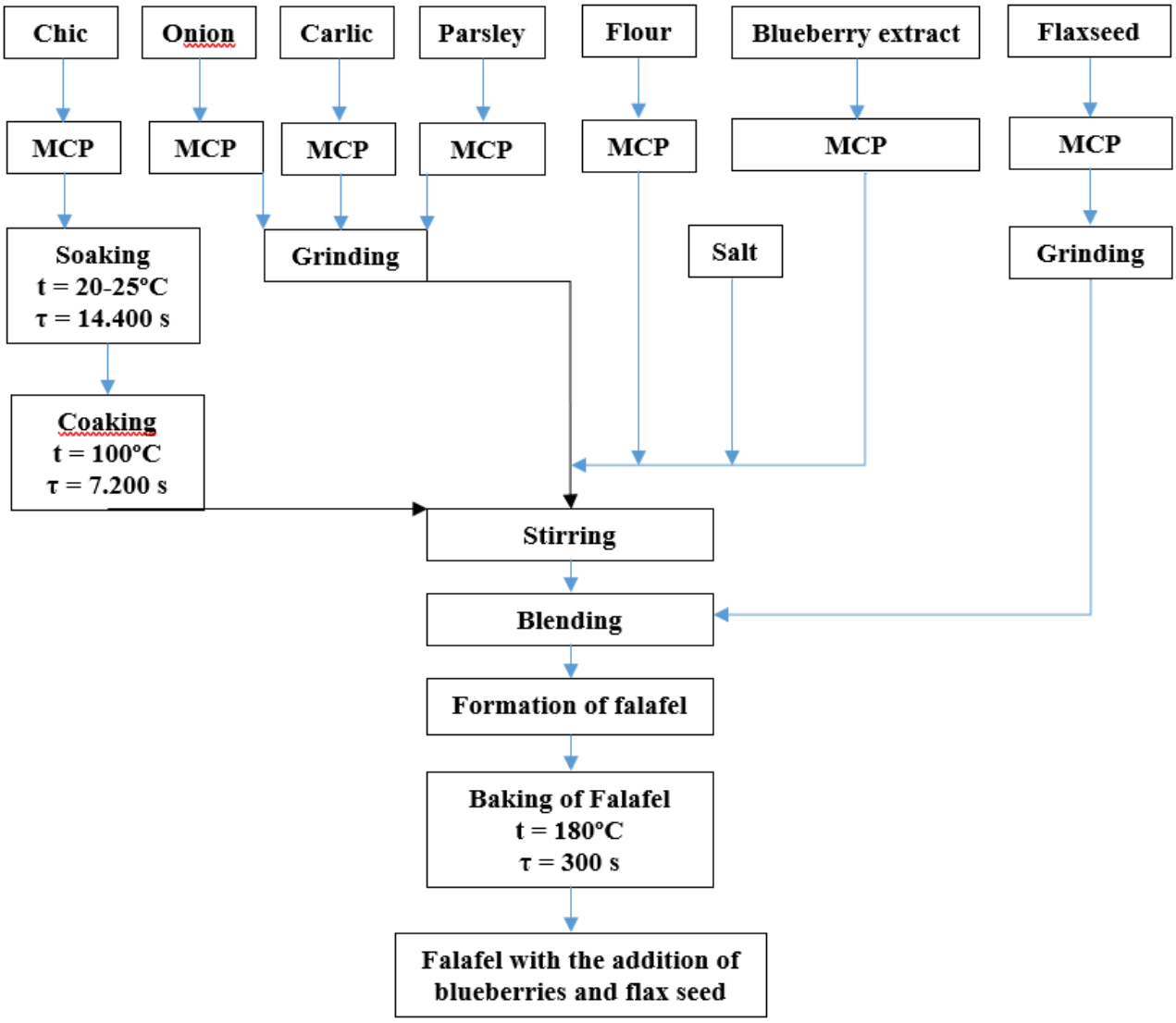


Figure 5 Technological scheme of cooking 'Falafel Active' dish for vegan cuisine.

The chemical composition of 'Falafel Active' based on chickpeas with the addition of dry blueberry extract and flax seeds was analyzed compared to the traditional recipe (Table 6). The content of biologically active substances increased in the developed 'Falafel Active' dish with the addition of 5% of dry blueberry extract and 5% of flax seeds: vitamin A – by 12%, vitamin C – by 17%, magnesium – by 31%, iron – by 40%, zinc – by 31%, phosphorus – by 53%. Vitamins B_1 and B_2 are almost absent in the control, and the amount of these vitamins in the developed product is 0.14 and 0.08 μg per 100 g of the product, respectively. It has been determined that, due to the use of a composition of flax seeds and blueberry extract in the traditional technology of cooking falafel, the developed dish has an attractive appearance, and the flavour of the dish has become more saturated. Most importantly, the content of biologically active substances has been significantly increased, namely the content of vitamins and micronutrients.

Table 6 Comparison of the chemical compositions of control and experimental samples of falafel.

Name of indicator	Control	Experiment 1	Difference, units	Difference, %
Energy value, kcal	321.00	294.00	-27.00	91.59
Proteins, g	13.00	14.00	1.00	107.69
Dietary fiber, g	6.90	7.40	0.50	107.25
Mono- and disaccharides	3.10	4.20	1.10	135.48
Saturated fatty acids	1.10	0.80	-0.30	72.73
Unsaturated fatty acids	2.50	3.50	2.50	126.43
Potassium, mg	738.20	771.30	33.10	104.48
Calcium, mg	143.70	146.40	2.70	101.88
Magnesium, mg	89.00	116.90	27.90	131.35
Sodium, mg	89.00	90.10	1.10	101.24
Phosphorus, mg	289.60	445.10	155.50	153.69
Iron, mg	2.20	3.10	0.90	140.91
Copper, μg	417.30	495.10	77.80	118.64
Zinc, mg	1.90	2.50	0.60	131.58
Vitamin A, μg	64.20	67.20	8.00	112.46
Beta-carotene, μg	0.40	0.40	0.00	100.00
Thiamine, B_1 μg	Traces	0.14	0.14	140.00
Riboflavin, B_2	Traces	0.08	0.08	8.00
Ascorbic acid, C, mg	9.40	11.00	1.60	117.02

The developed 'Falafel Active' dish, based on chickpeas using dry blueberry powder and flax seeds, is suitable for vegans and other categories of the population, namely for elderly people who need dietary nutrition.

Given that chickpeas are the main component of the 'Falafel Active' dish, research on the possibilities of using chickpeas for healthy nutrition was analyzed. The preclinical and clinical studies conducted **[44]** show that some components of chickpeas have several health benefits, including antioxidant capacity and antifungal, antibacterial, analgesic, anticancer, anti-inflammatory and hypocholesterolemic properties. Thus, developing new products with high biological activity creates new opportunities for research and application of chickpeas in food products.

The use of bioactive compounds of chick chickpeas ingredients in food products is also a promising area regarding the availability of their health benefits **[45]**. The results of research **[46]** on the production of chickpeas, trends of consumption, applications in the food industry, and most importantly, theologically active compounds and functional properties of the ingredients of chickpeas that need to be taken into account when developing new products, in particular when developing chickpea plant-based snacks, taking into account the biologically active compounds associated with healthy nutrition of patients with type 2 diabetes (DM2) **[47]**, are generalised.

It has been established that flax seeds are widely used to manufacture bakery products, cheeses, and sauces. Because flaxseed is a source of dietary fibre, complete protein, unsaturated fatty acids, minerals, and vitamins, it enriches bakery products of various assortments **[48]**. According to the research results, it has been established that the technologically possible dosage of crushed flax seeds in the wheat bread recipe is up to 20% by weight of flour. Products with developed porosity, a pleasant light-yellow colour of the crumb, and a pleasant nutty flavour are obtained at this dosage. In our research, the use of flax seeds is 7.5% of the product weight, which contributes to the fact that the 'Falafel Active dish has a pleasant nutty flavour and aroma.

Blueberries are widely used in the technology of cooking desserts and beverages **[49]**. The prospect of using dry blueberry extract is that blueberries contain many natural antioxidants. Modern research proves **[50]** that natural antioxidants of plant raw materials are also considered preservatives in various food products due to

innovative extraction methods and processes for stabilising their properties. The research has linked regular moderate consumption of blueberries or their anthocyanins to a reduced risk of cardiovascular disease and type 2 diabetes and improved weight maintenance and neuroprotection. These results are supported by data from human clinical trials based on biomarkers. Blueberries' most important beneficial properties are their anti-inflammatory and antioxidant effects and their beneficial effect on vascular and glucoregulatory function **[51]**. Blueberries' phytochemicals can affect the gastrointestinal tract's microflora and promote health.

"Falafel Active" is an innovative vegan product that offers numerous benefits. It combines the high content of omega-3 fatty acids from flaxseed and antioxidants from blueberries, providing a nutritious and delicious snack. Environmental and economic sustainability:

Locally grown ingredients: using raw materials grown on local farms reduces the carbon footprint by reducing transport costs and greenhouse gas emissions. It also helps preserve biodiversity and support local agricultural practices.

Supporting the local economy: purchasing ingredients from local producers contributes to the development of local businesses, job creation, strengthening the region's economic stability and increasing its well-being. Innovativeness:

The proposed combination of "Falafel Active" is unique on the market, offering a new approach to healthy eating, so there is no doubt that such a product will be able to satisfy the growing demand for healthy, sustainable and locally produced products among vegans and supporters of a healthy lifestyle. Thus, this product improves consumers' nutrition and contributes to the preservation of the environment and the support of the local economy.

The search for scientific research on using the composition of blueberries and flax seeds in food technologies showed no results. In our work, flax seeds and blueberry extract in vegan products have been investigated for the first time. Given that blueberries, like flax seeds, are the products that are grown in Ukraine and have long been used for the nutrition of Ukrainians, we can conclude that the research in the direction of applying the composition of such components as dry blueberry extract and flax seeds to develop the technologies of cooking various dishes is quite timely and promising.

CONCLUSION

The result of the research is the creation of the 'Falafel Active' *dish based on chickpeas* with the addition of dry blueberry extract and flax seeds. The optimal amount of dry blueberry extract and flax seeds, which is why the 'Falafel Active' dish has an increased content of minerals and vitamins and meets the specific needs of vegans, has been determined. It is established that the best results, according to the organoleptic evaluation, have been obtained for the experimental sample containing 2.5% dry blueberry extract and 7.5% flax seeds per 100 g of the product. The research results show an improvement in the organoleptic parameters of falafel. It is proved that the biological value of the 'Falafel Active' dish based on chickpeas with the addition of dry blueberry extract and flax seeds increases, as the content of phosphorus, potassium, calcium, zinc, magnesium, iron and vitamins A, C, B_1 and B_2, which are considered deficient for vegans, increases. It can be concluded that the developed 'Falafel Active product differs from the control by increasing the proportion of deficient vitamins and minerals, which makes it possible to increase the biological value of the new product. At the same time, the energy value of falafel decreases. Vegetable oil is wholly excluded from the falafel composition, and the flour amount is reduced by a third. Consequently, it can be concluded that it is advisable to use dry blueberry extract and flax seeds to cook a vegan dish – falafel based on chickpeas. The use of these nutritional supplements is a promising solution, and it allows for the expansion of the range of vegan dishes, enriching the flavour of a dish, contributing to ensuring the daily need for deficient minerals and vitamins, which contributes to improving the vegan diet and expanding the range of dishes in catering establishments. Scientists urgently need to develop technologies for cooking various health-improving dishes that use a composition of components useful for human health, such as dry blueberry extract and flax seeds.

REFERENCES

1. Bal-Prylypko, L., Nikolaenko, M., Stepasyuk, L., Cherednichenko, O., & Lialyk, A. (2022). Forecasting the sale price of pork in agricultural enterprises. In Agricultural and Resource Economics: International Scientific E-Journal (Vol. 8, Issue 4, pp. 170–187). Institute of Eastern European Research and Consulting. https://doi.org/10.51599/are.2022.08.04.08
2. Silveira Rossi, J. L., Barbalho, S. M., Reverete de Araujo, R., Bechara, M. D., Sloan, K. P., & Sloan, L. A. (2021). Metabolic syndrome and cardiovascular diseases: Going beyond traditional risk factors. In Diabetes/Metabolism Research and Reviews (Vol. 38, Issue 3). Wiley. https://doi.org/10.1002/dmrr.3502
3. Karpenko, P. O., & Hryshchenko, I. M. (2011). Basics of rational and medical nutrition. Kyiv: KNTEU.
4. Naumenko, O., Hetman, I., Chyzh, V., Gunko, S., Bal-Prylypko, L., Bilko, M., Tsentylo, L., Lialyk, A., Ivanytska, A., & Liashenko, S. (2023). Improving the quality of wheat bread by enriching teff flour. In Eastern-European Journal of Enterprise Technologies (Vol. 3, Issue 11 (123), pp. 33–41). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2023.279286
5. Shpahat, R. (2017). Osnova praktychnoyi teoriyi dlya rozuminnya perekhodu na vehanstvo. In Zhurnal doslidzhen′ tvaryn. Retrieved from http://health.gov/dietaryguidelines/2017/guidelines/.
6. Kim, G., Oh, J., & Cho, M. (2022). Differences between Vegetarians and Omnivores in Food Choice Motivation and Dietarian Identity. In Foods (Vol. 11, Issue 4, p. 539). MDPI AG. https://doi.org/10.3390/foods11040539
7. Zagorodneva, O., & Ponomaryova, L. (2021). The role of the World Health Organization in solving international public health problems: history, modernity and development prospects. (2021). In Actual problems of modern medicine (Issue 7). V. Karazin Kharkiv National University. https://doi.org/10.26565/2617-409x-2021-7-10
8. Leitzmann, C. (2014). Vegetarian nutrition: past, present, future. In The American Journal of Clinical Nutrition (Vol. 100, pp. 496S-502S). Elsevier BV. https://doi.org/10.3945/ajcn.113.071365
9. Sobiecki, J. G., Appleby, P. N., Bradbury, K. E., & Key, T. J. (2016). High compliance with dietary recommendations in a cohort of meat eaters, fish eaters, vegetarians, and vegans: results from the European Prospective Investigation into Cancer and Nutrition–Oxford study. In Nutrition Research (Vol. 36, Issue 5, pp. 464–477). Elsevier BV. https://doi.org/10.1016/j.nutres.2015.12.016
10. Bezsheiko, V. (2017). How does the brain affect cardiovascular health? In Psychosomatic Medicine and General Practice (Vol. 2, Issue 3, p. 020368). Private Publisher Chaban O.S. https://doi.org/10.26766/pmgp.v2i3.68
11. Anatoly. (2024). Vegetarianism in Ukraine is an important way to a healthy life and environmental sustainability. In Vegetable – Garden Magic. Retrieved from https://vegetable.com.ua/vegetarianstvo-v-ukraini-vikliki-ta-mozhlivost.
12. Dinu, M., Abbate, R., Gensini, G. F., Casini, A., & Sofi, F. (2017). Vegetarian, vegan diets and multiple health outcomes: A systematic review with meta-analysis of observational studies. In Critical Reviews in Food Science and Nutrition (Vol. 57, Issue 17, pp. 3640–3649). Informa UK Limited. https://doi.org/10.1080/10408398.2016.1138447
13. Brytek-Matera, A., Czepczor-Bernat, K., Jurzak, H., Kornacka, M., & Kołodziejczyk, N. (2018). Strict health-oriented eating patterns (orthorexic eating behaviours) and their connection with a vegetarian and vegan diet. In Eating and Weight Disorders - Studies on Anorexia, Bulimia and Obesity (Vol. 24, Issue 3, pp. 441–452). Springer Science and Business Media LLC. https://doi.org/10.1007/s40519-018-0563-5
14. Ditrykh, I., Bui, L., & Hanzha, A. (2017). Methods of improving vegetarian food based on replacing animal products by chia seeds. In Scientific Works of National University of Food Technologies (Vol. 23, Issue 4, pp. 207–214). National University of Food Technologies. https://doi.org/10.24263/2225-2924-2017-23-4-27
15. Zubar, N.(2018). Basicsofphysiologyandfoodhygiene: a textbook. Kyiv: Kondor Publishing House.
16. Cherevko, O. (2017). Innovative technologies of functional food products appointment. Kharkiv: KhDUHT.
17. DSTU 4492:2017 Oliya sonyashnikova. Technical minds
18. DSTU 46.004-99 Wheat flour.
19. Nikolaienko, M., & Bal-Prylypko, L. (2020). Development of an integrated food quality management system. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 862–873). HACCP Consulting. https://doi.org/10.5219/1434
20. Coxon, C., Hepsomali, P., Brandt, K., Vauzour, D., & Costabile, A. (2023). Personality, dietary identity, mental and sleep health in vegans and vegetarians: A preliminary cross-sectional study. In Health Science Reports (Vol. 6, Issue 8). Wiley. https://doi.org/10.1002/hsr2.1525

21. Rizzo, N. S., Jaceldo-Siegl, K., Sabate, J., & Fraser, G. E. (2013). Nutrient Profiles of Vegetarian and Nonvegetarian Dietary Patterns. In Journal of the Academy of Nutrition and Dietetics (Vol. 113, Issue 12, pp. 1610–1619). Elsevier BV. https://doi.org/10.1016/j.jand.2013.06.349
22. Dymchenko, A., Kirilenko, Y., Ochkolyas, O., & Bal-Prylypko, L. (2024). Gluten-free beer with unmalted millet. In European Food Research and Technology (Vol. 250, Issue 4, pp. 999–1005). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-023-04405-8
23. Onwezen, M. C., Bouwman, E. P., Reinders, M. J., & Dagevos, H. (2021). A systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plant-based meat alternatives, and cultured meat. In Appetite (Vol. 159, p. 105058). Elsevier BV. https://doi.org/10.1016/j.appet.2020.105058
24. Xiao, S., Li, Z., Zhou, K., & Fu, Y. (2022). Chemical composition of kabuli and desi chickpea (*Cicer arietinum L.*) cultivars grown in Xinjiang, China. In Food Science & Nutrition (Vol. 11, Issue 1, pp. 236–248). Wiley. https://doi.org/10.1002/fsn3.3056
25. Ereifej, K. I., Al-Karaki, G. N., & Hammouri, M. K. (2001). Seed chemical composition of improved chickpea cultivars grown under semiarid mediterranean conditions. In International Journal of Food Properties (Vol. 4, Issue 2, pp. 239–246). Informa UK Limited. https://doi.org/10.1081/jfp-100105190
26. Bober, A., Liashenko, M., Protsenko, L., Slobodyanyuk, N., Matseiko, L., Yashchuk, N., Gunko, S., & Mushtruk, M. (2020). Biochemical composition of the hops and quality of the finished beer. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 307–317). HACCP Consulting. https://doi.org/10.5219/1311
27. Łuszczki, E., Boakye, F., Zielińska, M., Dereń, K., Bartosiewicz, A., Oleksy, Ł., & Stolarczyk, A. (2023). Vegan diet: nutritional components, implementation, and effects on adults' health. In Frontiers in Nutrition (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fnut.2023.1294497
28. Samoilichenko, O., Priadko, O., Mokiichuk, V., Pashchenko, N., Bal-Prylypko, L., Slyva, Y., Tkachuk, V., Silonova, N., Nikolaienko, M., & Rozbytska, T. (2022). The impartiality of conformity assessment bodies is an integral component of the food safety management system. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 765–776). HACCP Consulting. https://doi.org/10.5219/1784
29. Vitriak, O. P., & Tkachenko, L. V. (2023). Section 17. Spicy and aromatic raw materials in the technology of healthy desserts and drinks. In Sustainable Food Chain And Safety Through Science, Knowledge and Business. Sustainable Food Chain and Safety Through Science, Knowledge and Business. Baltija Publishing. https://doi.org/10.30525/978-9934-26-328-6-17
30. Bal-Prylypko, L., Nikolaenko, M., Volkhova, T., Holembovska, N., Tyshchenko, L., Ivaniuta, A., Israelian, V., Menchynska, A., Shynkaruk, O., & Melnik, V. (2023). The study of functional and technological properties of vegetarian ice cream. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 110–121). HACCP Consulting. https://doi.org/10.5219/1798
31. Zheplinska, M., Mushtruk, M., Vasyliv, V., Sarana, V., Gudzenko, M., Slobodyanyuk, N., Kuts, A., Tkachenko, S., & Mukoid, R. (2021). The influence of cavitation effects on the purification processes of beet sugar production juices. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 18–25). HACCP Consulting. https://doi.org/10.5219/1494
32. Khomych, H. P., & Tkach, H. P. (2009). The use of wild raw materials for the provision of BAR food products. Poltava: RVV PUSKU.
33. Dmytrenko, A. A. (2022). Ethnocultural traditions of using blueberries in nutrition of the population of the right bank polissia: fillings for pie and varenyky. In Scientific notes of Taurida National V.I. Vernadsky University, series Historical Sciences (Issue 3, pp. 180–185). Publishing House Helvetica (Publications). https://doi.org/10.32838/2663-5984/2022/3.27
34. Casati, C. B., Baeza, R., & Sánchez, V. (2019). Physicochemical properties and bioactive compounds content in encapsulated freeze-dried powders obtained from blueberry, elderberry, blackcurrant and maqui berry. In Journal of Berry Research (Vol. 9, Issue 3, pp. 431–447). IOS Press. https://doi.org/10.3233/jbr-190409
35. Todorović, A., Šturm, L., Salević-Jelić, A., Lević, S., Osojnik Črnivec, I. G., Prislan, I., Skrt, M., Bjeković, A., Poklar Ulrih, N., & Nedović, V. (2022). Encapsulation of Bilberry Extract with Maltodextrin and Gum Arabic by Freeze-Drying: Formulation, Characterisation, and Storage Stability. In Processes (Vol. 10, Issue 10, p. 1991). MDPI AG. https://doi.org/10.3390/pr10101991
36. Rogoskii, I., Mushtruk, M., Titova, L., Snezhko, O., Rogach, S., Blesnyuk, O., Rosamaha, Y., Zubok, T., Yeremenko, O., & Nadtochiy, O. (2020). Engineering management of starter cultures in study of temperature of fermentation of sour-milk drink with apiproducts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 1047–1054). HACCP Consulting. https://doi.org/10.5219/1437
37. Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2020). Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. In Advances in Nutrition (Vol. 11, Issue 2, pp. 224–236). Elsevier BV. https://doi.org/10.1093/advances/nmz065

38. Koh, J., Xu, Z., & Wicker, L. (2020). Blueberry pectin and increased anthocyanins stability under in vitro digestion. In Food Chemistry (Vol. 302, p. 125343). Elsevier BV. https://doi.org/10.1016/j.foodchem.2019.125343
39. Chaikivskyi, T., & Zbarzhevetska, A. (2023). Use of flax processing products in the food industry. Zenodo. https://doi.org/10.5281/ZENODO.7989944
40. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of the dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/1711
41. Astrup, A., Magkos, F., Bier, D. M., Brenna, J. T., de Oliveira Otto, M. C., Hill, J. O., King, J. C., Mente, A., Ordovas, J. M., Volek, J. S., Yusuf, S., & Krauss, R. M. (2020). Saturated Fats and Health: A Reassessment and Proposal for Food-Based Recommendations. In Journal of the American College of Cardiology (Vol. 76, Issue 7, pp. 844–857). Elsevier BV. https://doi.org/10.1016/j.jacc.2020.05.077
42. Ustymenko, I., Bal-Prylypko, L., Nikolaenko, M., Ivaniuta, A., Tverezovska, N., Chumachenko, I., Pylypchuk, O., Rozbytska, T., Gruntovskyi, M., & Melnik, V. (2023). Development of sour cream with vegetable oils using a food emulsion stabilised by an emulsifying complex. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 159–169). HACCP Consulting. https://doi.org/10.5219/1849
43. Bal-Prylypko, L., Danylenko, S., Mykhailova, O., Nedorizanyuk, L., Bovkun, A., Slobodyanyuk, N., Omelian, A., & Ivaniuta, A. (2024). Influence of starter cultures on microbiological and physical-chemical parameters of dry-cured products. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 18, pp. 313–330). HACCP Consulting. https://doi.org/10.5219/1960
44. Bal-Prylypko, L., Berezina, L., Stepasyuk, L., Cherednichenko, O., & Lialyk, A. (2023). Developing dairy farming and improving product quality. In Scientific Horizons (Vol. 27, Issue 1, pp. 140–151). Scientific Journals Publishing House. https://doi.org/10.48077/scihor1.2024.140
45. Bhattacharya, S. (2023). Health and special snacks. In Snack Foods (pp. 461–524). Elsevier. https://doi.org/10.1016/b978-0-12-819759-2.00007-0
46. Pogarskaya, V., Pavlyuk, R., Kotuyk, T., Yurieva, O., Pogarskiy, A.,& Balabai, K. (2020). Development of a new method and nanotechnologies for the production of plant supplements from chickpeas for the production of health products. In Progressive equipment and technologies of food production, restaurant industry and trade. (Vol. 2, Issue 32, pp. 7–23). Baltija Publishing.
47. Acevedo Martinez, K. A., Yang, M. M., & Gonzalez de Mejia, E. (2021). Technological properties of chickpea (*Cicer arietinum*): Production of snacks and health benefits related to type-2 diabetes. In Comprehensive Reviews in Food Science and Food Safety (Vol. 20, Issue 4, pp. 3762–3787). Wiley. https://doi.org/10.1111/1541-4337.12762
48. Bondarenko, Yu., Bilyk, O., Kochubei-Lytvynenko, O., & Andronovich, G. (2020). Flax seeds as the prescription component of bakery products. In Scientific Works of National University of Food Technologies (Vol. 26, Issue 4, pp. 178–189). National University of Food Technologies. https://doi.org/10.24263/2225-2924-2020-26-4-20
49. Lapitska. N. (2021). The technology of infusions, extracts and concentrates. Chief assistant. Chernihiv: NUCHK was named after T.G. Shevchenko, p. 217.
50. Shanina, O., Minchenko, S., Gavrysh, T., Sukhenko, Y., Sukhenko, V., Vasyliv, V., Miedviedieva, N., Mushtruk, M., Stechyshyn, M., & Rozbytska, T. (2020). Substantiation of basic stages of gluten-free steamed bread production and its influence on quality of finished product. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 189–201). HACCP Consulting. https://doi.org/10.5219/1200
51. Miraghajani, M., Momenyan, S., Arab, A., Hasanpour Dehkordi, A., & Symonds, M. E. (2020). Blueberry and cardiovascular disease risk factors: A systematic review and meta-analysis of randomized controlled trials. In Complementary Therapies in Medicine (Vol. 53, p. 102389). Elsevier BV. https://doi.org/10.1016/j.ctim.2020.102389

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Dr. Larysa Bal-Prylypko

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Larysa Bal-Prylypko, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Management of Agricultural Products, Vystavkova Str., 16, studying building No 12, Kyiv, 03041, Ukraine,
Tel.: +38(067)401-86-72
E-mail: bplv@ukr.net
ORCID: https://orcid.org/0000-0002-9489-8610

***Inna Levytska**, National University of Life and Environmental Sciences of Ukraine, Educational and Scientific Institute of Continuing Education and Tourism, Department of Hotel and Restaurant Business and Tourism, Heroes of Defense Str., 10, 03040, Kyiv, Ukraine,
Tel.: +38(044) 527-80-61
E-mail: ilevytska@nubip.edu.ua
ORCID: https://orcid.org/0000-0003-3739-6662

Liubov Tkachenko, National University of Life and Environmental Sciences of Ukraine, Educational and Scientific Institute of Continuing Education and Tourism, Department of Hotel and Restaurant Business and Tourism, Heroes of Defense Str., 10, 03040, Kyiv, Ukraine,
Tel.: +38(044) 527-80-61
E-mail: lubashev28@gmail.com
ORCID: https://orcid.org/0000-0003-2731-1178

Yuliya Kryzhova, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Vistavkova Str., 16, Kyiv, 03041, Ukraine,
Tel.: +38(093)037-00-77
E-mail: name@domainname.sk
ORCID: https://orcid.org/0000-0000-0000-0000

Yaroslav Rudyk, National University of Life and Environmental Sciences of Ukraine, Faculty of Humanities and Pedagogy, Department of Management and Educational Technologies, Heroes of Defense Str., 15, 03041, Kyiv, Ukraine,
Tel.: +38(044)527-83-56
E-mail: rudyk.yaroslav@gmail.com
ORCID: https://orcid.org/0000-0001-5382-1505

Yevheniia Marchyshyna, National University of Life and Environmental Sciences of Ukraine, Mechanical and Technological Faculty, Department Occupational Safetyand Environmental Engineering, Heroyiv Oborony Str., 12B, Kyiv, 03041, Ukraine,
Tel.: +38(044)527-82-99
E-mail: marchyshyev@gmail.com
ORCID: https://orcid.org/0000-0001-8842-186X

Maxim Ryabovol, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Management of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Vystavkova Str., 16, studying building No 12, Kyiv, 03041, Ukraine,
Tel.: +38 (044) 527-89-50
E-mail: ryabovolmaksym@gmail.com
ORCID: https://orcid.org/0000-0003-0605-8708

Yuliia Slyva, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine, Faculty of Food Technologies and Quality Management of Agricultural Products, Department of Standardization and Certification of Agricultural Products, Heroiv Oborony St., 15, 03041 Kyiv, Ukraine
E-mail: yuliia_slyva@ukr.net
ORCID: https://orcid.org/0000-0003-2592-6822

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 13.2.2024
Revised: 31.5.2024
Accepted: 18.6.2024
Published: 13.9.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 792-806
https://doi.org/10.5219/1964
ISSN: 1337-0960 online
www.potravinarstvo.com

Innovative approach to the production of craft bread: A combination of tradition and innovation

Tetyana Semko, Mariia Paska, Olga Ivanishcheva, Liliia Kryzhak, Olena Pahomska, Alla Ternova, Olga Vasylyshyna, Serhii Hyrych

ABSTRACT

Today, the need to provide the population with high-quality food products, especially socially vulnerable citizens, requires significant efforts to find raw materials of high nutritional value available for mass consumption and their effective utilisation. This underscores the urgency of identifying reserves of available food nutrients to ensure balanced nutrition for the population of Ukraine, particularly as psycho-emotional stress increases in the conditions of martial law. These studies aim to scientifically justify and develop the latest technologies for bread incorporating sprouted wheat grain, hop sourdough, pumpkin puree, milk thistle, and fermented wort concentrate, which increase nutritional value. This article investigated the physicochemical and technological properties of dietary supplements and plant raw materials, scientifically substantiated the rational concentration and parameters of the technological process for the production of functional bakery products with their use, and studied the organoleptic, physicochemical properties, and nutritional value of the developed bakery products.

Keywords: rye-wheat bread, sprouted wheat grain, hop starter, pumpkin puree, milk thistle, dietary supplements

INTRODUCTION

An urgent contemporary issue is reducing environmental factors' negative impacts on health, particularly through improved nutrition. The unsatisfactory state of ecology and the deformed human diet lead to a decrease in the body's general resistance and the spread of several diseases, including those related to metabolism, such as diabetes, obesity, etc. **[1]**. Traditional nutrition does not provide a high preventive effect. According to the 'WHO Global Strategy in the Field of Nutrition, Physical Activity, and Health,' the gradual replacement of the traditional assortment of food products with functional ones, contributes to the maintenance of the normal functioning of all organs and systems of the human body, ensuring health and longevity, is the main direction of the civilised market **[2]**. Among the main principles is developing a strategy for creating new scientifically based technologies of ecologically clean food products, including functional purposes **[3]**.

In connection with the above, the formula for human nutrition at the beginning of the third millennium is the regular consumption of functional food products that, when consumed, specifically support and regulate specific physiological functions in the human body and reduce the occurrence of diseases **[4]**.

Due to their technological features, chemical composition, and biochemical characteristics of raw materials, bakery products have a high natural potential for correcting Ukrainians' nutritional status. Therefore, the efforts of domestic scientists and manufacturers are directed towards improving the assortment, developing, and implementing technologies of improved nutritional value with adjusted physiological properties **[5]**.

Bread is a product of mass regular consumption and occupies an average of 15% of the daily diet of Ukrainians. Increasing the nutritional value of bread and giving it functional properties is carried out by enriching it with natural products containing a significant amount of components, primarily biologically active substances **[29]**. It

contains carbohydrates, proteins, unsaturated fatty acids, and minerals **[6]**. However, despite having a fairly high caloric content, the chemical composition of bread needs to be more balanced in terms of vital components. Bread products have insufficient protein content. The ratio of proteins to carbohydrates is 1/6-1/7 compared to the optimal 1:4. Cereal proteins are inferior in amino acid composition. Bread proteins' main predominant amino acids are lysine, methionine, and tryptophan **[7]**.

Bread has high nutritional value. Unlike many other products, bread can give the human body significant energy and almost all vital substances: proteins, carbohydrates, vitamins, minerals, and fats. Thus, a bakery from high-grade wheat contains about 50% carbohydrates, 5-8% proteins, and 1% fats **[8]**. The energy value is 220 to 250 kcal per 100 g of the product. Additionally, wheat bakery products are important suppliers of some B vitamins (thiamine, riboflavin, niacin) and minerals (potassium, calcium, iron, magnesium, phosphorus) to the human body **[5]**.

Despite their rather high nutritional value according to modern requirements of nutritional science, bakery products need to improve their composition through enrichment with vegetable raw materials and dietary supplements **[6]**. In this regard, developing new functional bakery products using vegetable raw materials of increased biological value is relevant today **[25]**.

Due to their valuable chemical composition, pumpkin fruit processing products will allow for adjusting the bread recipe to obtain biologically valuable products with pronounced therapeutic and preventive properties **[9]**.

Milk thistle, which has been used in folk medicine for more than 2000 years as a remedy for various diseases, especially diseases of the liver, kidneys, and gall bladder, also has curative and preventive properties. Milk thistle is a rich source of amino acids (lysine, isoleucine, leucine, valine, and threonine), fatty acids, minerals, and phytochemicals, which, complementing the chemical composition and nutritional value of bread, will have a nutraceutical effect on human health (Table 1) **[10]**, **[11]**.

Table 1 Chemical composition of meals (per 100 g) **[30]**.

No	Indicator	Milk thistle meal
1	Protein, g	20
2	Fat, g	5.0
3	Carbohydrates, g	25.2
4	Fibre, g	35
5	Potassium, mg	920
6	Calcium, mg	1660
7	Magnesium, mg	420
8	Sodium, mg	4
9	Phosphorus, mg	960
10	Iron, mg	8
11	Iodine, mcg	9
12	Cobalt, mcg	10
13	Manganese, mcg	10
14	Copper, mcg	116
15	Vitamin A, mg	0.01
16	Vitamin C, mg	15
17	Vitamin B1, mg	0.3
18	Vitamin B2, mg	0.3
19	Vitamin B9, mcg	100
20	Vitamin E, mg	0.4
21	Vitamin PP, mg	2
22	Selenium, mcg	129

According to Table 2, milk thistle meal, in addition to high protein content, fat, and fibre, contains selenium, potassium, calcium, magnesium, iron, B vitamins, and vitamin E.

It has been established that such products can be used as food additives in products for medical and preventive purposes. Research data exists on the effect of plant additives on the properties of various types of dough and their components, as well as on the quality of finished products **[7]**.

Scientific Hypothesis

Dietary supplements can enhance the development of functional bread with increased nutritional value. After the addition of functional additives from sprouted wheat grain, pumpkin, fermented wort, and hops, we expect an increase in the amino acid composition and mineral substances in craft breads.

MATERIAL AND METHODOLOGY

Samples

The control sample of wheat yeast bread without additives was taken as a basis. The samples of semi-finished products made of dough for wheat bread with hops sourdough with sprouted wheat grain, wheat bread with hops sourdough with sprouted wheat grain with fermented wort concentrate, wheat bread with hops sourdough with sprouted wheat grain with pumpkin puree were tested.

Chemicals

All reagents (water, malt extract) were of USP purity and labelled LC/MS.

Animals, Plants and Biological Materials

This study used local raw materials from the Podillia region of Ukraine to produce craft bread.

Instruments

In the process of research, a MA 50.R weight moisture analyzer was used; laboratory scales TVE 3-0.05; hygrometer psychrometric VYT-2; 2-burner induction stove "ESPERANZA EKH008 St. Maria"; "Greentest" nitratometer; device for determining the humidity of food raw materials and products "Quartz" (Chyzhova's device); refractometer RPL-3; PH-262 pH meter; digital electronic thermometer with probe TP-101; laboratory tripod Bunsen SHL.

Laboratory Methods

Laboratory tests of raw materials were conducted at VITE's UTE laboratory of food technology, chemical, and microbiological research, which is certified by the quality management system (certificate No. UA.80050.063QMS-21). Indicators of raw materials and dough semi-finished products for craft bread were determined using physicochemical standard analysis methods following DSTU ISO 6820:2004 on Wheat and rye flour. General guidelines for the development of bread-making tests, corresponding to ISO 6820-1985 on Wheat flour and rye flour – General guidance on the development of bread-making tests, were followed as outlined in the relevant standards and instructions for technical and chemical control.

The preparation of the studied starter cultures was carried out following the recommendations **[12]**.

The rational amount of functional additives was determined experimentally. Organoleptic evaluation of the dough compositions was conducted on a 5-point scale.

The volume of starter cultures determined the physiological state of the microflora of liquid starter cultures. To one volume of spontaneously fermenting starter cultures, 3-5 volumes of water were added, shaken, and left for 1 minute. Afterward, the resulting suspension was used for the "crushed drop" preparation. In a stained preparation, a drop of Lugol's solution was added to the drop applied to the slide. The study was performed under a microscope at a magnification of *40 with a *10 or *15 eyepiece. The content of lactic acid bacteria and yeast was counted using standard methods with a Goryaev chamber **[3]**.

To determine the activity of lactic acid bacteria, 20 g of starter and 40 cm^3 of distilled water at 39 ±1 °C were stirred to a homogeneous consistency, and 10 cm^3 of the resulting suspension was taken into two test tubes. In one tube, 1 cm^3 of a 0.05% aqueous solution of methylene blue was added, and the other tube served as a control for color comparison. The tubes were closed with rubber stoppers, shaken, and placed in a thermostat at 40 °C. The time during which methylene blue discolored was recorded.

Humidity was determined by the drying method. The mass fraction of moisture was determined after baking by drying in an oven at 105 °C to a constant weight. The moisture content of semi-finished products (W), %, was calculated using the formula (1):

$$W = (m1-m2) \times 100 / m3; \quad (1)$$

Where:

m1, m2 – are the masses of the sample with the bag before and after drying, g; m3 – the weight of the semi-finished product, g.

The titratable acidity was determined using generally accepted methods (Figure 1).

Figure 1 Preparation of extract from the dough to determine acidity.

The lifting force of the dough was determined by the accelerated method by the floating of the dough ball, for which it was kneaded according to the recipes of the dough compositions. The dough food compositions were prepared by the dough batch method, and functional additives were introduced at the kneading stage.

Description of the Experiment

Number of samples analyzed: 5 samples.

Number of repeated analyses: All biochemical procedures were conducted in 15 repetitions.

Number of experiment replication: 2 times.

Design of the experiment: The experiment was carried out at the facilities of the craft bakery "Domashnya Vypichka Sonto France", Vinnytsia region, Ukraine.

The control sample of wheat yeast bread without additives was used as a basis. In the course of the experiment, semi-finished dough products were made for wheat bread on hop sourdough with germinated wheat grain "Semeyniy", wheat bread on hop sourdough from germinated wheat grain with fermented wort concentrate "Slovyanskiy", wheat bread on hop sourdough with germinated wheat grain with pumpkin puree "Selianskiy".

The specific measurement targets were the following indicators: active acidity for dough compositions, acidity of baked laboratory samples by the arbitration method, duration of fermentation of dough compositions, duration of proofing, spreading of the dough ball during fermentation and proofing, fermentation temperature, lifting power, mould stability, baking, specific volume of bread, porosity of crumb.

One method to enhance bakery products' microbiological purity is to utilize hop leavens in bread baking **[13]**. Hop-leavened bread has superior taste and aroma, undergoes less staling, and is less susceptible to foreign microflora **[14]**, **[15]**.

The studied starter cultures were prepared by fermenting 1st-grade wheat flour with a brew of hops (hops are poured with water and brewed for 15-20 minutes). After the hop brew has cooled, malt extract is added to it. The solution is poured into the fermentation vessel; additional flour is added and stirred. The starter is fermented at 30-32 °C to an acidity of 7-10 °T for 7-10 days until a specific "leaven" smell is obtained.

Arbitration method. Bread acidity was determined by the arbitration method. 25 g of crushed pulp was poured into a dry bottle with a capacity of 0.5 dm3, 250 cm^3 of room temperature water was added, and the pulp was thoroughly pounded with water with a wooden pestle. Shake vigorously for 2 minutes; stand for 10 min. Drained the extract through a sieve and pipetted 50 cm^3 into two conical flasks with a 100-150 cm^3 capacity and titrated. 0.1 mol/dm^3 sodium hydroxide solution. From 2-3 drops of phenolphthalein to a faint pink colour that does not disappear within 1 minute **[12]**.

To determine the rational amount of functional additives, experimental studies were conducted with the addition of germinated wheat grain in the amount of 10%, 20%, 30%, 40%, and 50% of the flour mass; pumpkin puree – 10%, 25%, 50%, 60%, 70% by mass of water; fermented wort concentrate – 0.1%, 0.5%, 1%, 2%, 4% by mass of sugar; milk thistle meal – 3%, 6%, 9%, 12%, 15% of the flour mass; hop starter – 100% of the mass of yeast. The influence of functional additives and plant raw materials on the structural and mechanical parameters of dough compositions is shown in Table 2.

The analysis of the research results showed that the addition of functional additives and vegetable raw materials in the production of dough food compositions helps to improve the elasticity of the dough for composition No. 1 – 4-10%, composition No. 2 – 9-12%, composition No. 3 – 12-18%; proofing of dough blanks - composition No. 1 – 4.9x103s., composition No. 2 – 4.6x103., composition No. 3 – 4.3x103s.; duration of fermentation on composition No. 1 – 7.5-8.6 x 103 s., composition No. 2 – 8.3-8.8 x 103 s., composition No. 3 – 6.6-7.8 x 103 s.; blurring of the dough ball during fermentation and proofing on THK No. 1 – 1-8%, THK No. 2 – 6-8%,

composition No. 3 – 8-17%; lifting force – composition No. 1 – 1.5 x 103 s, composition No. 2 – 1.4 x 103 s, composition No. 3 – 1.5 x 103 s. and dough properties improve during development.

Table 2 Structural and mechanical parameters of dough compositions.

Indexes	Control	Dough food composition No.1					Dough food composition No. 2					Dough food composition No. 3				
		Added SGW, % of the amount of flour					Added pumpkin puree, % of the amount of water					Added FWC, % of the amount of sugar, and RP, % of the amount of flour				
		D1	D2	D3	D4	D5	D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
		10	20	30	40	50	10	20	50	60	70	3	6	9	12	15
Duration of fermentation, min.	150	144	140	135	130	125	148	145	144	140	139	130	125	120	115	110
Duration of resistance, min.	120	83	81	80	81	82	77	76	75	74	73	73	72	70	68	66
Blurring of the dough ball during fermentation and proofing, %	170	169	167	165	160	158	157	158	160	159	158	145	150	158	156	154
Fermentation temperature, °C	28	27	26	26	27	28	27	25	26	25	24	23	25	26	25	24
Acidity, degrees	12	10	10	11	10	10.2	11	11	11	10	10.4	10.7	10	10	10.4	10.1
Lifting force, min.	30	25	24	23	23	23	24	23	23	23	23	25	24	23	23	23

The preparation of dough food compositions is given in Table 3.

Table 3 Dough food compositions.

Experiments		Experiment No. 1	Experiment No. 2	Experiment No. 3	Experiment No. 4	Experiment No. 5
Dough food composition No. 1, %	Wheat flour	90	80	70	60	40
	Sprouted grain of wheat	10	20	30	40	50
	Hop starter	100	100	100	100	100
Dough food composition No. 2, %	Wheat flour	90	80	70	60	40
	Sprouted grain of wheat	100	100	100	100	100
	Hop starter	100	100	100	100	100
	Water	90	80	50	40	30
	Pumpkin puree	10	20	50	60	70
Dough food composition No. 3, %	Wheat flour	90	80	70	60	40
	Sprouted grain of wheat	10	20	30	40	50
	Hop starter	100	100	100	100	100
	Wheat flour	97	94	91	88	85
	Spotted milk thistle	3	6	9	12	15
	Sugar	99.7	99.5	99	98	96
	Concentrate fermented wort	0.1	0.5	1	2	4

The dough was prepared using the steam method; functional additives and vegetable raw materials were introduced at the stage of kneading (Figure 2).

A . B. C.

Figure 2 The preparation of the dough. Note: A) displacement of dry ingredients with liquid; B) addition of hop starter; C) general view of the ooze.

When choosing the dosage of pumpkin puree when preparing dough composition No. 2, several factors were taken into account: the need for maximum enrichment of semi-finished products with vitamins and other biologically active substances; achieving optimal concentration in terms of their impact on the human body; obtaining baked semi-finished products with high organoleptic properties (color, taste, smell); economic feasibility. The concentration of pumpkin puree in the amount of 50% of the mass of water corresponds to these factors and is appropriate. Introducing pumpkin puree into the recipe allows you to increase the content of vitamins (A, B1, B2, and B6), and minerals (calcium, phosphorus, magnesium, potassium, and sodium) and reduce the energy value of baked semi-finished products. As a result of experimental studies, adding pumpkin puree to doughy food compositions improves the color, porosity, and elasticity of the pulp, and the shape of baked semi-finished products (Figure 3).

Figure 3 Determination of active acidity dough food composition No. 2 (pumpkin puree), dough food composition No. 3 (spotted milk thistle).

Statistical Analysis

Calculations were used on a computer. Experimental data was primarily processed using application programs for statistical analysis using criteria. The research was carried out in five repetitions. An application program for experiment planning and optimization was used to optimize the package of technological process parameters. The data were analyzed statistically using Microsoft Excel and Statistica 15. All experiments were performed in duplicate, and the results are presented as the results of these repeated determinations with standard deviations. The Students' t-test was used to analyze the results statistically. Data are presented as mean ± standard error of the mean (SEM). The minimum permissible difference for probes from the same sample was 5%. Test compositions with a larger difference were not considered.

RESULTS AND DISCUSSION

In the process of research, we used the following ingredients: wheat flour (DSTU 46.004-99 Wheat flour, Agrofirma Birkoff LLC, Ukraine), sprouted wheat grain (TM Dobra Ezhya Eco Choice, Ukraine), hop starter (SugrA-roma Durum Intense, Dr. Suwelack, Germany), pumpkin puree (local raw materials from Podillia region, Ukraine) **[24]**, milk thistle meal (Pharmacom Ltd., Ukraine), kvass wort concentrate (Kaniv Malt Extracts Plant Ltd., Ukraine), vegetable oil "Shchedryi Dar" DSTU 4292:2005 produced by Vinnytsia Oil and Fat Plant, Vinnytsia **[22]**.

Hop starters contain significantly more yeast cells than liquid rye starters, which affects their leavening power. Hop leavens rise in 18 minutes, compared to 25 minutes for rye leavens. The acidity of hop starters is typically 9.0 ±1.0 degrees **[13]** (Table 4).

Table 4 Comparative characteristics of sourdough acid accumulation **[13]**.

Time, min	Titrated sourdough acidity, degrees		
	hops	rye	grain
60	6.5	6.3	6.6
120	6.9	6.7	7.0
180	7.3	7.5	7.3
240	7.8	8.0	7.9
300	8.3	8.8	8.4
360	8.9	9.4	9.1
420	9.2	10.2	9.9
480	9.7	11.0	10.7
540	10.3	11.6	11.2
600	10.8	12.1	11.9
660	11.2	12.5	12.4
720	11.4	13.0	12.9

The data in Table 1 show that with an increase in fermentation time, the indicators of the hop leaven have the most optimal values, which will affect the acidity of the dough using this leaven and the quality of the finished products. Many scientists recommend including sprouted grain products in the diet, which cleanse the body, help remove toxins, and increase the immune system's resistance. The technology of making bread from sprouted wheat allows you to preserve a large amount of B vitamins and fiber in the final product, which are necessary for maintaining normal bodily functions **[23]**, **[26]**. This is achieved because grains are not ground into flour, and yeast is not added to the dough, allowing the healing properties of grain germs to be preserved **[14]**.

The use of fermented wort concentrate in the preparation of yeast-free bakery products allows not only to exclude sugar from the recipe while preserving the sweet taste of the product but also to enrich it with biologically active substances. Fermented wort concentrate is a brown, viscous liquid with a sweet-sour taste, maintained by kneading rye and barley malt with water, followed by clarification. It is well preserved due to its thick consistency (80%) **[16]**.

During the laboratory experiment, the tasting committee determined and analyzed the organoleptic indicators of all dough compositions **[30]**. The organoleptic evaluation on a 5-point scale is presented in Table 5.

After researching the rational amount of additives, the influence of sprouted wheat grain, hop sourdough, pumpkin puree, milk thistle, and fermented wort concentrate on the structural-mechanical, functional-technological, and physical properties of dough food compositions were determined **[28]**.

In the research process, laboratory baking of the developed dough compositions was conducted **[21]**. To fully characterize the nutritional value of baked samples, the chemical composition of bakery products with the addition of wheat sprouts and hop starter was investigated. Quantitative analysis showed that incorporating hop sourdough significantly improved dough quality, increasing volume by 5.7% and achieving more uniform porosity **[27]**.

The results of the experimental studies are presented in Table 6.

Comparing the chemical composition of the control and researched bakery products, it can be concluded that the nutritional value of the products increased according to the following indicators: proteins by 22.5% and 25.25% (Figure 4); potassium – by 32.6% and 32.65%; magnesium – by 186.2% and 188.18%, thiamine – by 285% and 585%, pyridoxine – by 180% and 344%; dietary fibers satisfy the daily requirement by 27% in experiments No. 1 and No. 2, respectively.

The organoleptic evaluation of dough composition No. 1 for “Family” bread is shown in Figure 4.

Table 5 Organoleptic evaluation of dough food compositions, points.

Experiment	Appearance	Color	Scent	Consistency	Taste	Porosity	Overall assessment
Dough food composition No. 1							
Control	4.79 ±0.10	4.82 ±0.10	4.74 ±0.10	4.65 ±0.10	4.46 ±0.10	4.45 ±0.10	4.65 ±0.10
Experiment No. 1	4.80 ±0.10	4.83 ±0.10	4.74 ±0.10	4.66 ±0.10	4.46 ±0.10	4.46 ±0.10	4.66 ±0.10
Experiment No. 2	4.81 ±0.10	4.82 ±0.10	4.75 ±0.10	4.65 ±0.10	4.45 ±0.10	4.46 ±0.10	4.66 ±0.10
Experiment No. 3	4.86 ±0.10	4.94 ±0.10	4.89 ±0.10	4.75 ±0.10	4.63 ±0.10	4.73 ±0.10	4.80 ±0.10
Experiment No. 4	4.76 ±0.10	4.80 ±0.10	4.72 ±0.10	4.60 ±0.10	4.44 ±0.10	4.43 ±0.10	4.62 ±0.10
Experiment No. 5	4.75 ±0.10	4.76 ±0.10	4.70 ±0.10	4.58 ±0.10	4.40 ±0.10	4.41 ±0.10	4.60 ±0.10
Dough food composition No. 2							
Control	4.79 ±0.10	4.82 ±0.10	4.74 ±0.10	4.65 ±0.10	4.46 ±0.10	4.45 ±0.10	4.65 ±0.10
Experiment No. 1	4.81 ±0.10	4.84 ±0.10	4.76 ±0.10	4.68 ±0.10	4.47 ±0.10	4.48 ±0.10	4.67 ±0.10
Experiment No. 2	4.83 ±0.10	4.85 ±0.10	4.77 ±0.10	4.67 ±0.10	4.48 ±0.10	4.49 ±0.10	4.68 ±0.10
Experiment No. 3	4.87 ±0.10	4.92 ±0.10	4.90 ±0.10	4.77 ±0.10	4.68 ±0.10	4.75 ±0.10	4.82 ±0.10
Experiment No. 4	4.77 ±0.10	4.82 ±0.10	4.74 ±0.10	4.62 ±0.10	4.47 ±0.10	4.47 ±0.10	4.69 ±0.10
Experiment No. 5	4.78 ±0.10	4.78 ±0.10	4.72 ±0.10	4.60 ±0.10	4.48 ±0.10	4.45 ±0.10	4.70 ±0.10
Dough food composition No. 3							
Control	4.79 ±0.10	4.82 ±0.10	4.74 ±0.10	4.65 ±0.10	4.46 ±0.10	4.45 ±0.10	4.65 ±0.10
Experiment No. 1	4.82 ±0.10	4.85 ±0.10	4.76 ±0.10	4.68 ±0.10	4.48 ±0.10	4.47 ±0.10	4.67 ±0.10
Experiment No. 2	4.84 ±0.10	4.84 ±0.10	4.77 ±0.10	4.67 ±0.10	4.47 ±0.10	4.48 ±0.10	4.68 ±0.10
Experiment No. 3	4.88 ±0.10	4.99 ±0.10	4.90 ±0.10	4.79 ±0.10	4.69 ±0.10	4.75 ±0.10	4.82 ±0.10
Experiment No. 4	4.78 ±0.10	4.82 ±0.10	4.73 ±0.10	4.62 ±0.10	4.45 ±0.10	4.45 ±0.10	4.64 ±0.10
Experiment No. 5	4.77 ±0.10	4.75 ±0.10	4.74 ±0.10	4.60 ±0.10	4.44 ±0.10	4.44 ±0.10	4.63 ±0.10

Table 6 Chemical composition of bakery products with the addition of sprouted wheat and hop leaven.

Nutrients	Control	Experiment No. 1	Experiment No. 2	Difference (experiment No. 1)	Difference (experiment No. 2)	Relative deviation, % (experiment No. 1)	Relative deviation, % (experiment No. 2)
Belky, g	16.0	19.16	20.04	3.16	4.04	22.50	25.25
Carbohydrates,g	70.0	76.24	76.7	6.24	6.70	8.90	9.57
Dietary fibers, g	0.3	7.94	7.96	7.64	7.66	2646	2653
K, mg	300	397.87	397.95	97.87	97.95	32.60	32.65
Ca, mg	250	261.55	261.87	11.55	11.87	4.62	4.75
P, mg	250	431.2	431.88	181.2	181.88	72.48	72.75
Fe, mg	2.0	4.45	11.8	2.45	9.8	2.22	5.90
Mg, mg	50	143.1	144.09	93.1	94.09	186.20	188.18
B_1, mg	0.2	0.77	1.37	0.57	1.17	285.0	585.0
B_3, mg	1.0	1.35	3.08	0.35	2.08	35.0	308.0
B_6, mg	0.5	0.9	1.72	0.40	1.22	180.0	344.0
PP, mg	5.0	5.35	5.39	0.35	0.37	7.0	7.8
E, mg	6.0	6.3	7.04	0.30	1.04	5.0	17.3
Folic acid, mg	-	0.11	0.18	0.11	0.18	-	-
Energetic value, kcal	335.5	395.1	445.91	59.60	110.41	17.8	32.9

Note: all values are expressed as the mean ±SD (standard deviation). The difference with the control is statistically significant, $p < 0.05$.

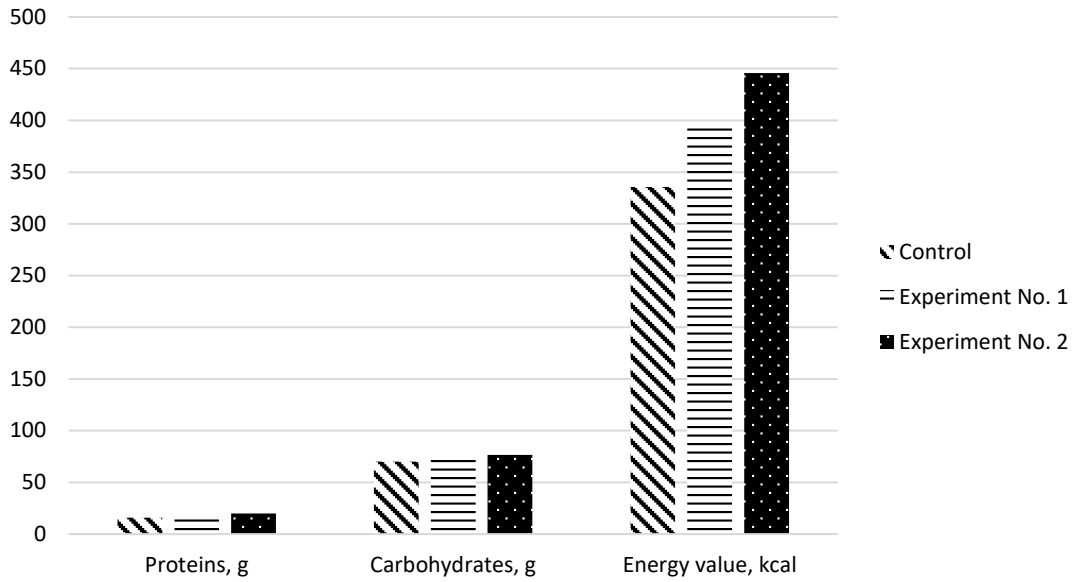


Figure 4 Composition and energy value of bakery products with the addition of sprouted wheat and hop starter.

The quality of baked laboratory samples according to organoleptic indicators corresponds to the regulatory and technological documentation – ISO 6820-1985.

Organoleptic and physicochemical indicators of the quality of experimental samples are given in Table 7.

Table 7 Organoleptic indicators of the quality of wheat bread with sprouted wheat grain.

Name indicator	Bread wheat (Control)	Wheat bread 'Family' (experiment)	Wheat bread 'Peasant' (experiment)	'Slavyansky' wheat bread (experiment)
Organoleptic indicators				
Surface	Smooth without pollution. large cracks and undermining	Coarse, without pollution, without large cracks and undermining, the grinding of soft wheat grains is visible		
Condition of the pulp	Baked, elastic, with developed porosity, without traces of unbaked and compacted pulp	Baked, compacted, soft without traces of unthreshing, on the cut you can see crushed soft grains of wheat		
The color of the crust	Light yellow	Yellow. without burning and pollution		
Taste and smell	Characteristic of this name of bread. without extraneous taste and smell	Pleasant without extraneous aftertaste and smell. characteristic of this type of bread and the raw materials and additives used		
Physico-chemical parameters				
Acidity, degrees	7.0	3.0	3.0	3.0
Humidity, %	45.0	44.0	42.0	43.0
Porosity, %	50.0	72.0	70.0	71.0

During experimental studies, it was established that a further increase in the concentration of functional additives leads to a decrease in quality, namely, a decrease in the volume of wheat bread, deterioration of the crumb structure, and loss of its elasticity, characteristics of products with such additives. The taste and smell of products with the recommended amount of additives acquire pleasant shades. Still, a further increase in the dosage of dietary supplements gives the products an excessively pronounced taste and smell **[3]**.

The introduction of germinated wheat grain practically does not affect the ough's initial titrated acidity. However, as the additive's dosage increases, the process of acid accumulation in dough samples with the additive is more intense than in the control one. The use of wheat germ processing products intensifies the process of acid accumulation in the dough, confirming the possibility of reducing the duration of its ripening **[17]**, **[12]**.

According to scientists' research, pumpkin fruit processing products (due to their valuable chemical composition) will allow for adjusting the bread recipe to obtain biologically valuable products with pronounced therapeutic and preventive properties **[9]**.

It was established that the addition of 70% pumpkin puree to the mass of water in the recipe of bread made from high-grade flour reliably reduces the baking of bread, its volume, specific volume, the ratio of the volume of bread to the volume of the dough, and convexity **[18]**.

Fermented wort concentrate is a brown, viscous liquid with a sweet-sour taste, maintained by kneading rye and barley malt with water, followed by clarification. It keeps well due to its thick consistency (80%). The use of fermented wort concentrate in the preparation of yeast-free bakery products makes it possible not only to exclude sugar from the recipe while preserving the sweet taste of the product but also to enrich it with biologically active substances **[16]**.

Milk thistle (Silybum marianum) contains fat-soluble vitamins such as vitamins A, D, E, and K, especially vitamin E, the main antioxidant among vitamins. This vitamin plays a significant role in the human body, protecting it from the effects of chemical and physical factors that contribute to the development of tumors **[11]**, **[19]**.

The plant contains the antioxidant silymarin, proteins (17%), which contain all essential amino acids, and water-soluble vitamins **[10]**, **[2]**. Based on the obtained data, it can be concluded that the baked semi-finished product, made with the addition of milk thistle in the amount of 9% of the flour mass and fermented wort concentrate in the amount of 1 % of the sugar mass, has the highest organoleptic indicators (Table 2, Figures 4-6).

The organoleptic evaluation of dough composition No. 1 for “Family” bread is presented in Figure 5.

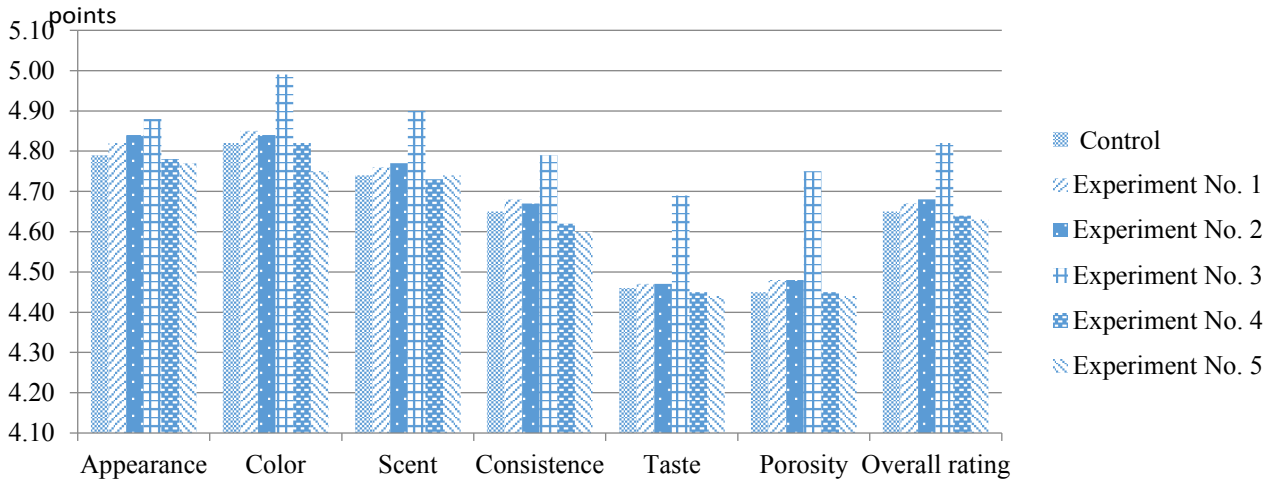


Figure 5 Organoleptic evaluation of dough food composition No. 1.

The organoleptic evaluation of dough composition No. 2 for “Selyansky” bread is presented in Figure 6.

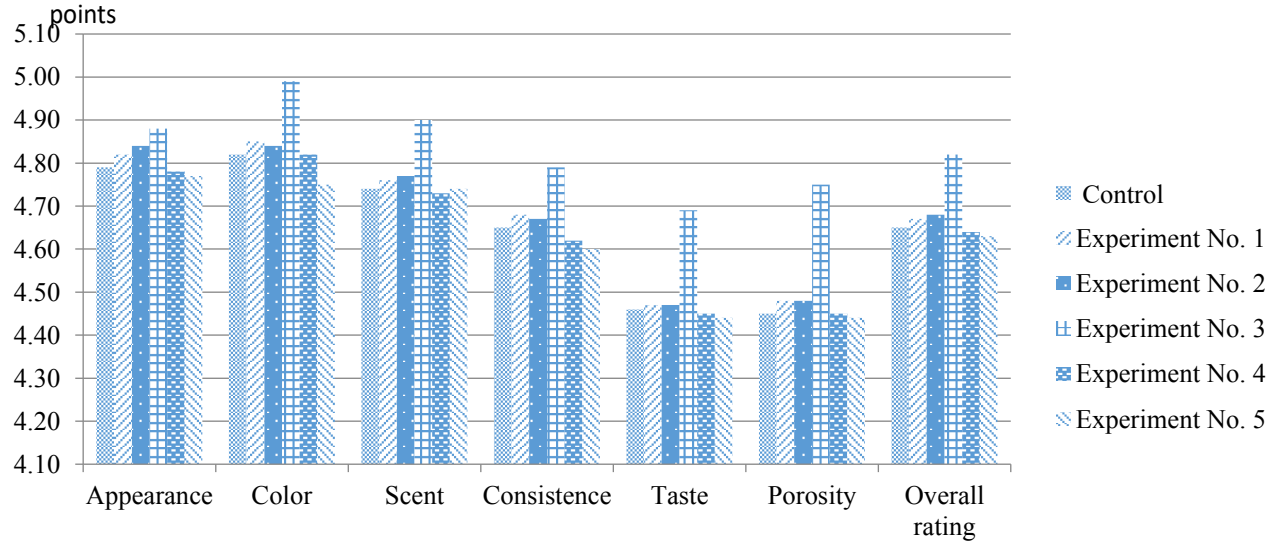


Figure 6 Organoleptic evaluation of dough food composition No. 2.

The organoleptic evaluation of dough composition No. 3 for "Slovyansky" bread is presented in Figure 7.

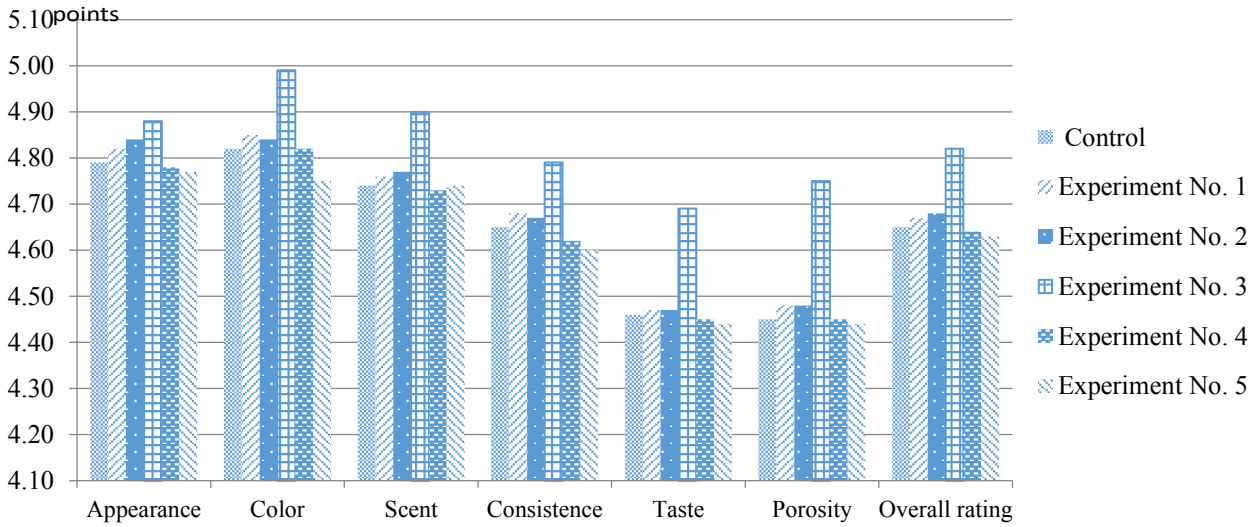
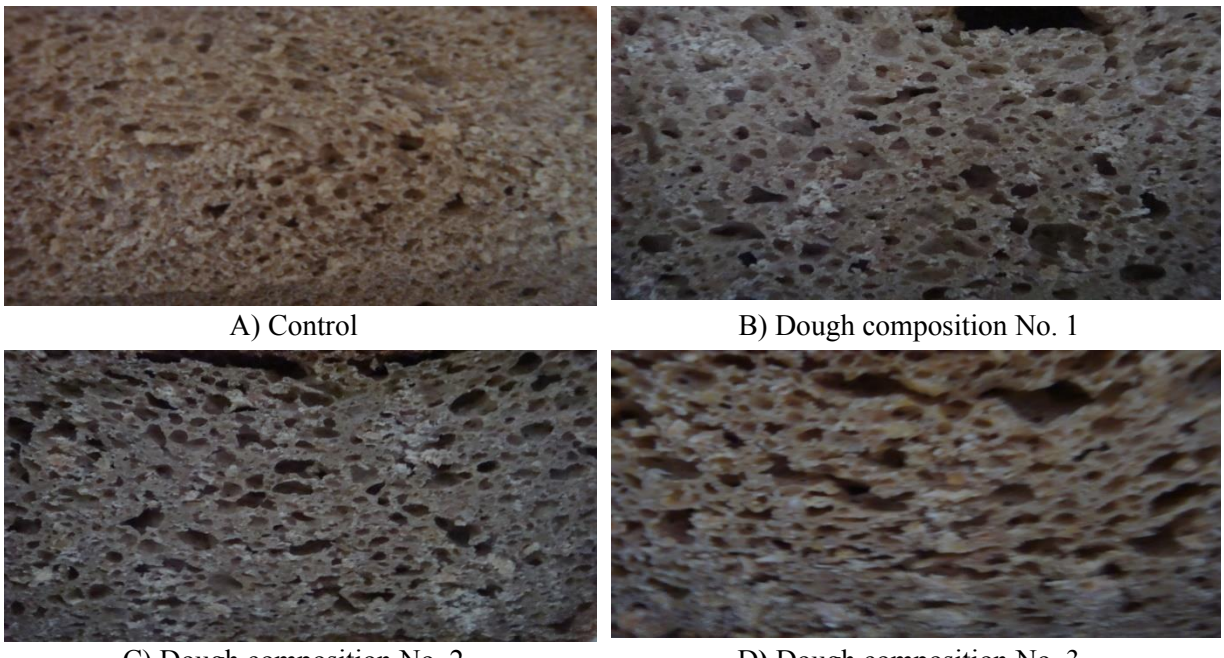


Figure 7 Organoleptic evaluation of dough food composition No. 3.

The results of the organoleptic evaluation of dough food compositions No. 3 established that the addition of milk thistle in the amount of 3-6% of the mass of flour and fermented wort concentrate in the amount of 0.1-0.5% of the mass of sugar does not significantly affect the organoleptic indicators of baked semi-finished products **[20]**.

Using hop leaven in dough food compositions improves the quality of baked semi-finished products: the pulp is more elastic, the porosity is more developed and uniform, and the baked semi-finished product acquires a specific pronounced taste and aroma. The time for preparing the dough and semi-finished product is reduced by 30 minutes **[15]**. The organoleptic evaluation of dough food compositions established that adding 10-20% sprouted wheat grain from the flour mass does not affect the organoleptic indicators of baked semi-finished products (Table 4). In 40-60%, sprouted wheat grain gives semi-finished products an excessively pronounced taste and smell and a too-dense consistency, reducing the organoleptic evaluation **[8]**. So, based on the obtained data, it can be concluded that the baked semi-finished product made with the addition of sprouted wheat grain in the amount of 30% of the flour mass has the highest organoleptic indicators.

After laboratory baking, photographic images were received of the structure of baked dough compositions using sprouted wheat grains, hop sourdough, pumpkin puree, sourdough wort concentrate, and milk (Figure 8).



A) Control B) Dough composition No. 1

C) Dough composition No. 2 D**)** Dough composition No. 3

Figure 8 Samples of laboratory baking of developed dough compositions.

As can be seen from Figure 8, the addition of dietary supplements and vegetable raw materials improves the structure of the porosity of the dough blanks compared to the control. In the first baking period, a crust's formation occurs due to thermal and moisture conductivity, and the amount of baking in this regard is insignificant. In contrast, the rate of moisture release gradually increases. In the second baking period, the rate of moisture release remains constant and equal to the maximum rate reached at the end of the first baking period. Therefore, the main baking loss occurs during the second baking period. The surface layer of the dough blanks is subjected to dehydration to a greater extent, while part of the moisture (approximately 80-85%) that evaporates passes into the gaseous environment of the baking chamber, and part – 20-15% moves due to thermal moisture conductivity into the soft dough blanks. As a result, to reduce baking losses, it is advisable to complete the baking of dough blanks at a reduced temperature of the baking chamber – 200 °C.

CONCLUSION

Our findings support the strategic inclusion of functional additives such as sprouted wheat grain, pumpkin puree, and milk thistle meal in craft bread dough formulations, marking a significant advance in nutrition and healthy eating. The rational amount of functional additives in dough food compositions was determined: sprouted wheat grain – 30%, milk thistle meal – 9%, pumpkin puree – 50% by weight of water, fermented wort concentrate – 1% by weight of sugar, hop sourdough starter – 100% by weight of yeast. It was found that a further increase in the concentration of functional additives leads to a decrease in bread quality, namely, a decrease in the volume of wheat bread, deterioration of the crumb structure, and loss of elasticity. As a result of the studies of the dough samples, it can be concluded that the nutritional value of the products increased by the following indicators: proteins by 22.5% and 25.25%; potassium by 32.6% and 32.65%; magnesium by 186.2% and 188.18%, thiamine by 285% and 585%, pyridoxine by 180% and 344%; dietary fibre satisfied the daily requirement by 27% in experiments No. 1 and No. 2, respectively. Developing dough compositions for producing Semeyniy, Slavyansky, and Selyansky bread will enable the production of products of high biological value for functional purposes that meet modern requirements for healthy eating. Our further scientific research aims to substantiate the technological modes of molding and baking the above types of bread.

REFERENCES

1. Pakhomska, O. V. (2020). Modern technologies of bakery products based on sprouted wheat grain and dietary supplements. In Quality and safety of food products and raw materials - today's problems: Materials of the international conference (pp. 143-144.) "Rastr-7" Publishing House.
2. Çelik, H. T., & Gürü, M. (2015). Extraction of oil and silybin compounds from milk thistle seeds using supercritical carbon dioxide. In The Journal of Supercritical Fluids (Vol. 100, pp. 105–109). Elsevier BV. https://doi.org/10.1016/j.supflu.2015.02.025
3. Vaskivska, A. O., & Peresichna, S. M. (2022). Technology of yeast-free bread using gluten-free raw materials. In Taurian Scientific Herald. Series: Technical Sciences (Issue 4, pp. 44–54). Publishing House Helvetica (Publications). https://doi.org/10.32851/tnv-tech.2022.4.6
4. Tebben, L., Shen, Y., & Li, Y. (2018). Improvers and functional ingredients in whole wheat bread: A review of their effects on dough properties and bread quality. In Trends in Food Science & Technology (Vol. 81, pp. 10–24). Elsevier BV. https://doi.org/10.1016/j.tifs.2018.08.015
5. Semko, T., & Pakhomska, O. (2023). Innovative craft bakery technologies. In Modern engineering and innovative technologies (Issues 27–01, pp. 68–73). Ukrainian Research and Design Institute of Building Materials and Products. https://doi.org/10.30890/2567-5273.2023-27-01-013
6. Nurgozhina, Z., Shansharova, D., Umirzakova, G., Maliktayeva, P., & Yakiyayeva, M. (2022). The influence of grain mixtures on the quality and nutritional value of bread. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 320–340). HACCP Consulting. https://doi.org/10.5219/1767
7. Simakova, O. O., & Nikiforov, R. P. (2018). Development of the latest technologies of flour products with specified properties: monograph. DonNUET, 146 p.
8. Wang, Y., Maina, N. H., Coda, R., & Katina, K. (2021). Challenges and opportunities for wheat alternative grains in breadmaking: Ex-situ- versus in-situ-produced dextran. In Trends in Food Science & Technology (Vol. 113, pp. 232–244). Elsevier BV. https://doi.org/10.1016/j.tifs.2021.05.003
9. Indrani, D., Soumya, C., Rajiv, J., & Venkateswara Rao, G. (2010). Multigrain Bread – Its Dough Rheology, Microstructure, Quality And Nutritional Characteristics. In Journal of Texture Studies (Vol. 41, Issue 3, pp. 302–319). Wiley. https://doi.org/10.1111/j.1745-4603.2010.00230.x
10. Saad-Allah, K. M., Fetouh, M. I., & Elhaak, M. A. (2017). Induction of milk thistle (Silybum marianum L. Gaertn) growth and phytochemicals production by natural stimulants. In Journal of Applied Research on

Medicinal and Aromatic Plants (Vol. 6, pp. 101–110). Elsevier BV. https://doi.org/10.1016/j.jarmap.2017.02.006

11. Abenavoli, L., Izzo, A. A., Milić, N., Cicala, C., Santini, A., & Capasso, R. (2018). Milk thistle (Silybum marianum): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. In Phytotherapy Research (Vol. 32, Issue 11, pp. 2202–2213). Wiley.
12. DSTU 7045:2009 Bakery products. Methods for determination of physical and chemical parameters. Kyiv: derzhspozhyvstandart Ukrainy, 2009. Natsionalnyi standart Ukrainy.
13. Olga, S., Olesya, S., Zhurok Iryna, Nadiya, L., & Olena, G. (2022). Investigation Of The Composition Of The Microflora Of Leavens' Spontaneous Fermentation For Rye-Wheat Bread. Zenodo. https://doi.org/10.5281/ZENODO.7110952
14. Shanina, O., Galyasnyj, I., Gavrysh, T., Dugina, K., Sukhenko, Y., Sukhenko, V., Miedviedieva, N., Mushtruk, M., Rozbytska, T., & Slobodyanyuk, N. (2019). Development of gluten-free non-yeasted dough structure as factor of bread quality formation. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 13, Issue 1, pp. 971–983). HACCP Consulting. https://doi.org/10.5219/1201
15. Papadimitriou, K., Zoumpopoulou, G., Georgalaki, M., Alexandraki, V., Kazou, M., Anastasiou, R., & Tsakalidou, E. (2019). Sourdough Bread. In Innovations in Traditional Foods (pp. 127–158). Elsevier. https://doi.org/10.1016/b978-0-12-814887-7.00006-x
16. Ivanishcheva, O., & Pakhomska, O. (2021). Trends in the quality of functional bakery products. In Young Scientist (Issue 5 (93), pp. 159–163). Publishing House Young Scientist. https://doi.org/10.32839/2304-5809/2021-5-93-30
17. DSTU 7044:2022 Bakery products. DSTU 7044:2022 Bakery products. Acceptance rules, sampling methods, methods for determining the weight of products. Kyiv: DP «UkrNDNTs».
18. Liubych, V. V., Zheliezna, V. V., & Hrabova, D. M. (2021). Quality of triticale cupcakes enriched with pumpkin paste. In Collected Works of Uman National University of Horticulture (Vol. 1, Issue 99, pp. 17–28). Uman National University of Horticulture. https://doi.org/10.31395/2415-8240-2021-99-1-17-28
19. Bojňanská, T., Vollmannová, A., & Musilová, J. (2020). Milk thistle flour effect on dough rheological properties. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 788–797). HACCP Consulting. https://doi.org/10.5219/1365
20. Melnyk, O., & Marenkova, T. (2023). The Use Of Milk Thistle Seed Flour In The Composition Of Yeast Dough For Cheese Pastry. In Grain Products and Mixed Fodder's (Vol. 22, Issue 3, pp. 45–50). Odesa National University of Technology. https://doi.org/10.15673/gpmf.v22i3.2460
21. Gao, J., Tay, S. L., Koh, A. H. S., & Zhou, W. (2017). Dough and bread made from high- and low-protein flours by vacuum mixing: Part 2. Yeast activity, dough proofing and bread quality. In Journal of Cereal Science (Vol. 77, pp. 275–283). Elsevier BV. https://doi.org/10.1016/j.jcs.2017.08.015\
22. Torbica, A., Belović, M., & Tomić, J. (2019). Novel breads of non-wheat flours. In Food Chemistry (Vol. 282, pp. 134–140). Elsevier BV. https://doi.org/10.1016/j.foodchem.2018.12.113
23. Zhemela, H. P., Bahan, A. V., Barabolia, O. V., Shakaliy, S. M., & Chaika, T. O. (2020). Ecological baking of wheat bread using hop sourdoughs and spirulina. In Scientific Progress & Innovations (Issue 1, pp. 100–106). Poltava State Agrarian Academy. https://doi.org/10.31210/visnyk2020.01.11
24. Barabolia O. V., Kalashnyk O. V., Moroz S. E., Zhemela G. P., Yudicheva O. P., Serhienko O. V. (2018). The use of pumpkin semi-product for wheat bread enrichment. In Scientific Progress & Innovations, (Issue 4, pp. 76–79). Poltava State Agrarian Academy. https://doi.org/10.31210/visnyk2018.04.11
25. Osokina, N., Kostetska, K., Gerasymchuk, H., Voziian, V., Telezhenko, L., Priss, O., Zhukova, V., Verkholantseva, V., Palyanichka, N., & Stepanenko, D. (2017). Development of recipes and estimation of raw material for production of wheat bread. In EUREKA: Life Sciences (Vol. 4, pp. 26–34). OU Scientific Route. https://doi.org/10.21303/2504-5695.2017.00381
26. Oliinyk, S., Samokhvalova, O., Lapitskaya, N., & Kucheruk, Z. (2020). Study of the influence of meals of wheat and oat germs and wild rose fruits on the fermenting microflora activity of rye-wheat dough. In Eureka: Life Sciences (Vol. 1, pp. 40–47). OU Scientific Route. https://doi.org/10.21303/2504-5695.2020.001114
27. Yudicheva, O. P., Kalashnyk, O. V., Moroz, S. E., Rybalko, O. A., & Korsun, A. V. (2020). Organoleptic assessment of wheat bread enriched with pumpkin processing products. In Herald of Lviv University of Trade and Economics. Technical sciences (Issue 23, pp. 136–144). Publishing House Helvetica (Publications). https://doi.org/10.36477/2522-1221-2020-23-18
28. Hospodarenko, H. M. (2022). Optimization of pumpkin flour in bread technology. In Bulletin of Uman National University of Horticulture (Vol. 1, pp. 82–88). Uman National University of Horticulture. https://doi.org/10.31395/2310-0478-2022-1-82-88

29. Pahomska, O. (2019). Scientific approach to the creation of bakery products of high functional purpose. In Scientific Works of National University of Food Technologies (Vol. 25, Issue 2, pp. 276–283). National University of Food Technologies. https://doi.org/10.24263/2225-2924-2019-25-2-30

30. Antonenko, A. V., & Mikhailik, V. S. (2016). Manufacturing process and the quality of cookies with the whole grains of the oil-bearing plants. In Food Science and Technology (Vol. 10, Issue 1). Odessa National Academy of Food Technologies. https://doi.org/10.21691/fst.v10i1.83

Funds:

This research received no external funding.

Acknowledgments:

We would like to express our gratitude to the Slobodianiuk family's craft bakery "Home Baking "Sonto Frans", Vinnytsia region, Ukraine, which was used for the research.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Tetyana Semko**, Vinnytsia Institute of Trade and Economics of State University of Trade and Economics, Faculty of Trade, Marketing and Services, Department of Tourism, Hotel and Restaurant Business, Soborna, 87, 21050, Vinnytsia, Ukraine,
Tel.: +380679625468
E-mail: semko1965@ukr.net
ORCID: https://orcid.org/0000-0002-1951-5384

Mariia Paska, Ivan Bobersky Lviv State University of Physical Culture, Faculty of Tourism, Department of Hotel and Restaurant Business, Kostyushka Street, 11, 79000, Lviv, Ukraine,
Tel.: +380673026120
E-mail: maria_pas@ukr.net
ORCID: https://orcid.org/0000-0002-9208-1092

Olga Ivanishcheva, Vinnytsia Institute of Trade and Economics of State University of Trade and Economics, Faculty of Trade, Marketing and Services, Department of Tourism, Hotel and Restaurant Business, Soborna, 87, 21050, Vinnytsia, Ukraine,
Tel.: +380933460220
E-mail: o.ivanishcheva@vtei.edu.ua
ORCID: https://orcid.org/0000-0002-0500-3652

Liliia Kryzhak, Vinnytsia Institute of Trade and Economics of State University of Trade and Economics, Faculty of Trade, Marketing and Services, Department of Tourism, Hotel and Restaurant Business, Soborna, 87, 21050, Vinnytsia, Ukraine,
Tel.: +380967242684
E-mail: liliakrizhak44@gmail.com
ORCID: https://orcid.org/0000-0002-4882-897X

Olena Pahomska, Vinnytsia Institute of Trade and Economics of State University of Trade and Economics, Faculty of Trade, Marketing and Services, Department of Tourism, Hotel and Restaurant Business, Soborna, 87, 21050, Vinnytsia, Ukraine,
Tel.: +380987876853
E-mail: olana1980@ukr.net
ORCID: https://orcid.org/0000-0002-0915-8811

Alla Ternova, Vinnytsia Institute of Trade and Economics of State University of Trade and Economics, Faculty of Trade, Marketing and Services, Department of Commodity Science, Expertise and Commercial Business, Soborna, 87, 21050, Vinnytsia, Ukraine,
Tel.: +380977605126
E-mail: a.ternova@vtei.edu.ua
ORCID: https://orcid.org/0000-0002-7447-137X

Olga Vasylyshyna, Vinnytsia Institute of Trade and Economics of State University of Trade and Economics, Faculty of Trade, Marketing and Services, Department of Commodity Science, Expertise and Commercial Business, Soborna, 87, 21050, Vinnytsia, Ukraine,
Tel.: +380982671583
E-mail: olga_vasilishina@ukr.net
ORCID: https://orcid.org/0000-0003-0955-511X

Serhii Hyrych, Vinnytsia Institute of Trade and Economics of State University of Trade and Economics, Faculty of Trade, Marketing and Services, Department of Commodity Science, Expertise and Commercial Business, Soborna, 87, 21050, Vinnytsia, Ukraine,
Tel.: +380673963114
E-mail: s.hyrych@vtei.edu.ua
ORCID: https://orcid.org/0000-0002-0342-092X

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 16.7.2024
Revised: 31.8.2024
Accepted: 3.9.2024
Published: 27.9.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 807-818
https://doi.org/10.5219/2000
ISSN: 1337-0960 online
www.potravinarstvo.com

Proximate and fatty acid analysis of goat and goat-cow mixed milk cheeses

Altynay Abdugamitova, Assiya Serikbayeva, Akmaral Mateyeva, Gulbagi Orymbetova, Sulushash Mateyeva, Ainur Matibaeyva, Akerke Toleugazykyzy

ABSTRACT

Goat milk is a valuable resource for food production thanks to its physical, chemical, and biological properties, easy digestibility, and lower allergenicity. The dairy product market in Kazakhstan is growing, leading to advancements in the industrial processing of goat milk. Consequently, this study aimed to analyse the proximate composition of raw goat milk, produce cheeses from goat milk alone and in combination with cow milk and examine the fatty acid profiles of the resulting cheeses. The findings indicated that goat milk contained a higher percentage of protein, while a 1:1 mixture of goat and cow milk exhibited increased levels of lactose and fat (p <0.001). A soft cheese prepared from goat milk exhibited faster coagulation, higher cheese yield and fat in dry matter. Furthermore, the saturated fatty acid (SFA) content was greater in cheeses made from the goat-cow milk blend compared to those made solely from goat milk (p <0.05). Notably, an increased presence of oleic acid and polyunsaturated fatty acids (PUFAs), such as linoleic, linolenic, and palmitoleic acids, was only detected in 100% goat milk cheeses. Overall, the soft cheese produced from goat milk showed improved nutritional qualities, particularly regarding fatty acid content. However, additional research is needed to assess sensory attributes and consumer acceptance.

Keywords: goat milk, cow milk, cheese yield, nutritional analysis

INTRODUCTION

At present, the domestic market of dairy products is developing and industrial processing of goat milk is evolving. Goat breeds such as Zaanen goat, Alpine goat, and Nubian goat are the most used dairy breeds in Kazakhstan. Goat's milk is of interest because of its physicochemical and biological properties, as it is easier to digest and is considered less allergenic **[1]**. The fat content of goat's and cow's milk is almost at the same level at around 3.5-4% **[2]**. Previous research has shown that goat and camel milk is better digested than buffalo and cow milk because of the small size of the fat globules **[3]**. The fat content of goat's milk has higher short- and medium-chain triglycerides than cow's milk fat **[4]**. Creating biotechnologies of nutritious food products is a priority and the most important area of development of the dairy industry. Currently, the range of goat milk products is limited **[5]**. The peculiarity of goat's milk cheese production is due to its lower ability to be coagulated by enzymes, which, to some extent, can be explained by the fractional composition of the protein and low titratability **[6]**. Therefore, cow milk or the introduction of increased doses of bacterial starter and calcium chloride are often used to ripen goat milk when processing goat milk into cheese **[7]**. Using goat's milk in producing soft cheeses, possessing hypoallergenic and special biological properties, is of certain scientific and practical interest. López-Villafaña et al. analysed the antioxidant activity, which was stronger in Mexican Panela cheeses manufactured from goat milk **[8]**. In addition, the Akavi and Halloumi cheeses from goat milk have been studied previously **[9]**.

Recent efforts have focused on creating goat milk cheese with altered texture properties. For example, fresh goat cheese was developed by incorporating polysaccharides to meet consumer desires and production requirements **[10]**. The strain of *Lactococcus lactis* has been utilised to enhance the quality and safety of goat fresh cheese while maintaining natural farming methods **[11]**. Thus, the research addressed growing consumer interest in the health benefits and nutritional differences between goat and goat-cow milk cheese, particularly regarding their fatty acid profiles, which can influence dietary choices and health outcomes. The paper presents the possibility of using goat's milk as the main component in cheesemaking in a ratio to cow's milk. This work aimed to develop soft brine cheeses from 100% goat and mixed (1:1) goat and cow milk and to study their approximate composition. The practical impact of this study lies in providing valuable insights for consumers, nutritionists, and food producers, enabling informed decisions that cater to health preferences and dietary needs.

Scientific Hypothesis

We expect significant differences in proximal composition and fatty acid composition between goat and cow milk blends and soft cheeses prepared on their basis. We hypothesise that goat milk will exhibit a higher concentration of PUFAs than cow milk blends, which are expected to contain more SFAs.

MATERIAL AND METHODOLOGY

Samples

The studies were conducted in the laboratory of the "Aq Altyn" cow milk processing dairy enterprise. Goat milk from Alpine and Zaanen breeds in the Zhambyl region of Kazakhstan was used as raw materials to make soft cheeses.

Chemicals

Isoamyl alcohol (purity ≥99.9%), distilled water, and sodium hydroxide (purity ≥99%) were retrieved from LPP Labhimprom (Almaty, Kazakhstan). 37-component FAME Mix standard from Supelco, Merck (Darmstadt, Germany), nitrogen (purity ≥99%) (Hauppauge, NY, USA), sodium methylate powder (Sigma-Aldrich, St. Louis, MO, USA), absolute methanol (Sigma Aldrich, St. Louis, MO, USA), MikroMilk YO 100 cultures, MicroMilk MF2.250 enzyme (microMilk S.r.l., Italy), $CaCl_2$ (Russia). All chemicals were of analytical grade quality.

Instruments

Milk Analyzer Ekomilk BOND (Bultech2000, Bulgaria), Shimadzu GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan), laboratory liquid thermometer (Termex, Russia), flasks (K-1-250-29/32) and glasses (V-1-150 TS) (Kazlabpribor, Kazakhstan), class 4 laboratory scales (Mertech, Kazakhstan), water bath (Stegler, US).

Laboratory Methods

Samples of milk and finished products were analysed using the standard methods. The technical conditions for raw goat and cow milk were analysed according to the **[12]** GOST 32940-2014 and GOST 31449-2013 **[13]**. Fat content, acidity, and milk density were carried out based on the **[14]** GOST 5867-90, **[15]** GOST 3624-92 and **[16]** ST RK 1483-2005, respectively. Technical conditions for brine cheeses were performed according to the **[17]** GOST-33959-2016. The determination of fatty acid content was analysed by gas chromatography **[18]**. The following formula determined the cheese yield:

$$Vpr = (Mg.pr)/Ms \times 100\%,$$

Where:

Vpr – product yield; %; Mg.pr – mass of the finished product; g; Ms is mass of initial raw material, g.

Description of the Experiment

Sample preparation: The goat milk was cooled to 4 °C and transported in a thermal container capable of maintaining product temperature for 48 hours.

Number of samples analyzed: 8

Number of experiment replication: 3

Design of the experiment:

Soft goat cheese manufacture: All cheese samples were obtained according to the traditional technology of brine cheese production. Milk filtration was carried out to purify from mechanical impurities. After that, milk samples were sent for separation at 40 °C and subjected to normalization by mass fraction of fat. A milk ratio of 1:1 was produced for mixed milk samples. Next, the milk samples were homogenized to obtain a homogeneous mixture at 15 MPa, pasteurized at 72-74 °C with a holding time of 20-25 sec, and cooled to leavening temperature

34 °C. Furthermore, to give taste characteristics lyophilized starter cultures of direct application of MikroMilk YO 100 (pure cultures of *Streptococcus thermophilus*, *Lactobacillus bulgaricus* in the ratio of 1:1) in the amount of 1-3% of the weight of milk was introduced. Then, calcium chloride (CaCl2) was used to restore calcium balance after pasteurization and improve rennet coagulation. MicroMilk MF2.250 was used to coagulate the mixture, and a standardised powder of 100% chymosin was obtained by fermentation of Rhizomycor Miehei using a VNIIMS mug. Milk coagulation was carried out for 30-40 minutes until a dense clot was obtained; staging was done by cutting the clot into cubes of equal size. We carried out moulding and self-pressing, salting in brine for a day at a salt concentration of 20%, incubation in brine at a concentration of 18% for 4 days, realisation in brine (in a barrel or in any other container) or packing in vacuum bags. The shelf life of the finished product in brine is 75 days, and in vacuum packaging, it is 50 days at a temperature of no more than 6 °C.

Determination of fatty acid content by gas chromatography: The cheese samples were securely sealed and kept in a refrigerator at a temperature of +2 degrees for 8 hours during its transfer from the workshop to the laboratory. Before being shipped, the cheese samples were soaked in brine for 24 hours, containing a table salt concentration of 19-21%. Around 100-150 g of shredded cheese was combined with a solution of n-hexane and sodium methylate in methanol. After allowing the methylation reaction mixture to settle for 5 minutes, it was centrifuged at 3000 rpm for an additional 5 minutes. Following this, 1 mL of the resulting supernatant was transferred to a vial for gas chromatography analysis. The measurements were then conducted using chromatography. The fatty acid methyl esters (FAMEs) were analysed using a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu, Kyoto, Japan) featuring a flame ionisation detector **[18]**. A high-polarity CP-Sil 2560 fused silica column (100 m × 0.250 mm × 0.20 µm; Agilent Technologies, Santa Clara, CA, USA) was employed for the chromatographic analysis of FAMEs. The gas chromatography experiment was conducted with the following settings: an injector temperature of 250 °C and a detector temperature of 260 °C. The analysis was performed in split mode with a ratio of 1:40, and the total flow rate was set at 95.5 mL/min. The temperature program for the column began at 100 °C, maintained for 5 minutes. The temperature was then increased at a rate of 4 °C/min until it reached 210 °C, where it was held for 8 minutes. Subsequently, the temperature was raised by 10 °C/min to reach 240 °C, which was then held for an additional 16.5 minutes. An injection volume of 1.0 µL was used, resulting in a total analysis duration of 60 minutes. The carrier gas used was nitrogen generated by a Parker Domnick Hunter G1110E nitrogen generator (Hauppauge, NY, USA). The hydrogen flow rate was set at 30 mL/min, while the air flow rate was 300 mL/min, with the remaining flow also at 30 mL/min. To quantify the FAMEs, the 37-component FAME Mix standard from Supelco, Merck (Darmstadt, Germany) was utilised. Each component's concentration was calculated by normalising its peak area against the total peak area, thus expressing the relative abundance of each compound **[19]**. The analysis of fatty acids (FAs) using the gas chromatograph equipped with a flame ionisation detector (GC/FID) method was validated following International Conference on Harmonisation (ICH) guidelines **[20]**. Each FAME component was identified based on the retention times and chromatograms obtained from the standard mix. The method's precision was evaluated through repeatability, which involved performing the procedure five times with the standard mix solution. The precision of the chromatographic system was confirmed by calculating the %RSD for retention times and peak areas, with five injections made across three different days.

Statistical Analysis

The fatty acid composition was analysed using Hierarchical Cluster Analysis (HCA), employing Euclidean distances and Ward's method. Since our study compares the mean between two independent groups, we used independent samples t-test in the SPSS v25.0 software (IBM Corporation, New York, USA). Outcomes with $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Organoleptic and physicochemical properties of tested milk samples

Goat's milk has been demonstrated as an alternative for people sensitive or allergic to cow milk **[21]**. It can be seen from the data presented in Table 1 that the organoleptic data of the mixture of cow and goat milk (GCM) was similar to the goat milk (GM) samples. The fat mass fraction and lactose content of the GCM were 5.2% and 4.6%, which are significantly higher than that of GM ($p < 0.05$). Compared to pure goat milk, the higher fat mass fraction and lactose content in goat and milk blends can be attributed to the composition and properties of the additional milk used, often cow's milk, which typically has a higher lactose concentration and fat content than goat milk. Previous studies detected higher lactose content in cow or human milk samples than in goat milk **[22]**, **[23]**. According to the meta-analysis, the average goat milk lactose was 4.44% **[24]**. Importantly, the mass fraction of protein was higher (3.21%) in GM than in GCM ($p < 0.001$). Goat milk has a higher protein content than cow milk due to its unique casein profile, which contributes to better digestibility and a higher concentration of

essential amino acids. Differently, greater protein content was observed in sheep milk cheese than in goat milk cheese **[25]**. In addition, GM showed enhanced TA (p <0.001), while no significant changes were found for pH. In general, titratable acidity indicates the freshness of the milk **[26]**.

Table 1 Organoleptic and physicochemical properties of tested milk samples.

Indicators	100% GM	1:1 GCM
Color	Yellowish tinge	Pale yellow
Taste and odor	Clean, without any foreign odors or flavors not characteristic of fresh goat's milk	Clear, without any odors or tastes not typical of fresh goat's milk
Consistency and appearance	Homogeneous liquid without sediment and protein flakes	Homogeneous liquid without sediment and protein flakes
Mass fraction of fat, %	^{b}4.91 ±0.02	^{a}5.2 ±0.12
Mass fraction of protein, %	^{A}3.21 ±0.01	^{B}2.98 ±0.008
Density, kg/m3	1.29 ±0.008	1.29 ±0.001
Titratable acidity (TA), °T	^{A}18 ±0.12	^{B}16.91 ±0.04
Lactose, %	^{B}4.06 ± 0.01	^{A}4.6 ±0.12
Acidity, pH	6.61 ±0.004	6.5 ±0.15
Freezing point, °C	^{A}56.2 ±0.07	^{B}54.08 ±0.06
Temperature, °C	^{A}24.2 ±0.2	^{B}22.95 ±0.02

Note: different letters indicate significance, a, b (p <0.05); A, B (p <0.001).

Physicochemical properties of tested brine cheese samples

The technological properties of the cheese samples are given in Table 2 and Figure 1 depicts the ready cheeses. It should be noted that coagulation in GMB was faster than in GCMB (Figure 2) (p <0.001). These data are important for cheese production because if the coagulation time is increased more than necessary, it will significantly affect the consistency of the cheese. This might be due to the higher concentration of certain coagulation factors, such as specific casein fractions and a more favourable micelle structure in goat milk, which promote more efficient aggregation and formation of curds during the cheese-making process **[27]**. In contrast, according to a previous study, buffalo and sheep milk demonstrate superior cheese-making qualities, including coagulation and curd firming properties, nutrient retention in the curd, and overall cheese yield, in comparison to bovine and caprine milk **[28]**. However, both breed and nutrition are critical in determining the coagulation properties of milk and the overall yield and quality of cheese produced. For instance, Italian local goats demonstrated better coagulation characteristics than Saanen goats **[29]**. Regarding cheese yield, cheese made from pure goat milk resulted in significantly higher cheese yield (p <0.001) (Figure 3). Meanwhile, high-temperature treatments of goat milk were observed to have a notable impact on the fundamental composition and protein composition of goat cheese whey **[30]**. Elgaml et al. reported that cheese prepared from 100% goat milk yielded higher Halloumi cheese yields than cow and mixed cheese samples **[31]**. Conversely, Algerian Bouhezza soft cheese yield was highest when made from sheep's milk, followed by goat's milk and cow's milk. This was notable despite the initial volume of milk used in the cheese-making process being nearly identical, suggesting that the processing methods significantly impact the final yield. **[32]**. The Nubian breed has been shown to produce more cheese than the Alpine breed, indicating that breed and other factors can influence the quality and quantity of dairy products **[33]**. Moreover, the colour of the cheese samples varied from white to yellowish, which indicated a difference in the mass fraction of fat in the samples (Table 2).

In our study, GMB showed higher fat content (45.13%) than GCMB (43.05%), which may be affected by cheese-making. In contrast, higher fat content was revealed in traditionally smoked cheeses made from cow milk than those made from sheep and goat milk **[34]**. Significantly greater moisture (53.06%) was found in GCMB samples (p <0.001). This result agrees with Gebreyowhans et al., who also revealed reduced moisture content in pure goat milk Camembert cheese than cheese made from cow milk **[35]**. Besides, our study did not detect differences in salt percentage and pH among samples (p >0.05).

Figure 1 Finished brine cheeses: top left 50:50% goat and cow's milk cheese and right 100% goat's milk cheese; bottom left 100% goat's milk cheese and right 50:50% goat and cow's milk cheese.

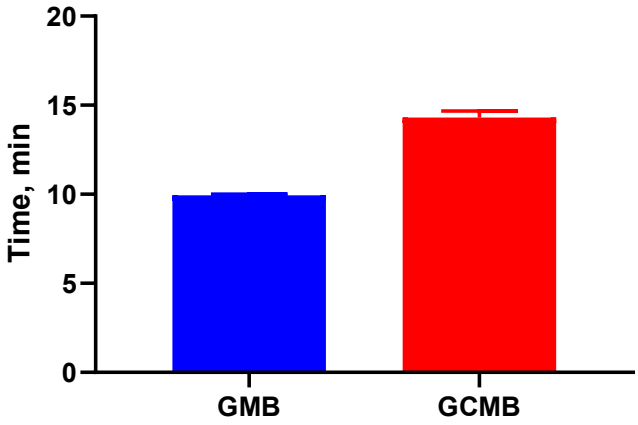


Figure 2 Beginning of coagulation time. GMB: based on goat milk, GCMB: based on 1:1 ratio of cow and goat milk.

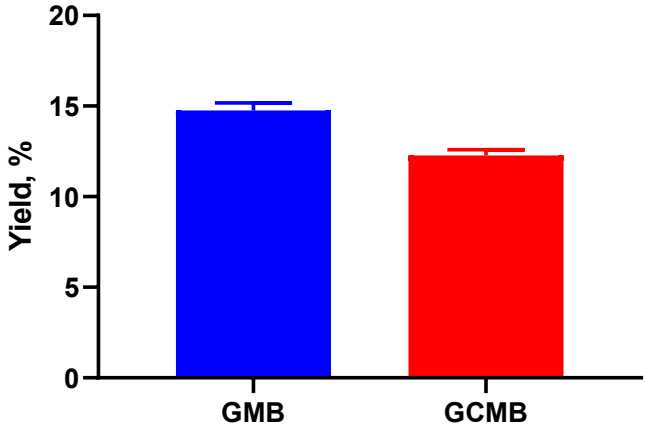


Figure 3 Cheese yield**.** GMB: based on goat milk; based on the ratio (1:1) of cow and goat milk.

Table 2 Technological properties of tested cheese samples.

Parameters	100% GMB	1:1 GCMB
Color	Yellowish	Faint yellow
Taste and odor	Pure, characteristic of goat's milk, creamy	Pure, characteristic of goat's milk, moderately acidic
Consistency	Homogeneous, slightly tender	moderately dense
Appearance	The outer layer is compacted, and the surface of the cheese is flat and slightly rough.	The outer layer is compacted, and the surface of the cheese is flat and slightly rough.
Drawing	A small number of irregularly shaped eyes throughout the mass	A small number of irregularly shaped eyes throughout the mass
Mass fraction of fat in dry matter, %	^{A}45.13 ±0.1	^{B}43.05 ±0.08
Mass fraction of moisture, %	^{B}49.98 ±0.05	^{A}53.06 ±0.06
Mass fraction of salt, %	1.51 ±0.01	1.50 ±0.007
Active acidity, pH	5.37 ±0.06	5.22 ±0.05

Note: different letters indicate significance, A, B (p <0.001).

Fatty acid profile of produced cheeses

Fatty acid composition is one of the most important indicators of the nutritional quality of goat milk and milk products. Table 3 and Figure 4 presents the data on cheeses' fatty acid composition. The amount of saturated fatty acids (SFAs) was higher in GCMB cheese than in pure goat milk cheese (p <0.05). In a previous study, the content of $C_{16:0}$ and $C_{18:0}$ was about 33.60-31.68% and 10.25-8.82% in goat cheese samples, respectively **[36]**. In addition, less $C_{18:0}$ was detected in hard goat's milk cheeses than in hard cheeses made from cow's or sheep's milk **[37]**. These results were also confirmed in Egyptian fresh soft cheese samples by Ibrahim et al. **[38]**. Therefore, the increase in saturated fatty acids is likely due to the mixing of cow milk with goat milk. Szterk et al. found that the levels of SFAs in the analysed commercial goat cheeses are relatively low. The authors also suggested that the type of cheese significantly influences the fatty acid composition, with soft cheeses being more likely to contain essential fatty acids **[39]**. In addition, it is suggested that palmitic acid should be consumed in a certain ratio with PUFAs to prevent the acceleration of deleterious effects **[40]**.

Regarding monounsaturated fatty acids (MUFAs), significantly more oleic acid ($C_{18:1n9c}$) was detected in goat milk cheese compared to mixed milk cheese (p <0.05). $C_{18:1n9}$ contents were higher in goat milk than human milk **[41]**. In contrast, the levels of MUFAs, particularly oleic acid, in goat and sheep cheese samples were similar, whereas cow cheese samples showed significantly higher amounts **[42]**. It is well-documented that oleic acid lowers lipid levels in the blood and has a positive effect that positively affects the cardiovascular system **[43]**.

Importantly, $C_{18:3n3c}$, $C_{16:1}$, $C_{18:2n6c}$ fatty acids were identified only in 100% goat milk sample cheeses. Commercial goat cheeses had the highest n-3 PUFA content, according to a study by Paszczyk and Łuczyńska **[37]**. However, it has been shown that sheep cheese exhibited a higher concentration of polyunsaturated fatty acids (PUFAs), including n-3 PUFAs, compared to cow or goat cheese **[44]**. PUFAs are involved in the structure and functioning of cell membranes **[45]**. Linoleic acid is an omega-6 fatty acid part of membrane phospholipids and serves as a structural component of membrane fluid to maintain the epidermal transdermal water barrier **[46]**.

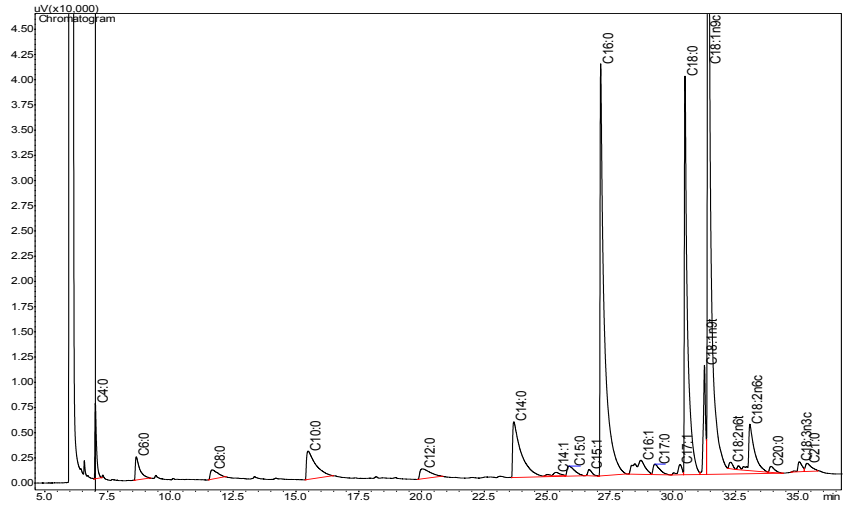
Furthermore, increased levels of linoleic acid were found in cheese-containing cultures in goat cheese **[47]**. It is also reported that incorporating extruded linseed into goats' diets enhanced the fatty acid composition of Padraccio cheese **[48]**. Thus, the fatty acid profile of goat cheeses can be significantly influenced by the goats' breed and diet, particularly the types of forage and supplements they consume. For instance, grazing led to notably increased levels of n-3 fatty acids in goat milk and cheese samples and a reduced n-6/n-3 ratio **[49]**. Additionally, factors such as the stage of lactation and the processing methods employed during cheese production can also alter the composition and ratios of fatty acids in the final product. Currò et al. reported that SFAs were abundant in early lactation, while unsaturated fatty acids were abundant in late lactation. Also, local goat breeds produced milk with a lower concentration of SAFs than the cosmopolitan breeds **[50]**.

Table 3 The fatty acid composition of cheese.

Fatty acid composition of fat phase, µg/mL	100% GMB	1:1 GCMB (50G:50C)
Saturated fatty acids		
$C_{16:0}$ palmitic acid	^{B}21.86 ±0.21	^{A}32.51 ±0.19
$C_{18:0}$ stearic acid	^{B}15.44 ±0.02	^{A}20.66 ±0.19
Monounsaturated fatty acids		
$C_{18:1n9c}$ oleic acid	^{A}35.29 ±0.05	^{B}31.87 ±0.04
$C_{21:0}$ henicosanoic acid	0.64 ±0	ND
Denatured fatty acids		
$C_{18:2n6t}$ linolelaidic acid	0.32 ±0.006	ND
Polyunsaturated fatty acids		
$C_{18:2n6c}$ linoleic acid	3.47 ±0.03	ND
$C_{18:3n3c}$ linolenic acid	0.56 ±0.008	ND
$C_{16:1}$ palmitoleic acid	1.9 ±0.02	ND

Note: ND – not detected.

A)



B)

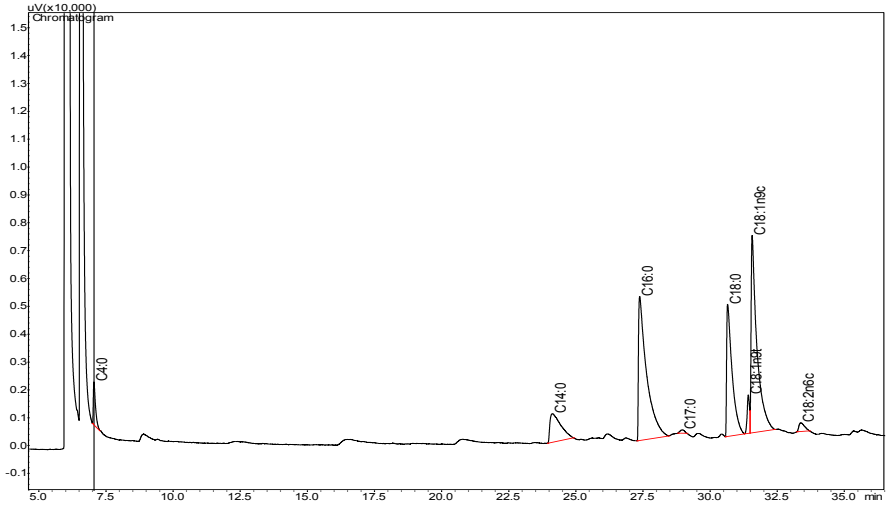


Figure 4 Chromatogram of cheese samples: A) 100% goat milk cheese; B) 1:1 goat and cow milk blend cheese.

CONCLUSION

The present results showed that goat milk samples exhibited a higher percentage of protein, while goat and cow milk blends showed greater lactose and fat content. Soft cheese prepared from goat milk exhibited faster coagulation, higher cheese yield and fat content in dry matter. The content of SFAs was higher in goat and cow milk cheeses than in those made from pure goat milk cheeses. In addition, increased content of oleic acid as well as PUFAs such as linoleic, linolenic and palmitoleic acids were found only in 100% goat milk cheeses. Soft cheese made from goat milk exhibited improved nutritional characteristics, especially concerning fatty acids. However, further research is required on sensory analyses and consumer acceptability.

The study revealed that goat milk-based cheeses offer significant nutritional benefits. The goat milk samples contained a higher protein content (3.21%) compared to the goat-cow milk mixture (2.98%), while the goat-cow milk blend had a higher fat content (5.2%) and lactose content (4.6%) compared to goat milk alone (4.91% fat, 4.06% lactose). The cheese yield was notably higher for goat milk cheeses ($p < 0.001$), and these cheeses also had a greater fat content in dry matter (45.13%) compared to cheeses made from a goat-cow milk mixture (43.05%).

The fatty acid analysis showed that saturated fatty acids (SFAs) were significantly higher in the goat-cow milk cheeses, with palmitic acid (C16:0) reaching 32.51 µg/mL and stearic acid (C18:0) at 20.66 µg/mL, compared to 21.86 µg/mL and 15.44 µg/mL, respectively, in pure goat milk cheeses. Meanwhile, pure goat milk cheeses contained higher levels of oleic acid (C18:1n9c, 35.29 µg/mL). They were the only ones to exhibit polyunsaturated fatty acids (PUFAs) such as linoleic acid (C18:2n6c, 3.47 µg/mL), linolenic acid (C18:3n3c, 0.56 µg/mL), and palmitoleic acid (C16:1, 1.9 µg/mL).

These findings highlight the superior nutritional profile of 100% goat milk cheeses, particularly regarding their higher PUFA content, contributing to improved health benefits. Further research is necessary to explore sensory qualities and consumer preferences.

REFERENCES

1. Park, Y. W. (2017). Goat Milk – Chemistry and Nutrition. In Handbook of Milk of Non-Bovine Mammals (pp. 42–83). Wiley. https://doi.org/10.1002/9781119110316.ch2.2
2. Nayik, G. A., Jagdale, Y. D., Gaikwad, S. A., Devkatte, A. N., Dar, A. H., & Ansari, M. J. (2022). Nutritional Profile, Processing and Potential Products: A Comparative Review of Goat Milk. In Dairy (Vol. 3, Issue 3, pp. 622–647). MDPI AG. https://doi.org/10.3390/dairy3030044
3. Meena, S., Rajput, Y. S., & Sharma, R. (2014). Comparative fat digestibility of goat, camel, cow and buffalo milk. In International Dairy Journal (Vol. 35, Issue 2, pp. 153–156). Elsevier BV. https://doi.org/10.1016/j.idairyj.2013.11.009
4. Turkmen, N. (2017). The Nutritional Value and Health Benefits of Goat Milk Components. In Nutrients in Dairy and their Implications on Health and Disease (pp. 441–449). Elsevier. https://doi.org/10.1016/b978-0-12-809762-5.00035-8
5. Miller, B. A., & Lu, C. D. (2019). Current status of global dairy goat production: an overview. In Asian-Australasian Journal of Animal Sciences (Vol. 32, Issue 8, pp. 1219–1232). Asian Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.19.0253
6. Akshit, F. N. U., Deshwal, G. K., Sharma, H., Kumar, P., Maddipatla, D. K., Singh, M. P., & Goksen, G. (2023). Technological challenges in production of goat milk products and strategies to overcome them: a review. In International Journal of Food Science & Technology (Vol. 59, Issue 1, pp. 6–16). Wiley. https://doi.org/10.1111/ijfs.16782
7. Popović Vranješ, A. (2017). Production of hard goat cheese and goat whey from organic goat's milk. In Mljekarstvo (pp. 177–187). Croatian Dairy Union. https://doi.org/10.15567/mljekarstvo.2017.0302
8. López-Villafaña, B. P., Rojas-González, S., Elías-Román, R. D., & Rodríguez-Hernández, G. (2023). The evolution of antioxidative properties of protein-derived peptides of Mexican Panela goat and cow milk cheese during its shelf life. In CyTA - Journal of Food (Vol. 21, Issue 1, pp. 57–63). Informa UK Limited. https://doi.org/10.1080/19476337.2022.2152100
9. Sakr, H., Mohamed, E., & Attalla, N. (2011). Using goat's milk in making three types of soft cheese. In Journal of Food and Dairy Sciences (Vol. 2, Issue 5, pp. 251–260). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/jfds.2011.81950
10. Wang, W., Jia, R., Hui, Y., Zhang, F., Zhang, L., Liu, Y., Song, Y., & Wang, B. (2023). Utilization of two plant polysaccharides to improve fresh goat milk cheese: Texture, rheological properties, and microstructure characterization. In Journal of Dairy Science (Vol. 106, Issue 6, pp. 3900–3917). American Dairy Science

Association. https://doi.org/10.3168/jds.2022-22195
11. Remini, H., Remini-Sahraoui, Y., Benbara, T., & Sadoun, D. (2024). From farm to cheeseboard: Harnessing the biopreserving performance and enhancing safety of Lactococcus lactis KJ660075 in goat's milk cheese. In International Dairy Journal (Vol. 157, p. 105977). Elsevier BV. https://doi.org/10.1016/j.idairyj.2024.105977
12. GOST 32940-2014 Raw goat milk. Technical conditions.
13. GOST 31449-2013 Raw cow's milk. Technical conditions.
14. GOST 5867-90 Milk and milk products. Methods of determination of fat.
15. GOST 3624-92 Milk and milk products. Titrimetric methods for determination of acidity.
16. ST RK 1483-2005 Cow's milk. Test methods for determining the composition and density of milk.
17. GOST-33959-2016 Brine cheeses. Technical conditions.
18. Toishimanov, M., Nurgaliyeva, M., Serikbayeva, A., Suleimenova, Z., Myrzabek, K., Shokan, A., & Myrzabayeva, N. (2023). Comparative Analysis and Determination of the Fatty Acid Composition of Kazakhstan's Commercial Vegetable Oils by GC-FID. In Applied Sciences (Vol. 13, Issue 13, p. 7910). MDPI AG. https://doi.org/10.3390/app13137910
19. Joensen, H., & Grahl-Nielsen, O. (2000). Discrimination of Sebastes viviparus, Sebastes marinus and Sebastes mentella from Faroe Islands by chemometry of the fatty acid profile in heart and gill tissues and in the skull oil. In Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology (Vol. 126, Issue 1, pp. 69–79). Elsevier BV. https://doi.org/10.1016/s0305-0491(00)00172-3
20. Borman, P., & Elder, D. (2017). Q2(R1) Validation of Analytical Procedures. In ICH Quality Guidelines (pp. 127–166). Wiley. https://doi.org/10.1002/9781118971147.ch5
21. ALKaisy, Q. H., Al-Saadi, J. S., AL-Rikabi, A. K. J., Altemimi, A. B., Hesarinejad, M. A., & Abedelmaksoud, T. G. (2023). Exploring the health benefits and functional properties of goat milk proteins. In Food Science & Nutrition (Vol. 11, Issue 10, pp. 5641–5656). Wiley. https://doi.org/10.1002/fsn3.3531
22. Prosser, C. G. (2021). Compositional and functional characteristics of goat milk and relevance as a base for infant formula. In Journal of Food Science (Vol. 86, Issue 2, pp. 257–265). Wiley. https://doi.org/10.1111/1750-3841.15574
23. Stergiadis, S., Nørskov, N. P., Purup, S., Givens, I., & Lee, M. R. F. (2019). Comparative Nutrient Profiling of Retail Goat and Cow Milk. In Nutrients (Vol. 11, Issue 10, p. 2282). MDPI AG. https://doi.org/10.3390/nu11102282
24. Akshit, F. N. U., Mao, T., Kaushik, R., Poswal, V., & Deshwal, G. K. (2024). Global comprehensive review and meta-analysis of goat milk composition by location, publication year and lactation stage. In Journal of Food Composition and Analysis (Vol. 127, p. 105973). Elsevier BV. https://doi.org/10.1016/j.jfca.2024.105973
25. Kawęcka, A., & Pasternak, M. (2022). Nutritional and dietetic quality of milk and traditional cheese made from the milk of native breeds of sheep and goats. In Journal of Applied Animal Research (Vol. 50, Issue 1, pp. 39–46). Informa UK Limited. https://doi.org/10.1080/09712119.2021.2020125
26. Huang, W., Fan, D., Li, W., Meng, Y., & Liu, T. C. (2022). Rapid evaluation of milk acidity and identification of milk adulteration by Raman spectroscopy combined with chemometrics analysis. In Vibrational Spectroscopy (Vol. 123, p. 103440). Elsevier BV. https://doi.org/10.1016/j.vibspec.2022.103440
27. Zhao, X., Wang, C., Cheng, M., Zhang, X., & Jiang, H. (2021). Influence of calcium on the properties of micellar casein in goat milk. In LWT (Vol. 150, p. 111935). Elsevier BV. https://doi.org/10.1016/j.lwt.2021.111935
28. Bittante, G., Amalfitano, N., Bergamaschi, M., Patel, N., Haddi, M.-L., Benabid, H., Pazzola, M., Vacca, G. M., Tagliapietra, F., & Schiavon, S. (2022). Composition and aptitude for cheese-making of milk from cows, buffaloes, goats, sheep, dromedary camels, and donkeys. In Journal of Dairy Science (Vol. 105, Issue 3, pp. 2132–2152). American Dairy Science Association. https://doi.org/10.3168/jds.2021-20961
29. Currò, S., Manuelian, C. L., De Marchi, M., Goi, A., Claps, S., Esposito, L., & Neglia, G. (2020). Italian local goat breeds have better milk coagulation properties than cosmopolitan breed. In Italian Journal of Animal Science (Vol. 19, Issue 1, pp. 593–601). Informa UK Limited. https://doi.org/10.1080/1828051x.2020.1772130
30. Miloradovic, Z., Tomic, N., Kljajevic, N., Levic, S., Pavlovic, V., Blazic, M., & Miocinovic, J. (2021). High Heat Treatment of Goat Cheese Milk. The Effect on Sensory Profile, Consumer Acceptance and Microstructure of Cheese. In Foods (Vol. 10, Issue 5, p. 1116). MDPI AG. https://doi.org/10.3390/foods10051116
31. Elgaml, N., Moussa, M. A. M., & Saleh, A. E. (2017). Comparison of the Properties of Halloumi Cheese

Made from Goat Milk, Cow Milk and Their Mixture. In Journal of Sustainable Agricultural Sciences (Vol. 0, Issue 0, pp. 77–87). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/jsas.2017.1065.1006

32. Boudalia, S., Boudebbouz, A., Gueroui, Y., Bousbia, A., Benada, M., Leksir, C., Boukaabene, Z., Saihi, A., Touaimia, H., Aït-Kaddour, A., & Chemmam, M. (2020). Characterization of traditional Algerian cheese "Bouhezza" prepared with raw cow, goat and sheep milks. In Food Science and Technology (Vol. 40, Issue suppl 2, pp. 528–537). FapUNIFESP (SciELO). https://doi.org/10.1590/fst.35919
33. Muñoz-Salinas, F., Andrade-Montemayor, H. M., De la Torre-Carbot, K., Duarte-Vázquez, M. Á., & Silva-Jarquin, J. C. (2022). Comparative Analysis of the Protein Composition of Goat Milk from French Alpine, Nubian, and Creole Breeds and Holstein Friesian Cow Milk: Implications for Early Infant Nutrition. In Animals (Vol. 12, Issue 17, p. 2236). MDPI AG. https://doi.org/10.3390/ani12172236
34. Filipczak-Fiutak, M., Pluta-Kubica, A., Domagała, J., Duda, I., & Migdał, W. (2021). Nutritional value and organoleptic assessment of traditionally smoked cheeses made from goat, sheep and cow's milk. In B. T. Šiler (Ed.), PLOS ONE (Vol. 16, Issue 7, p. e0254431). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0254431
35. Gebreyowhans, S., Zhang, S., Pang, X., Yang, B., Wang, T., Wu, Z., Lu, J., & Lv, J. (2020). Changes in texture, composition and sensory characteristics of Camembert cheese made from a mixture of goat milk and cow milk during ripening. In International Journal of Dairy Technology (Vol. 73, Issue 3, pp. 604–615). Wiley. https://doi.org/10.1111/1471-0307.12699
36. Klir Šalavardić, Ž., Novoselec, J., Ronta, M., Čolović, D., Šperanda, M., & Antunović, Z. (2021). Fatty Acids of Semi-Hard Cheese Made from Milk of Goats Fed Diets Enriched with Extruded Linseed or Pumpkin Seed Cake. In Foods (Vol. 11, Issue 1, p. 6). MDPI AG. https://doi.org/10.3390/foods11010006
37. Paszczyk, B., & Łuczyńska, J. (2020). The Comparison of Fatty Acid Composition and Lipid Quality Indices in Hard Cow, Sheep, and Goat Cheeses. In Foods (Vol. 9, Issue 11, p. 1667). MDPI AG. https://doi.org/10.3390/foods9111667
38. Ibrahim, A., Zahran, H., Awaad, S., & Hegab, O. (2023). Comparative evaluation of fatty acid profiles and lipid nutritional indexes in Egyptian fresh cow, buffalo, goat soft cheeses and their mixtures. In Egyptian Journal of Chemistry (Vol. 0, Issue 0, pp. 0–0). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/ejchem.2023.206528.7879
39. Szterk, A., Ofiara, K., Strus, B., Abdullaev, I., Ferenc, K., Sady, M., Flis, S., & Gajewski, Z. (2022). Content of Health-Promoting Fatty Acids in Commercial Sheep, Cow and Goat Cheeses. In Foods (Vol. 11, Issue 8, p. 1116). MDPI AG. https://doi.org/10.3390/foods11081116
40. Carta, G., Murru, E., Banni, S., & Manca, C. (2017). Palmitic Acid: Physiological Role, Metabolism and Nutritional Implications. In Frontiers in Physiology (Vol. 8). Frontiers Media SA. https://doi.org/10.3389/fphys.2017.00902
41. Liao, G., Han, H., Wang, T., Li, H., Qian, Y., Zhu, M., Jia, Q., & Qiu, J. (2024). Comparative analysis of the fatty acid profiles in goat milk during different lactation periods and their interactions with volatile compounds and metabolites. In Food Chemistry (Vol. 460, p. 140427). Elsevier BV. https://doi.org/10.1016/j.foodchem.2024.140427
42. Ali, A. H., Khalifa, S. A., Gan, R.-Y., Shah, N., & Ayyash, M. (2023). Fatty acids, lipid quality parameters, and amino acid profiles of unripened and ripened cheeses produced from different milk sources. In Journal of Food Composition and Analysis (Vol. 123, p. 105588). Elsevier BV. https://doi.org/10.1016/j.jfca.2023.105588
43. Lu, Y., Zhao, J., Xin, Q., Yuan, R., Miao, Y., Yang, M., Mo, H., Chen, K., & Cong, W. (2024). Protective effects of oleic acid and polyphenols in extra virgin olive oil on cardiovascular diseases. In Food Science and Human Wellness (Vol. 13, Issue 2, pp. 529–540). Tsinghua University Press. https://doi.org/10.26599/fshw.2022.9250047
44. Kawęcka, A., Radkowska, I., Kawęcka, A., & Sikora, J. (2020). Concentratıons of selected bıoactıve components ın tradıtıonal cheeses made from goat's, cow's and sheep's mılk. In Journal of Elementology (Issue 2/2020). Polish Society for Magnesium Research. https://doi.org/10.5601/jelem.2019.24.3.1907
45. Mercola, J., & D'Adamo, C. R. (2023). Linoleic Acid: A Narrative Review of the Effects of Increased Intake in the Standard American Diet and Associations with Chronic Disease. In Nutrients (Vol. 15, Issue 14, p. 3129). MDPI AG. https://doi.org/10.3390/nu15143129
46. Hamilton, J. S., & Klett, E. L. (2021). Linoleic acid and the regulation of glucose homeostasis: A review of the evidence. In Prostaglandins, Leukotrienes and Essential Fatty Acids (Vol. 175, p. 102366). Elsevier BV. https://doi.org/10.1016/j.plefa.2021.102366
47. Taboada, N., Van Nieuwenhove, C., Alzogaray, S. L., & Medina, R. (2015). Influence of autochthonous

cultures on fatty acid composition, esterase activity and sensory profile of Argentinean goat cheeses. In Journal of Food Composition and Analysis (Vol. 40, pp. 86–94). Elsevier BV. https://doi.org/10.1016/j.jfca.2014.12.013

48. Cosentino, C., Colonna, M. A., Musto, M., Dimotta, A., Freschi, P., Tarricone, S., Ragni, M., & Paolino, R. (2021). Effects of dietary supplementation with extruded linseed and oregano in autochthonous goat breeds on the fatty acid profile of milk and quality of Padraccio cheese. In Journal of Dairy Science (Vol. 104, Issue 2, pp. 1445–1453). American Dairy Science Association. https://doi.org/10.3168/jds.2020-18805

49. Bodnár, Á., Egerszegi, I., Kuchtik, J., Penksza, K., Póti, P., & Pajor, F. (2021). Effect of grazing on composition, fatty acid profile and nutritional indices of the goat milk and cheese. In Journal of Animal and Feed Sciences (Vol. 30, Issue 4, pp. 320–328). The Kielanowski Institute of Animal Physiology and Nutrition, PAS. https://doi.org/10.22358/jafs/144843/2021

50. Currò, S., Manuelian, C., De Marchi, M., Claps, S., Rufrano, D., & Neglia, G. (2019). Effects of Breed and Stage of Lactation on Milk Fatty Acid Composition of Italian Goat Breeds. In Animals (Vol. 9, Issue 10, p. 764). MDPI AG. https://doi.org/10.3390/ani9100764

Funds:

The current study did not receive any financial support.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Altynay Abdugamitova**, S. Seifullin Kazakh Agrotechnical Research University, Zhenis avenue, 62, 010011, Astana, Kazakhstan,
Tel.: +7 (702) 293 6680
E-mail: abdugamitova@inbox.ru
ORCID: https://orcid.org/0000-0001-7402-2016

Assiya Serikbayeva, Kazakh National Agrarian Research University, Abay avenue, 8, 050010, Almaty, Kazahstan,
Tel.: +7 (777) 256 5375
E-mail: serikbayeva@yandex.kz
ORCID: https://orcid.org/0000-0003-4632-7343

Akmaral Mateyeva, "LF Company" LLP, Seifullin st., 460, 050000, Almaty, Kazakhstan,
Tel.: +7 (701) 981 2173
E-mail: akmaral79@list.ru
ORCID: https://orcid.org/0000-0001-7146-4951

Gulbagi Orymbetova, South Kazakhstan Medical Academy, Al-Farabi square, 1, 160019, Shymkent, Kazakhstan,
Tel.: +7 (701) 257 1021
E-mail: orim_77@mail.ru
ORCID: https://orcid.org/0000-0001-8987-3366

Sulushash Mateyeva, M.Kh.Dulaty Taraz Regional University, Suleymenov, 7, 080012, Taraz, Kazakhstan,
Tel.: +7 (702) 343 4843
E-mail: mateeva73@mail.ru
ORCID: https://orcid.org/0000-0003-4416-1124

Ainur Matibaeyva, Almaty Technological University, Tole bi st., 100, 050000, Almaty, Kazakhstan,
Tel.: +7 (775) 444 0081
E-mail: matibaeva@bk.ru
ORCID: https://orcid.org/0000-0001-8945-0873

Akerke Toleugazykyzy, S.Seifullin Kazakh Agrotechnical Research University, Zhenis avenue, 62, 010011, Astana, Kazakhstan,
Tel.: +7 (775) 391 5632
E-mail: akerke.0192t@gmail.com
ORCID: https://orcid.org/0000-0002-2061-1699

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 18.7.2024
Revised: 30.8.2024
Accepted: 3.9.2024
Published: 27.9.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 819-833
https://doi.org/10.5219/2004
ISSN: 1337-0960 online
www.potravinarstvo.com

Evaluating heavy metal contamination in paper-based packaging for bakery products: a HACCP approach

Almas Zhanbolat, Ulbala Tungyshbayeva, Zhanar Nabiyeva, Raushangul Uazhanova, Madina Yakiyayeva, Abdysemat Samodun

ABSTRACT

Food quality and safety are among the most important aspects of the food industry. The object of this study was packaging materials intended for packaging bakery products. One of the key factors for food safety is provided by packaging. The increase in food packaging materials creates a demand for promoting products and brands that are safe for consumption. Food spoilage due to poor-quality packaging causes huge losses not only to businesses but also to consumers. Despite the effectiveness of existing practices, retailers still face many challenges, including the materials used and their possible interaction with food. In addition, the transfer of harmful materials from packaging to food is still an issue. This study presents the results of an assessment of the migration of heavy metals used in different types of food packaging in the Republic of Kazakhstan. Determination of heavy metal migration was carried out in aqueous extract by atomic absorption spectrometry. The practical value of the above research is to study the content of lead, zinc and chromium in the composition of various types of paper food packaging. The assessment of the average migration level of heavy metals following the current requirements revealed that all types of paper contain significant amounts of lead and zinc, which do not meet the requirements for paper intended for food packaging. Thus, specific samples should be considered a potential risk to human health if reused without pre-treatment as a source of recycled cellulose fibre for producing packaging used in direct contact with foodstuffs.

Keywords: food packaging, food safety, food shelf life, heavy metals, permissible migration of heavy metals

INTRODUCTION

Safe food is a major determinant of human health. Access to safe, nutritious, healthy food is a basic human right. To guarantee this right, public policy ensures that available food meets safety standards. This is not an easy task, as developments in food production, management, delivery, and consumption have rapidly improved in various ways over the last two decades **[1]**. These factors call for a new global approach to improving food safety and strengthening national food safety systems while improving national and international cooperation **[2]**. The baking industry is one of the leading food industries in the Republic of Kazakhstan's agro-industrial complex. It fulfils the task of producing products of prime necessity for the following reasons: availability, nutritional value, convenience, etc. Accordingly, their sales in packaged form are increasing. According to the statistics of the Republic of Kazakhstan for January-June 2024, the main share in the structure of the manufacturing industry is the production of flour (37%) and about 16% production of bread and bakery products **[3]**. Of all the packaging materials for bakery products, paper packaging is the most common and best option. However, since bakery products, compared to other foodstuffs, have a relatively high moisture content and low pH level, as well as the increasing tendency for the packaged realisation of this type of product, it is of great importance to investigate the permissible migration of heavy metals into the food product. Like all food products, bakery products are a complex

matrix; therefore, the analysis of migrating substances can cause analytical difficulties. Therefore, test media (food simulants) that reflect the basic physicochemical properties of food products are used. Specific migration tests can sometimes be performed directly in food, but general migration cannot be tested in food. Packaging and materials for direct food contact must meet various food safety requirements. Materials and articles must not transfer their components to food in quantities that may endanger human health and/or unacceptably alter food composition or cause deterioration of bakery products' colour, taste or odour. Packaging plays an important role in ensuring the quality and safety of food products. Packaging usually performs basic functions such as protecting, containing and informing. In addition to these functions, the food packaging process needs to guarantee the transportation of food products. The chemical indicator of the quality of a product during its shelf life is mainly determined by contaminants and toxic components, whose presence in certain quantities makes it possible to assess the current quality of the product or, more preferably, to estimate its shelf life. For an organism to be suitable for use as such an indicator, it must meet the following requirements as much as possible (Figure 1):

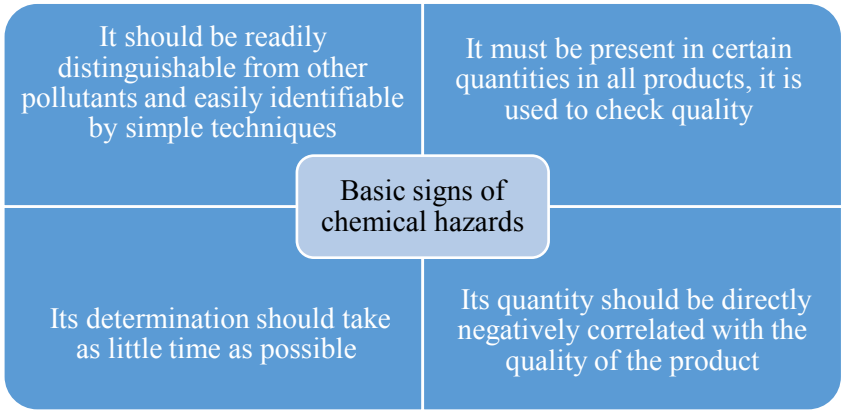


Figure 1 Basic signs of chemical hazards.

Packaging materials include packaging for direct contact with food substances, for which contaminants and toxic components mustn't migrate into the product during contact **[4]**. Paper, cardboard, and corrugated board are considered traditional packaging materials for bakery products, processed by different operations, and have different properties depending on their application. Let's analyse the sources of chemical contaminants. We can assume that the structure of the paper is formed from pulp, which, during production, goes through processes such as bleaching and adding dyes, pigments, and chemicals that strengthen the paper **[5]**. To improve paper's strength and other properties, recycled paper is produced using chemical additives **[6]**. In addition, during these processes, the inclusion of chemical components of printing inks is allowed, which increases the amount of contaminants and toxic components in food paper and cardboard packages **[7]**. Food packaging also provides easy handling and transportation by preventing chemical contamination and extending shelf life, providing consumers convenience **[8]**. The increase in food packaging materials creates a demand for promoting products and brands that are environmentally friendly **[9]**. Despite the effectiveness of existing practices, retailers still face many challenges, including the materials used and their possible interaction with food. The transfer of harmful materials from packaging to food is still an issue, especially when reusing food-grade plastic **[10]**. A significant issue is reusability, including how much packaging waste is recyclable (disposal). To reduce the toxicity of packaging waste, it is necessary to reduce the content of heavy metals in the packaging and/or ensure that such substances are not released into the environment, including the food product. Reusing organic materials for food contact is undesirable because of the difficulty in controlling the constituents present in such materials due to other types of materials other than packaging materials. Several carcinogenic and non-carcinogenic diseases may occur due to exposure to heavy metals above the safety limit **[11]**. Therefore, European legislation sets quality standards for food contact packaging materials and emphasises that materials used for food should not pose a risk to human health. According to the RASFF report, 219 notifications of food contact materials were transmitted in 2022, about half involving the migration of a wide range of substances. Most concern primary aromatic amines, followed by formaldehyde and lead **[12]**. In the Republic of Kazakhstan, there is a Register of products that do not comply with the requirements of normative legal acts in the sphere of sanitary and epidemiological well-being of the population. The grounds for inclusion of products in the Register are:

1) results of sampling and sanitary-epidemiological examination of products in cases revealing violations of the requirements of the legislation of the Republic of Kazakhstan in the sphere of sanitary-epidemiological well-being of the population poses a threat to life, human health and habitat;

2) results of sampling and sanitary-epidemiological examination of products, confirming information

from international organisations, from member states of the Eurasian Economic Union or third countries on the detection of products controlled by the state sanitary and epidemiological supervision (control), which do not meet the requirements of technical regulations **[13]**. According to the available data of the register, there was no information on violations of food contact materials.

In Europe, paper fibre has recycled an average of 3.5 times, but up to 7 cycles is technically feasible. Recycled material is often preferable to paper and board made from fresh fibres because recycling reduces waste and saves raw materials and energy. For successful recycling, separate collection systems for paper and cardboard have been created to prevent contamination by, for example, food waste. However, unlike some types of plastic, it is not customary to fractionate the collected material into food-grade and non-food-grade streams before recycling. Safety limit values set by different international standards are listed in Table 1 **[14]**.

Table 1 Variation of heavy metal concentrations among three different types of recycled papers (mg/kg).

Controlled indicators	EPA, 2012 paper-based food packaging regulations	Metal Content Directive 2002/72/EC limited values	Council of Europe specific release limits (SRLs) for food packaging
Lead (Pb)	3	2	0.01
Zinc (Zn)	50	100	5
Chromium (Cr)	3.05	1	0.250

On the territory of the Republic of Kazakhstan, there is Technical Regulation (hereinafter TR) 005/2011, "On the safety of packaging", which specifies sanitary and hygienic safety indicators and standards of substances emitted from packaging (closures) in contact with food products. According to this Regulation, the permissible migration quantity (hereinafter called DCM) of heavy metals is indicated in Table 2 **[15]**.

Table 2 Permissible migration amount (PMA, mg/l) of heavy metals, according to TR TS 005/2011 "On the safety of packaging".

Product material name	Controlled indicators	PMA, mg/L	Hazard class
Paper	Lead (Pb)	0.030	2
	Zinc (Zn)	1.000	3
	Chromium (Cr3+)	Total 0.100	3
	Chromium (Cr6+)		3
Cardboard	Lead (Pb)	0.030	2
	Zinc (Zn)	1.000	3
	Chromium (Cr3+)	Total 0.100	3
	Chromium (Cr6+)		3
Paraffinized paper and cardboard	Lead (Pb)	0.030	2
	Zinc (Zn)	1.000	3
	Chromium (Cr3+)	Total 0.100	3
	Chromium (Cr6+)		3

The study of heavy metal migration in paper food packaging makes it possible to assess their compliance with the requirements established by current legislation, thus developing a monitoring system for enterprises. Also, the scientific novelty of this study is the approbation of the method based on the measurement of resonance absorption of light by free atoms of the determined metal during the passage of light through the atomic pair of the sample under study, formed in electrothermal atomisation for the determination of heavy metals in food packaging used in the process of packaging bakery products.

Scientific Hypothesis

The main scientific hypothesis of the study is that paper-based packaging materials used for the production of bakery products exhibit varying levels of heavy metal migration, with some types likely to exceed the permissible limits set by current requirements for the safety of packaging materials. This assumption is based on the fact that paper fibre is recycled on average 3.5 times, but technically feasible up to 7 cycles, in turn recycling can increase the level of potentially hazardous chemicals in packaging and – after migration – In food.

MATERIAL AND METHODOLOGY

Samples

The samples were obtained from the manufacturer prior to certification that they conformed with the Republic of Kazakhstan's packaging safety legislation. The samples were paper bags for bakery products, laminated cardboard sheets and boxes for confectionery products, cardboard boxes, and pizza corners. Table 3 shows samples of packaging paper intended for contact with food products.

Table 3 Samples of packaging paper intended for contact with food products.

Sample code	Type of packaging paper material
1	White paper bag for bakery products.
2	Brown paper bag for bakery products.
3	White paper bag for confectionery products.
4	Brown paper bag for confectionery products.
5	Laminated white cardboard box for confectionery products.
6	Laminated brown cardboard box for confectionery products.
7	White cardboard with lamination for cakes
8	Brown cardboard with cake lamination
9	White cardboard corner for pizza
10	Brown cardboard corner for pizzas
11	White cardboard pizza box
12	Brown cardboard pizza box

Chemicals

The research utilised 65% nitric acid of EMSURE® Reag. Ph Eur, ISO.

Animals, Plants and Biological Materials

This study did not use any biological or animal components.

Instruments

Experimental studies were carried out using the atomic absorption spectrometer KVANT-Z.ETA (KORTEK LLC, Russia) The instrumental parameters of the methods are given in Table 4, while the description of the thermal programs followed for the determination of each single metal is reported in Table 5, Table 6 and Table 7.

Table 4 Instrumental Parameters of atomic absorption spectrometer KVANT-Z.ETA.

Metal	Wavelength (nm)	Slit Width (mm)	Lamp Current (mA)
Zn	307.6	0.5	20
Cr	357.9	0.5	25
Pb	283.3	0.5	10

Table 5 Graphite Furnace program for the determination of Zn.

Step	Temperature (°C)	Ramp (s)	Hold (s)
1	100	3	5
2	120	3	5
3	300	3	5
4*	2000	0	1
5	2600	0	2

Note: *The atomization occurs at step 4.

Table 6 Graphite Furnace program for the determination of Cr.

Step	Temperature (°C)	Ramp (s)	Hold (s)
1	100	3	5
2	120	3	5
3	600	3	5
4*	2400	0	1
5	2650	0	2

Note: *The atomization occurs at step 4.

Table 7 Graphite Furnace program for the determination of Pb.

Step	Temperature (°C)	Ramp (s)	Hold (s)
1	100	3	5
2	120	3	5
3	300	3	5
4*	1900	0	1
5	2600	0	2.2

Note: *The atomisation occurs at step 4.

Table 8 Calibration curve information and detection limits of atomic absorption spectrometer KVANT-Z.ETA.

Metal	Concentrateion (µg/L)	Number of calibration points	Curve type	Detection Limits (mg/dm^3)
Zn	30	3	non-linear	0.04 - 2
Cr	5	7	non-linear	0.02 - 10
Pb	5	3	non-linear	0.02 - 0,5

Calibration of atomic absorption spectrometer KVANT-Z.ETA was carried out following the operating instructions. The wavelength and slit width are selected according to the recommendations of the manufacturer of a particular model of a spectrophotometer, and the gas flow rate and spraying speed are optimized for each individual instrument, and the metal to be detected.

Laboratory Methods

The migration levels of heavy metals from food packaging materials were analysed according to ST RK 1788 1-2008 "Packaging. Requirements for measuring and identifying four heavy metals and other hazardous substances in the package and their entry into the environment. Part 1. Requirements for measuring and identifying four heavy metals in package" **[16]**.

Description of the Experiment

Sample preparation: Samples of food packaging paper were purchased from local dealers, cut into samples and stored at 24 °C (humidity: 50-55%). Experimental studies were conducted under repeatable conditions, at 22 °C; humidity 69% and atmospheric pressure 93.0 kPa, 5 series of measurements were obtained, 3 blank samples and 2 repetitions were run for each analyzed parameter to ensure accuracy and precision.

Number of samples analyzed: 12

Number of repeated analyses: 5

Number of experiment replications: 2

Design of the experiment: The studies were carried out by measuring the resonance absorption of light by free atoms of the determined metal when light passes through the atomic pair of the sample under study, formed in electrothermal atomisation. Preparation of the main calibration solution. The main calibration solution (solution A) of the metals to be determined is prepared from the corresponding Standard Reference Materials (SSR) of aqueous solutions of metal ions following the recommendation of their instructions for use. Carefully transfer a pipette 5 cm^3 of SSR into a 50 cm^3 measuring flask, bring the volume to the mark with 5% (v/v) nitric acid solution and mix. The main graded solution A contains 100 mg/dm^3 metal. The shelf life of solution A is 2 months at a temperature of 2-10 °C. Preparation of working calibration solution with a concentration of 10 mg/dm^3 (solution B). 10 cm^3 of solution A is transferred with a pipette into a measuring flask with a capacity of 100 cm^3, brought to the mark with 5% (v/v) nitric acid solution and mixed. The metal concentration in the resulting calibration solution B is 10 mg/dm^3. Preparation of working calibration solution with a concentration of 1 mg/dm^3 (solution C). 10 cm^3 of solution B with a concentration of 10 mg/dm^3 is transferred into a measuring flask with a capacity of 100 cm^3, bring the volume to the mark with 5% (v/v) nitric acid solution and stir. The resulting calibration solution C contains 1 mg/dm^3 of metal. A series of calibration solutions of the metals to be determined is prepared by adding the volumes of working calibration solutions to 100 cm^3 measuring flasks and bringing to the mark with 5% (v/v) nitric acid solution. Preparation of aqueous extract from the sample using nitric acid. The package samples weighing 30 g, weighed with an error of no more than 0.001 g, were placed in conical flasks (Figure 2).To the samples, pour 99 cm^3 of distilled water into a pipette or cylinder. The sample with water is stirred for 3 min, after adding 1 cm^3 of nitric acid (65%) by pipette and left for 24 hours. Prepared samples were decanted and filtered through a fritted-glass filter with porosity 4 (nom. size 90).

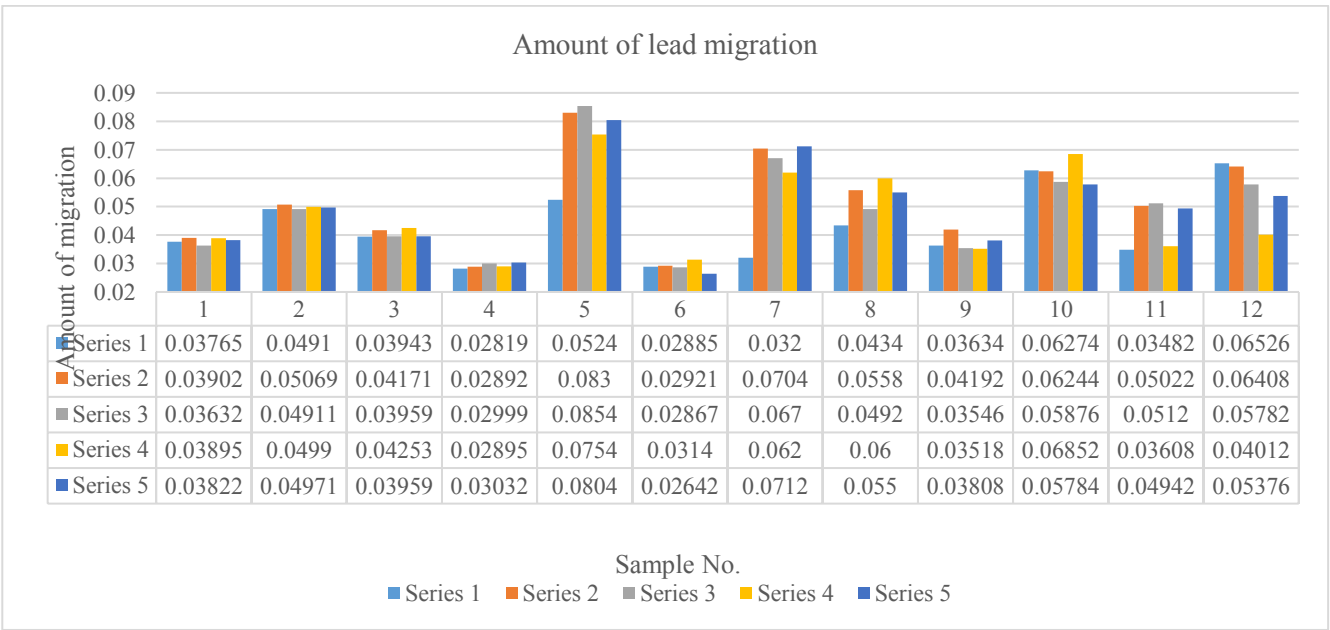
Figure 2 Samples of samples after preparation in aqueous fume hood.

Statistical Analysis

Statistical analysis was performed to evaluate the amount of migration of lead, chromium and zinc by one-way ANOVA and Tukey HSD as post hoc tests using Excel and SPSS for Windows V27.0.1.0 software (SPSS, Inc., Chicago, IL, USA, 2020). Results were considered statistically significant at a p-level equal to or less than 0.05 ($p \leq 0.05$).

RESULTS AND DISCUSSION

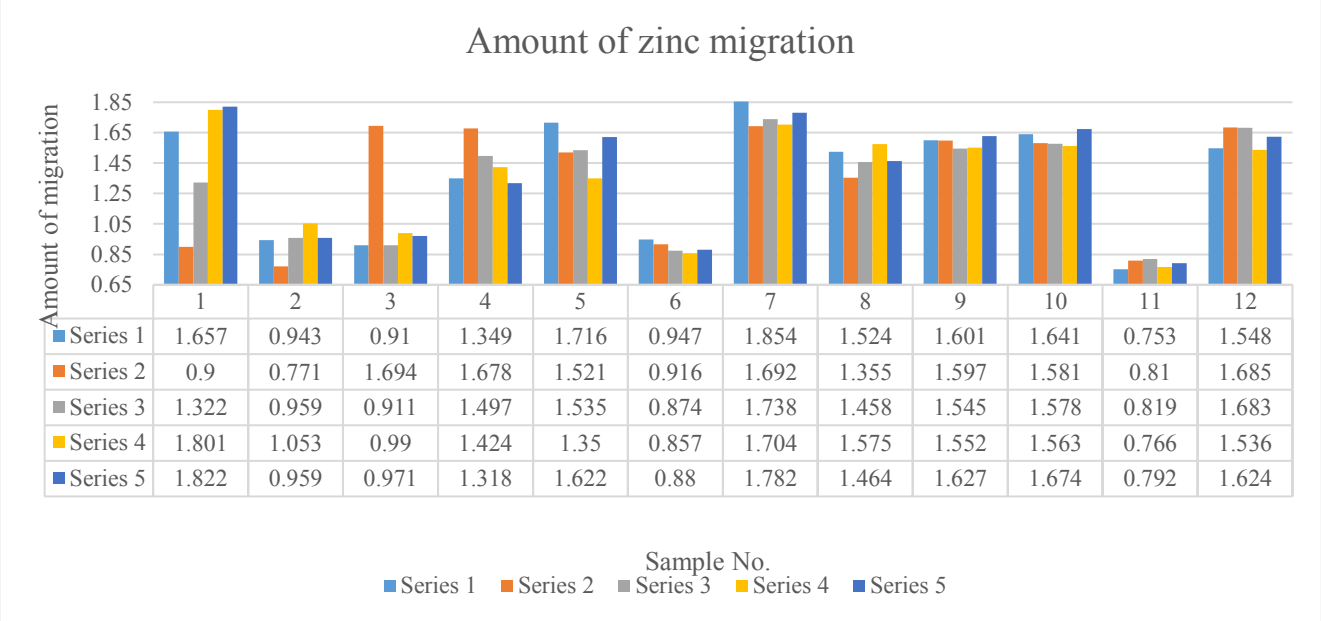
Low cost, lightweight, availability, printability and durability make paper and paperboard products popular as food packaging materials worldwide **[17]**. However, a limitation of using paper and paperboard as food packaging material is its permeability to moisture, which can promote the migration of undesirable compounds from paper and paperboard packaging into food products. The study **[18]** shows that heavy metals in food products are water soluble, and the heavy metal content of food products will decrease if the products are handled or cooked with water. In addition, it is pointed out that it is necessary to determine the migration rate in other cooked foods and to evaluate the risk of heavy metals in concentrations calculated from the migration rate. In this work, the migration of zinc and its effect on the functionality of the nanocomposite film has been studied. In contrast, the migration of zinc from paper packaging materials for food contact still needs to be studied. Natural pigments are the main source of lead in paper and board **[19]**. Lead content should be no more than 0.050 mg/L and can be found in paper and cardboard, especially those intended for use in the printing industry. Lead content in paper and cardboard intended for packaging will be reduced to 0.020 mg/L **[20]**. The results of the study of lead migration during water extraction are presented in Figure 3.



	1	2	3	4	5	6	7	8	9	10	11	12
Series 1	0.03765	0.0491	0.03943	0.02819	0.0524	0.02885	0.032	0.0434	0.03634	0.06274	0.03482	0.06526
Series 2	0.03902	0.05069	0.04171	0.02892	0.083	0.02921	0.0704	0.0558	0.04192	0.06244	0.05022	0.06408
Series 3	0.03632	0.04911	0.03959	0.02999	0.0854	0.02867	0.067	0.0492	0.03546	0.05876	0.0512	0.05782
Series 4	0.03895	0.0499	0.04253	0.02895	0.0754	0.0314	0.062	0.06	0.03518	0.06852	0.03608	0.04012
Series 5	0.03822	0.04971	0.03959	0.03032	0.0804	0.02642	0.0712	0.055	0.03808	0.05784	0.04942	0.05376

Figure 3 Results on the study of the amount of lead migration.

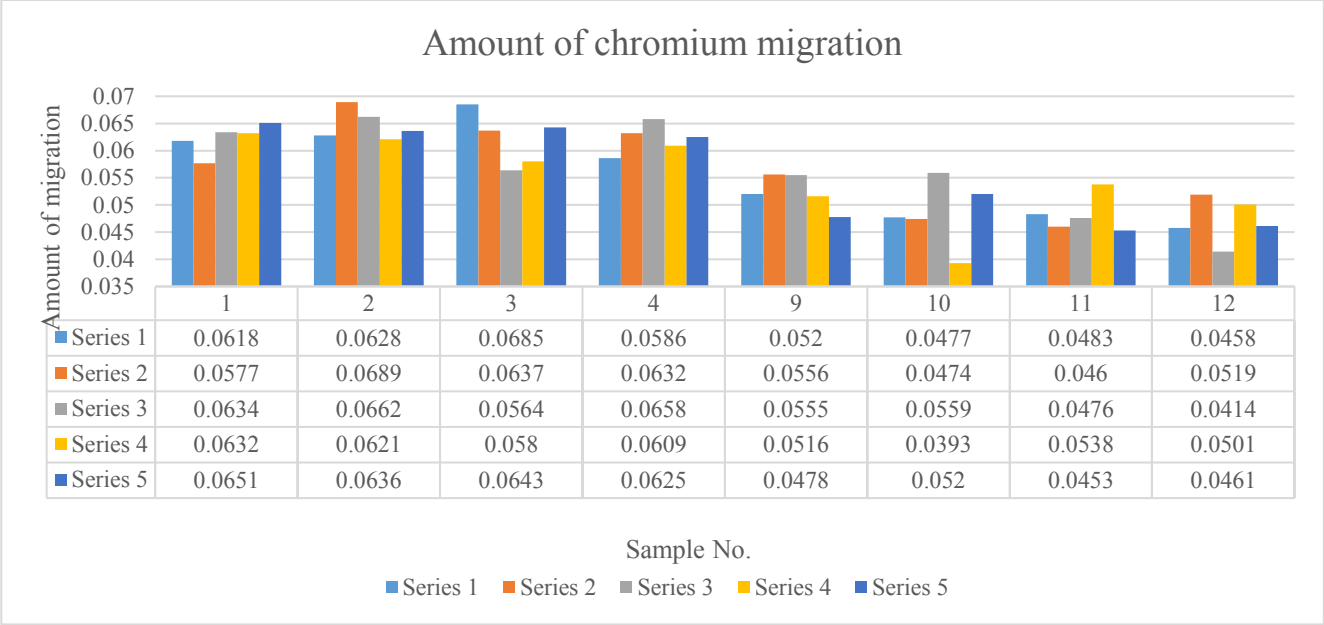
The main reason for high migration rates is using various synthetic and natural pigments, which contain various heavy metal compounds transferred to paper and cardboard packaging **[14]**. The use of recycling is also one of the important reasons for the formation of large amounts of heavy metal compounds. Sood and Sharma used an inductively coupled plasma-optical emission spectrometry technique to determine the content of various metals in paper food packages. They found high lead levels in similar packages **[21]**. These results indicate the lead content problem and potential lead migrations into food products. Interest in incorporating nanoparticles into food contact materials is growing due to their attractive functions, such as ultraviolet (UV) blocking and antimicrobial activity **[11]**. When using nanoparticles, it should be considered that heavy metal migration affects food packaging, especially acidic foods, as Zn is likely to migrate. Hence, their UV blocking and antimicrobial functions may no longer be effective. Zinc oxide or zinc sulfate compounds are sometimes added to paper and are used to increase opacity and to produce copying and packaging paper **[22]**. In the final product, these compounds, including food, can migrate into the environment. The results of the study of zinc migration during aqueous extraction are presented in Figure 4.



	1	2	3	4	5	6	7	8	9	10	11	12
Series 1	1.657	0.943	0.91	1.349	1.716	0.947	1.854	1.524	1.601	1.641	0.753	1.548
Series 2	0.9	0.771	1.694	1.678	1.521	0.916	1.692	1.355	1.597	1.581	0.81	1.685
Series 3	1.322	0.959	0.911	1.497	1.535	0.874	1.738	1.458	1.545	1.578	0.819	1.683
Series 4	1.801	1.053	0.99	1.424	1.35	0.857	1.704	1.575	1.552	1.563	0.766	1.536
Series 5	1.822	0.959	0.971	1.318	1.622	0.88	1.782	1.464	1.627	1.674	0.792	1.624

Figure 4 Results on the study of the amount of zinc migration.

Zinc oxide and zinc sulfate are used in papermaking to increase the opacity of speciality papers. According to Erkan and Malayoglu, when strong mineral acids were used as food simulants, the paper's maximum, minimum and average values of Zn content were below the limit values compared to the EC requirements. In addition, it was observed that the colour of paper packaging has an important role in zinc content, especially red and blue colours affected zinc formation at about the same rate **[23]**. Due to recycling, hexavalent chromium cannot be present in paper or cardboard as it is immediately converted to trivalent chromium. The chromium content should be a maximum of 0.1 mg/L **[23]**. The source is mainly natural white pigments (kaolin, clay, calcium carbonate, etc.) used as paper-making fillers and surface coating to improve printing quality **[24]**. The amount of chromium migration was not detected in samples No. 5-8. The results of the chromium migration study in water extraction are presented in Figure 5.



	1	2	3	4	9	10	11	12
Series 1	0.0618	0.0628	0.0685	0.0586	0.052	0.0477	0.0483	0.0458
Series 2	0.0577	0.0689	0.0637	0.0632	0.0556	0.0474	0.046	0.0519
Series 3	0.0634	0.0662	0.0564	0.0658	0.0555	0.0559	0.0476	0.0414
Series 4	0.0632	0.0621	0.058	0.0609	0.0516	0.0393	0.0538	0.0501
Series 5	0.0651	0.0636	0.0643	0.0625	0.0478	0.052	0.0453	0.0461

Figure 5 Results of the study of the amount of chromium migration.

The results of this study suggest that laminated surface treatment of paper packaging helps reduce chromium migration in the aqueous extract. High chromium content was found in confectionery boxes in paper food packaging in India. In addition, chromium ingestion is suspected to be caused by printing inks and wood treated with chromate copper arsenate as a preservative **[21]**. Compared to the above studies, the method used in this work provides a more stable process for obtaining results from aqueous extracts by using an atomic absorption spectrometer. The authors **[25]** used drying food packages after preparing an aqueous extract to measure the total migration of heavy metals. The main difference of this work is the possibility of determining different types of heavy metals and the possibility of application in other types of packaging materials. The limitation of this study is the dependence of the results on the detection limits of the atomic absorption spectrometer, which may affect the study of the content of small amounts of compounds in food packages. Assessment of heavy metal migration results according to sanitary-hygienic requirements for food packages in the Republic of Kazakhstan. Material properties such as mechanical and other physical properties, permeability, sealing and migration of substances in contact with food determine food quality, shelf life and safety. Therefore, food packaging materials need to be tested to ensure that they have the correct properties in terms of permeability to gases, water vapour, contaminants, mechanical and other physical properties, and thickness of the main components and coating layers **[26]**. In investigating plastic-based food contact materials **[24]**, the paper showed that plastic-based materials potentially threaten the environment and public health by releasing toxic heavy metals. This study aimed to identify the types of plastic commonly used as food contact materials (FCM) in Bangladesh and assess the migration of heavy metals from these FCMs. As the study shows that paper packaging material is also a potential threat to public health if a certain level of migration is not controlled. The average amount of metal migration plays a major role in food safety. The levels of similarity and/or difference, as well as the significance level between the groups of samples studied, were identified for each requirement of sanitary and hygienic safety indicators and standards of substances emitted from food contact packaging. The average amount of heavy metal migration from food packages intended for contact with food products is shown in Table 9.

The study showed that in all types of paper, there was a significant amount of heavy metals, which did not meet the requirements established by the Technical Regulation for paper intended for food packaging. In the study of the amount of zinc values close to the limits established by TR CU 005/2011. As a result of the evaluation of the presence of heavy metals: zinc (Zn), chromium (Cr) and lead (Pb) in different recycled papers, the variation of the mean value with standard deviations of the different heavy metals present in each type of paper and the safety limits set by different international standards are shown in Table 1. Compliance of the research results with the requirement of sanitary and hygienic safety indicators and norms of substances migrating from the packaging is an important factor for regulating the safety level of food packaging in the countries that have adopted this Technical Regulation. Compliance with the safety requirement of the average amount of migration of heavy metals for each sample of food packages, according to TR TS 005/2011 “On the safety of packaging”, is shown in Figure 6.

Table 9 Average amount of heavy metal migration from food packages.

Sample	Amount of migration		
	Pb	Cr	Zn
1	0.0380 ±0.00111^{ab}	0.0525 ±0.00323^{a}	1.5004 ±0.39071^{b}
2	0.0497 ±0.00066^{bc}	0.0485 ±0.00620^{a}	0.9370 ±0.10249^{a}
3	0.0406 ±0.00145^{ab}	0.0482 ±0.00335^{a}	1.0952 ±0.33663^{a}
4	0.0293 ±0.00087^{a}	0.0471 ±0.00410^{a}	1.4532 ±0.14356^{b}
5	0.0753 ±0.01334^{d}	Not detected	1.5488 ±0.13583^{b}
6	0.0289 ±0.00177^{a}	Not detected	0.8948 ±0.03624^{a}
7	0.0605 ±0.01635^{c}	Not detected	1.7540 ±0.06592^{b}
8	0.0527 ±0.00646^{bc}	Not detected	1.4752 ±0.08248^{b}
9	0.0374 ±0.00277^{ab}	0.0622 ±0.00280^{b}	1.5844 ±0.03483^{b}
10	0.0621 ±0.00421^{c}	0.0647 ±0.00281^{b}	1.6074 ±0.04769^{b}
11	0.0443 ±0.00816^{ab}	0.0622 ±0.00494^{b}	0.7880 ±0.02815^{a}
12	0.0562 ±0.01014^{bc}	0.0622 ±0.00268^{b}	1.6152 ±0.07130^{b}
Effect of types of packaging materials on migration of heavy metals from packaging			
Sanitary and hygienic safety indicators		***F***	***p***
Pb		17.313	<0.001
Cr		384.415	<0.001
Zn		64.967	<0.001

Note: Data are expressed as mean (n = 5) ± standard deviation (SD). The effect of type of packaging material on migration of heavy metals from packaging is significant at $p \leq 0.05$. $^{a-b}$ Mean values in the column with different letters are significantly different ($p \leq 0.05$).

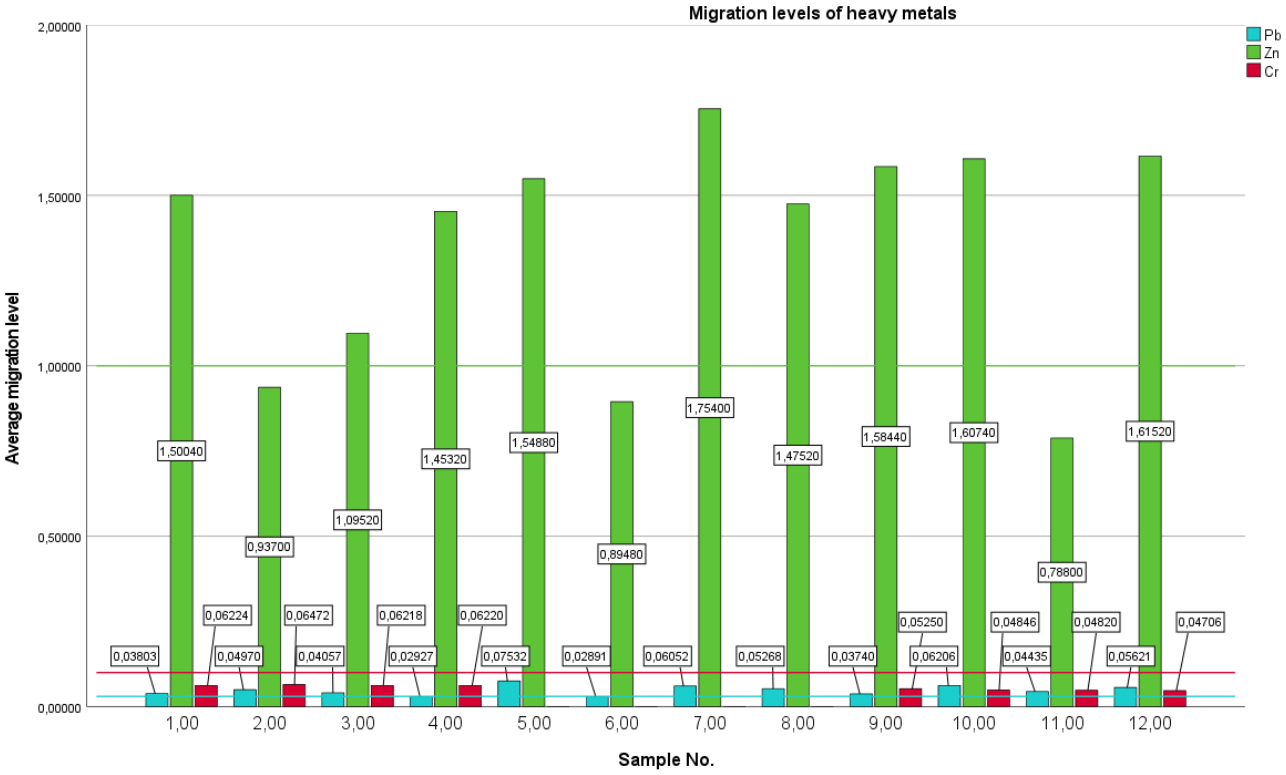


Figure 6 Average migration level of heavy metals according to the requirement of TR TS 005/2011 "On the safety of packaging".

A study **[27]** analysed heavy metals, including lead (Pb), cadmium (Cd), arsenic (As) and aluminium (Al) in oilseeds, noodles, tea leaves and their processed or cooked products to study the effect of food processing methods on the migration of heavy metals. The paper indicates ways to reduce heavy metals naturally present in food products. Recycling is currently seen as an important measure to manage packaging waste. However, recycling can increase potentially hazardous chemicals in packaging and - after migration – In food **[28]**. Chemicals of various origins are commonly present in recovered paper and may eventually end up in the recycled product. The

paper shows that these include additives introduced during manufacturing and often intended to be retained in the paper product, such as fillers, retention agents, adhesives, coatings, biocides and synthetic binders. In addition, paper is typically printed, dyed, glued or labeled, resulting in printing inks, adhesives, photoinitiators, solvents, plasticisers, surfactants and pigments in the wastepaper. Contaminants can also be introduced during the use or waste disposal process, as paper and paperboard tend to absorb chemicals. Thus, recycled paper and paperboard used as primary or secondary food packaging is usually derived from rather uncertain sources. A study **[29]** examined food contact materials, items of any type intended to come into contact with food, thus representing a potential source of human exposure to chemicals. The authors point out that information on chemical constituents and potential human health effects remains scarce for materials made from paper and cardboard, making safety assessment difficult. The paper describes a guided exposure strategy for identifying and characterising new chemicals for paper and paperboard. That is, practical regulation remains unlearned.

Recommended interim production control measures

Food safety is considered in European legislation within the framework of an integrated approach based on the principle "from the field (stall) to the table" as a single continuous chain that starts with the production of animal feed includes (but is not limited to) the production of primary products, processing, packaging, transportation and marketing, and ends with the consumption of the food product by the final consumer **[30]**. The international document **[31]** states that food business operators must ensure that food contact materials and articles are used in the production or preparation, storage and distribution of food in a manner that does not compromise compliance with applicable Council of Europe Technical Guidelines, EU and Member State legislation or recommendations on food contact materials and articles.

In the production of bakery products, there are significant critical control points such as baking and cooling processes **[32]**. Naturally, every food processing plant has a certificate of conformity check when purchasing packaging materials. It is proposed that purchasing packaging materials should be included as a control point **[33]**. A control point is any point in a particular food system where loss of control does not result in an unacceptable health risk **[34]**. To these intermediate control processes, according to the results of this study, monitoring the procurement process of the packaging can be added as a control point, as shown in Table 10.

Table 10 Recommended monitoring activities for the implementation of interim production control.

	No. CP	CP No. 3 – procurement process for packaging materials
The monitoring procedure	**Dangerous factor**	Chemical Hazard Factor - absence of a certificate of conformity if the requirements for evaluating the choice of packaging materials for contact with bakery products are not met. Permissible migration amount (PMA) of heavy metals, according to TR TS 005/2011 "On the safety of packaging".
	Procedure (what will be measured, how, how often)	Compliance with the requirements for evaluating the choice of packaging materials for contact with bakery products.
	Frequency (how often)	Once a year, an independent examination by an accredited testing laboratory is recommended.
	Responsible	Technologist
	Correction, corrective actions	Product inspection, rejection, isolation and disposal if necessary. Additional staff training on purchasing of packaging materials
	HACCP records	Records in the logbook of control of technological parameters test protocols of packaging materials. Records of confirmation of competence and responsibility of personnel

Chemical hazards in food products usually result from unintentionally including certain ingredients during food production **[35]**. A scientific paper **[36]** presents the results of studies on the kinetics of food deterioration. The paper provides an in-depth study of shelf life and various evaluations of approaches to determining the shelf life of finished products. Based on the nature of food products as physically, chemically and biologically active systems, food quality is a dynamic state constantly changing at ever lower levels **[37]**. In general, the overall quality of food products is better described by organoleptic evaluation. Consequently, for each product, there is a certain finite time from the moment of its production, during which, under given storage conditions, it retains the required level of organoleptic properties **[38]**. Metals and alloys are used in food contact materials and articles, food-processing equipment, containers, household utensils, and foil used to wrap food. These materials are frequently used as a safety barrier between the food and the environment. They are often covered by a coating to

reduce ion release into foods. Metal ions can be released from materials and put into food. They may endanger the health of the 89 consumers if the intake exceeds the toxicological reference value or may unacceptably alter the 90 composition of the food or its organoleptic characteristics. Consequently, it was decided to establish 91 technical guidance in this area **[39]**. The results of this study indicate the importance of regulating the safety levels of food packaging at the level of technical documents, as the obtained data exceeded/complied close to the maximum permissible requirements of TR TS 005/2011. Thus, these samples should be considered as a potential risk to human health if they can be reused without any pre-treatment as a source of recycled cellulose fibre for the production of packaging to be used in direct contact with food. One tonne of recycled newspapers will save one tonne of wood while recycling printing or copy paper will save more than 2 tonnes **[40]**.

This finding presents authorities with a dilemma: recycling is supported for the sustainable use of materials, but based on current toxicological assessments, migration often goes far beyond what is acceptable **[41]**. Using recycled cardboard and corrugated cardboard for food packaging is in the interest of resource sustainability, but in most cases, food must be protected from contamination by these materials **[42]**. Due to increased consumer health awareness, the importance of transferring harmful materials from packaging to food is of great concern **[43]**. Chemical compounds incorporated into packaging materials to improve functionality can interact with food components during processing or storage and migrate into the food. Food quality and safety can be jeopardised once these compounds reach a certain limit **[44]**.

In general, it is necessary to identify and restrict the amount of migrating chemicals from the coating into food to a level that does not present risks to human health. An evaluation of starting substances can serve as a basis for the safety assessment of coatings, provided that the evaluation considers the foreseeable reactions that may occur during the manufacturing process **[45]**. Ideally, the evaluation should consider both the starting material AND the type of process to guarantee that the use of such material will always lead to the same reaction or degradation products (evaluated as of no safety concern when the substance was assessed) **[46]**.

According to the study's results, we can conclude that the main scientific hypothesis is confirmed. Paper-based packaging materials used for the production of bakery products show different levels of heavy metal migration, and some types exceed the permissible limits established by the requirements for the safety of packaging materials.

CONCLUSION

1. Heavy metals in food packages used in bakery products were determined by atomic absorption spectrometry: zinc migration (13.6% less than PMA for sample No. 9, 9.8% less than PMA for sample No. 10, 11.3% less than PMA for sample No. 11, 12.9% less than PMA for sample No. 12, respectively). Samples No. 1 to No. 8 had no significant difference, with the lowest zinc content in sample No. 8 (45.1% less than PMA).

a) Lead. As a result of the study, the average amount of lead migration from food packaging was below the PMA specified in TR/TC 005 2011 'On the safety of packaging' in samples No. 4 and No. 6 (2.42% less than the PMA for sample No. 4 and 3.63% less than the PMA for sample No. 6, respectively). The results of the remaining samples exceeded the permissible migration level. The highest amount of lead was detected in samples No. 5 (151.06% more than the permissible level), No. 7 (101.73% more than the permissible level), No. 8 and No. 10 (75.6% and 106.86% more than the PMA, respectively).

b) Zinc. The average amount of zinc migration from samples No. 2, No. 6, and No. 11 met the acceptable migration level specified in the Technical Regulations (6.3% less than the PMA for sample No. 2, 10.52% less than the PMA for sample No. 6, and 21.2% less than the PMA for sample No. 11, respectively). On average, the other samples exceeded the PMA by 1.5 times. The highest amount of zinc migration was found in samples No. 5, No. 7, No. 9, No. 10, and No. 12 (54.9% more than the PMA for sample No. 5, 75.4% more than the PMA for sample No. 7, 58.4% more than the PMA for sample No. 9, 60.7% more than the PMA for sample No. 10, 61.5% more than the PMA for sample No. 12, respectively).

c) Chromium. No chromium content was detected in the laminated cartons. The values of the other samples were within the permissible limits of the Technical Regulations. The average amount of chromium migration in paper packages and cartons was significantly different, with the highest value in sample No. 1 for paper packages and in sample No. 10 for cartons (47.5 per cent less than the PMA for sample No. 1 and 35.3 per cent less than the PMA for sample No. 10, respectively).

2. According to the results of the assessment of the average migration level of heavy metals according to the requirement of TR TS 005/2011 'On the safety of packaging' (Figure 2), it can be concluded that in all types of paper, there is a significant amount of lead and zinc, the level of which did not meet the requirements established by the Technical Regulations for paper intended for food packaging. In the study of the amount of zinc, values were found to be close to the limits set by TR CU 005/2011. Thus, these particular samples should be considered as a potential risk to human health in case they can be reused without any pre-treatment as a source of recycled cellulose fibre for the production of packaging to be used in direct contact with food products.

Further research is needed to study in more depth the influence of food simulator parameters such as temperature and acidity, as well as the effect of storage time, on the degree of migration of heavy metals directly to the food product.

REFERENCES

1. Leks-Stępień, J. (2011). Paper packaging materials and food safety. In International Circle of Educational Institutes for Graphic Arts: Technology and Management (Vol. 4, pp. 49–51). The International Circle of Educational Institutes for Graphic Arts: Technology and Management.
2. Duran, A., & Soylak, M. (2013). Evaluation of Metal Concentrations in Food Packaging Materials: Relation to Human Health. In Atomic Spectroscopy (Vol. 34, Issue 3, pp. 99–103). Atomic Spectroscopy Press Limited. https://doi.org/10.46770/as.2013.03.004
3. Resources and utilization of certain types of products (goods) and raw materials in the Republic of Kazakhstan. (2024). In Official site of the Bureau of National Statistics of the Republic of Kazakhstan.
4. Xue, M., Wang, S. & Huang, C. (2010). Determination of Heavy Metals (Pb, Cd, Cr and Hg) in Printed Paper as Food Packaging Materials and Analysis of Their Sources. In CIESC Journal (Vol. 12, pp. 32). Chemical Industry Press Co., Ltd.
5. Conti, M. E. (2006). Heavy Metals in Food Packagings. In Mineral Components in Foods (pp. 339–362). CRC Press. https://doi.org/10.1201/9781420003987-9
6. Tiggelman, I. (2012). Migration of Organic Contaminants through Paper and Plastic Packaging [Master's Thesis, Stellenbosch University, Stellenbosch, South Africa].
7. Elmas, G. M., & Çınar, G. (2018). Toxic Metals in Paper and Paperboard Food Packagings. In BioResources (Vol. 13, Issue 4). BioResources. https://doi.org/10.15376/biores.13.4.7560-7580
8. Cederberg, D. L., Ekroth, S., Engman, J., Fabech, B., Guðjónsdóttir, K., Håland, J. T., Jónsdóttir, I., Kostaomo, P., Legind, C., Mikkelsen, B., Ólafsson, G., & Svensson, K. (2015). Food contact materials – metals and alloys (Vol. 2015522). Nordic Council of Ministers. https://doi.org/10.6027/tn2015-522
9. Hutton, T. (2003). Food packaging: An introduction. Key topics in food science and technology–Number 7. Chipping Campden, Gloucestershire, UK: Campden and Chorleywood Food Research Association Group, 108 p.
10. Ahmadkhaniha, R., & Rastkari, N. (2017). Identification of suspected hazardous chemical contaminants in recycled pastry packaging. In Acta Scientiarum Polonorum Technologia Alimentaria (Vol. 16, Issue 1, pp. 33–41). Uniwersytet Przyrodniczy w Poznaniu (Poznan University of Life Sciences). https://doi.org/10.17306/j.afs.2017.0444
11. Mengistu, D. A. (2021). Public health implications of heavy metals in foods and drinking water in Ethiopia (2016 to 2020): systematic review. In BMC Public Health (Vol. 21, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12889-021-12189-3
12. 2022 Annual Report. Alert and Cooperation Network. (2024, August 25). In Health and Food Safety Department of European Commission.
13. Rules for maintaining a register of products not complying with the requirements of regulatory legal acts in the field of sanitary and epidemiological welfare of the population KR DSM-229/2020.
14. Bandara, R., & Indunil, G. M. (2022). Food packaging from recycled papers: chemical, physical, optical properties and heavy metal migration. In Heliyon (Vol. 8, Issue 10, p. e10959). Elsevier BV. https://doi.org/10.1016/j.heliyon.2022.e10959
15. Technical Regulations of the Customs Union 005/2011 On Packaging Safety.
16. ST RK 1788-1-2008 Packaging. Requirements for measuring and identification of four heavy metals and other hazardous substances in the package and their entry into the environment. Part 1. Requirements for measurement and identification of four heavy metals in package.
17. Elmas, G. M., & Çınar, G. (2018). Toxic Metals in Paper and Paperboard Food Packagings. In BioResources (Vol. 13, Issue 4). BioResources. https://doi.org/10.15376/biores.13.4.7560-7580
18. Bumbudsanpharoke, N., Choi, J., Park, H. J., & Ko, S. (2019). Zinc migration and its effect on the functionality of a low density polyethylene-ZnO nanocomposite film. In Food Packaging and Shelf Life (Vol. 20, p. 100301). Elsevier BV. https://doi.org/10.1016/j.fpsl.2019.100301
19. Regulation (EC) No 94/62 of the Council of 20 December 1994 on packaging and packaging waste (2013 edition).
20. Regulation (EC) No 94/62 of the Council of 20 December 1994 on packaging and packaging waste (2015 edition).

21. Sood, S., & Sharma, C. (2019). Levels of Selected Heavy Metals in Food Packaging Papers and Paperboards Used in India. In Journal of Environmental Protection (Vol. 10, Issue 03, pp. 360–368). Scientific Research Publishing, Inc. https://doi.org/10.4236/jep.2019.103021
22. Mertoglu-Elmas, G. (2017). The Effect of Colorants on the Content of Heavy Metals in Recycled Corrugated Board Papers. In BioResources (Vol. 12, Issue 2). BioResources. https://doi.org/10.15376/biores.12.2.2690-2698
23. Uazhanova, R., Tungyshbaeva, U., Kazhymurat, A., & Mannino, S. (2018). Evaluation of the Effectiveness of Implementing Control Systems in the Increasing of Food Safety. In Journal of Advanced Research in Dynamical and Control Systems (Vol 10, Special Issue 13, pp 649–656). JARDCS .
24. Industry Guideline for the Compliance Paper and Board Materials and Articles for Food Contact. Issue No. 2. (2024, March 4). In The International Confederation of Paper and Board Converters in Europe (CITPA).
25. Eti, S. A., Islam, M. S., Shourove, J. H., Saha, B., Ray, S. K., Sultana, S., Ali Shaikh, M. A., & Rahman, M. M. (2023). Assessment of heavy metals migrated from food contact plastic packaging: Bangladesh perspective. In Heliyon (Vol. 9, Issue 9, p. e19667). Elsevier BV. https://doi.org/10.1016/j.heliyon.2023.e19667
26. Singh, P., Wani, A. A., & Langowski, H.-C. (2017). Food Packaging Materials. CRC Press. https://doi.org/10.1201/9781315374390
27. Lee, J.-G., Hwang, J.-Y., Lee, H.-E., Kim, T.-H., Choi, J.-D., & Gang, G.-J. (2019). Effects of food processing methods on migration of heavy metals to food. In Applied Biological Chemistry (Vol. 62, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s13765-019-0470-0
28. Geueke, B., Groh, K., & Muncke, J. (2018). Food packaging in the circular economy: Overview of chemical safety aspects for commonly used materials. In Journal of Cleaner Production (Vol. 193, pp. 491–505). Elsevier BV. https://doi.org/10.1016/j.jclepro.2018.05.005
29. Rosenmai, A. K., Bengtström, L., Taxvig, C., Trier, X., Petersen, J. H., Svingen, T., Binderup, M.-L., Barbara Medea Alice, van V.-L., Dybdahl, M., Granby, K., & Vinggaard, A. M. (2017). An effect-directed strategy for characterizing emerging chemicals in food contact materials made from paper and board. In Food and Chemical Toxicology (Vol. 106, pp. 250–259). Elsevier BV. https://doi.org/10.1016/j.fct.2017.05.061
30. Uazhanova, R. U., Kazhymurat, A. T., Tungyshbayeva, U. O., & Cepeda Saez, A. (2019). Development and implementation of HACCP plan based on FMEA in collagen hydrolizate industry. In Journal of Hygienic Engineering and Design (Issue 28, pp 13–21). Journal of Hygienic Engineering and Design (JHED).
31. Resolution CM/Res. (2020). 9 of the European Directorate for the Quality of Medicines and HealthCare of 7 Octover 2020 on the safety and quality of materials and articles for contact with food.
32. Tungyshbayeva, U., Mannino, S., Uazhanova, R., Adilbekov, M. A., Yakiyayeva, M. A., & Kazhymurat, A. (2021). Development of a methodology for determining the critical limits of the critical control points of the production of bakery products in the Republic of Kazakhstan. In Eastern-European Journal of Enterprise Technologies (Vol. 3, Issue 11 (111), pp. 57–69). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2021.234969
33. ST RK ISO 22000-2019. (2019). Food safety management systems. Requirements for organisations involved in the food chain.
34. Tungyshbayeva, U. (2019). Development of the model of the integrated system of safety and quality of bakery products [Doctoral dissertation, Almaty Technological University].
35. Batt, C. A. (2016). Chemical and Physical Hazards in Food. In Reference Module in Food Science. Elsevier. https://doi.org/10.1016/b978-0-08-100596-5.03437-5
36. Wallace, C. A., Sperber, W. H., & Mortimore, S. E. (Eds.). (2018). Food Safety for the 21st Century. Wiley. https://doi.org/10.1002/9781119053569
37. Marriott, N. G., Schilling, M. W., & Gravani, R. B. (2018). Principles of Food Sanitation. In Food Science Text Series. Springer International Publishing. https://doi.org/10.1007/978-3-319-67166-6
38. Mortimore, S., & Wallace, C. (2013). HACCP. Springer US. https://doi.org/10.1007/978-1-4614-5028-3
39. Technical Guide on Metals and Alloys used in food contact materials and articles, 2nd edition (2024, August 25). In European Committee for Food Contact Materials and Articles.
40. Soni, R., Suryawanshi, N., Khidkikar, P., Thakur, S., Kshtriya, S., Gajbhiye, R., & Wadbudhe, R. (2020). Paper Recycling and Paper Plate Forming Machine. In International Journal of Innovative Research in Technology (Volume 6, Issue 10, pp 314–316). IJIRT.
41. Biedermann, M., & Grob, K. (2010). Is recycled newspaper suitable for food contact materials? Technical grade mineral oils from printing inks. In European Food Research and Technology (Vol. 230, Issue 5, pp. 785–796). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-010-1223-9

42. Biedermann-Brem, S., Biedermann, M., & Grob, K. (2016). Required barrier efficiency of internal bags against the migration from recycled paperboard packaging into food: a benchmark. In Food Additives & Contaminants: Part A (pp. 1–16). Informa UK Limited. https://doi.org/10.1080/19440049.2016.1160744
43. Alamri, M. S., Qasem, A. A. A., Mohamed, A. A., Hussain, S., Ibraheem, M. A., Shamlan, G., Alqah, H. A., & Qasha, A. S. (2021). Food packaging's materials: A food safety perspective. In Saudi Journal of Biological Sciences (Vol. 28, Issue 8, pp. 4490–4499). Elsevier BV. https://doi.org/10.1016/j.sjbs.2021.04.047
44. Bhunia, K., Sablani, S. S., Tang, J., & Rasco, B. (2013). Migration of Chemical Compounds from Packaging Polymers during Microwave, Conventional Heat Treatment, and Storage. In Comprehensive Reviews in Food Science and Food Safety (Vol. 12, Issue 5, pp. 523–545). Wiley. https://doi.org/10.1111/1541-4337.12028
45. European Food Safety Authority. (2011). Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment [JB]. EFSA Journal, 9(3). https://doi.org/10.2903/j.efsa.2011.2097
46. EFSA Task Force On Varnishes And Coatings For Food Contact Materials. (2019). Evaluation of materials intended for varnishes and coatings on food contact materials. Zenodo. https://doi.org/10.5281/ZENODO.3583381

Funds:

The study was carried out with financial support of JSC 'Almaty Technological University' project No. 10677508-1 to provide an experimental base and to conduct laboratory and instrumental research to develop standards.

Acknowledgments:

-

Conflict of Interest:

The authors declare that they have no conflict of interest in connection with this research, whether financial, personal, author or otherwise, that could influence the research and its results presented in this paper.
No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Almas Zhanbolat, Almaty Technological University, Department of Food Safety and Quality, Tole bi str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77083870617
E-mail: zhanbolatalmas@gmail.com
ORCID: https://orcid.org/0009-0001-7983-3245

***Ulbala Tungyshbayeva**, Almaty Technological University, Department of Food Safety and Quality, Tole bi str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77015038906
E-mail: ulbala_84@mail.ru
ORCID: https://orcid.org/0000-0003-0593-1130

Raushangul Uazhanova, Almaty Technological University, Department of Food Safety and Quality, Tole bi str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +7 777-130-17-41
E-mail: raushanu 67@mail.ru
ORCID: http://orcid.org/0000-0002-5156-5322

Zhanar Nabiyeva, Almaty Technological University, Research Institute of Food Safety, Tole bi str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77759771901
E-mail: atu_nabiyeva@mail.ru
ORCID: https://orcid.org/0000-0001-7258-746X

Madina Yakiyayeva, Almaty Technological University, Research Institute of Food Technologies, Department of Bread Technology and Processing Industries, Tole bi str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +7 701 162 6749
E-mail: yamadina88@mail.ru
ORCID: https://orcid.org/0000-0002-8564-2912

Abdysemat Samadun, Almaty Technological University, Research Institute of Food Safety, Tole bi str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77073871595
E-mail: abdu.93_93@mail.ru
ORCID: https://orcid.org/0000-0002-5286-5175

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 31.7.2024
Revised: 4.9.2024
Accepted: 17.9.2024
Published: 27.9.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 834-846
https://doi.org/10.5219/2003
ISSN: 1337-0960 online
www.potravinarstvo.com

Formation of the biological value of beef protein depending on the age and breed of bulls

Anatoliy Paliy, Stepan Michalchenko, Igor Korkh, Kateryna Rodionova, Svetlana Tkachuk, Mariia Khimych, Nina Dankevych, Nataliia Boiko

ABSTRACT

The article substantiates the expediency of assessing the content of essential amino acids in the samples obtained during the controlled slaughter of bulls for the protein of chicken eggs as an effective means of improving the quality of the management processes for producing biologically complete products. To ascertain the biological value of beef, samples of the longest back muscle were obtained from bulls of six domestic dairy breeds (Black-and-White, Red Steppe, and Angler dairy breeds and Simmental, Lebedyn, and Gray Ukrainian dairy breeds of combined productivity) at the ages of 3, 6, 9, 12, 15, 18, and 21 months. The experiments were conducted using the ion-exchange liquid column chromatography method on an automatic amino acid analyser (T-339 M) manufactured by Microtechna (Czech Republic). It has been demonstrated that the biological value of meat from bulls of the studied breeds is limited during ontogeny, primarily due to age-related factors. As bulls grow older and gain weight, their meat proteins exhibit increased biological value, approaching the reference index of chicken egg proteins. The first peak in the average values of the amino acid index in beef of bulls of 12 months of age (0.89%) was followed by a consistent decrease to 15 months (0.68%) and a repeated increase in values in animals of 21 months of age (0.83%). This is mainly due to the rise in the scores for methionine by 0.16%, isoleucine by 0.16%, histidine by 0.42%, arginine by 0.18%, and threonine by 0.20%. The increase in the biological value of the remaining amino acid scores in the age trend of changes did not exceed 0.15%. The identified patterns indicate the presence of additional reserves in the near-term scenario, which can be utilised to ensure the production of high-grade beef while optimising the age parameters of slaughtering bulls of different productivity directions.

Keywords: amino acid score, age, breed, beef

INTRODUCTION

The significance of protein deficiency in human diets is increasing today. A lack of protein can result in many adverse effects, including a decline in metabolic function, an impairment of the immune system, and the malfunction of specific organs or systems. One potential solution is the development of animal agriculture, a strategy to alleviate protein deficiency **[1]**, **[2]**. Concurrently, the enhancement of production and the assurance of the quality and safety of meat, from its initial production to its final consumption, including beef, represents the fundamental instrument for its implementation **[3]**. Meat production, in general, and beef, in particular, depends on many factors, such as religious and cultural preferences, convenience, accessibility, etc. **[4]**. The term "beef" comes from the common Slavic word "govedo," which means "bull". Beef meat is an excellent source of lipids, carbohydrates, biologically active substances, and micro- and trace elements **[5]**, **[6]**, **[7]**. However, its most important component is high-quality protein, which provides the body with plastic material **[8]**. Protein consists of amino acids. All the essential amino acids contained in beef make it a complete and balanced protein

[9], the bioavailability of which depends on the proper organization of feeding, and housing conditions **[10]**, **[11]**, **[12]**.

Breed is one of the key factors influencing muscle tissue characteristics **[13]**, **[14]**, **[15]**. First of all, it affects the meat yield and the ratio of muscle, bone, and fat tissue. These components determine not only the quality of the meat but also its nutritional value. This is well illustrated in the study **[16]**. The researchers proved that the best indicators for the content of valine – by 7.4%, isoleucine – by 45.3% (p <0.001), leucine – by 15.2% (p <0.001), lysine – by 7.8%, threonine and phenylalanine + tyrosine by 6.5% (p <0.05) and 7.5% (p <0. 01) in 6-7-month-old bulls of the Charolais breed compared to Aberdeen-Angus and Black-and-White mixed breed, raised according to the technology of beef cattle breeding, compared to their counterparts of the Black-and-White breed. On the other hand, the amino acid score for most amino acids within all breeds exceeded 100%, indicating the high biological and nutritional value of veal. On the other hand, no significant differences were found between the breeds except for the histidine content when studying the characteristics of the amino acid profile of beef from Hungarian Grey and Holstein-Friesian bulls **[17]**. We cannot ignore the detailed studies **[18]**, which registered a significant difference in the content of essential amino acids in beef samples ranging from 30.16% in Montbéliard bulls to 34.50% in Menck-Anjou bulls and from 30.22% in Hereford bulls to 33.75% in Aberdeen Angus bulls, which was mainly due to the content of lysine – 7.85-8.73% and 7.76-8.75%, respectively. Age-related characteristics, which also affect the biological value of beef, have been well studied **[19]**, **[20]**. However, **[21]** found no significant differences in the amino acid content of beef due to the age of Qinchuan cattle.

Information on the content of amino acids in food products, including beef, can be used to determine their ability to meet human protein needs **[22]**. It is worth noting that with the development of the latest analytical methods for quality assessment, there is a growing interest in fundamental and applied research aimed at reviewing the role of amino acids in the formation of the biological value of livestock products, including beef, which is determined by chemical, biochemical and biological methods. At present, the most informative biochemical method is determining the amino acid number – the "score". Its fundamentally important essence lies in consistently comparing the concentration of a specific essential amino acid in a product with the content of the same amino acid in an ideal protein, which is taken as the amino acid composition of chicken egg white **[23]**, **[24]**. The proper implementation of this methodological assessment apparatus aims to increase the efficiency of quality management of final products during their industrial processing.

Over the past decade, Ukraine has significantly increased its understanding of the leading role of healthy eating in stimulating and activating the human body's defences through consuming nutritious products, especially beef. However, the production of this type of product is in a state of recovery. That is why one of the vectors for the development of this area in the context of protein deficiency is to produce high-protein meat products at the optimal age, to achieve scientifically sound pre-slaughter live weight of young animals, and to use breeds with increased genetic potential for increasing protein in carcasses. With the implementation of this task, the problem of vitamin nutrition can be completely solved, since the absorption of vitamins in the human body directly depends on the provision of biologically complete protein. Instead, the generalization of domestic literature sources that have begun to address this issue shows insufficient attention from scientists who only partially disclose it, encouraging the experimental development of appropriate recommendations.

Based on these considerations, the research aimed to determine the parameters that form beef protein's biological value depending on the bulls' age and breed.

Scientific Hypothesis

The breed and age of bulls can influence the formation of the biological integrity of the longest back muscle, which may deviate from the general trends of its formation in other breeds of the corresponding direction of productivity.

MATERIAL AND METHODOLOGY

Samples

During the growing period (from 30 days to 21 months of age), the experimental animals were fed diets of the same nutritional value, taking into account detailed feeding standards, which provided for 900-1000 g of average daily gain. The level of feeding during the growing period was high. It was designed to identify potential opportunities for increasing meat productivity and achieving a live weight of 550-650 kg by bulls at 18-21 months. The formation of meat productivity of bulls was evaluated at 3, 6, 9, 12, 15, 18, 21 months of age. The control slaughter was performed in the Kharkiv Meat Processing Plant, which met the requirements of DSTU 4673:2006: "Cattle for slaughter. Technical Conditions" **[25]**. The live weight of bulls formed into groups for slaughter corresponded to the average live weight of animals at the end of a certain age period. In order to determine the content of amino acids, samples of the longest muscle of the back were taken from three heads of each breed and

of each age.

Chemicals

All chemicals were purchased from reputable brands on the market and met the highest analytical standards: alcohol solution of ninhydrin (grade A, chemically pure, Private Enterprise "Systema Optimum", Ukraine); Nessler's reagent (TU 6-09-2089-77, Joint Stock Company Kyiv Plant of Reagents, Indicators and Analytical Preparations "RIAL", Ukraine), sulfuric acid, H_2SO_4 (grade A, chemically pure, Limited Liability Company "Khimlaborreactiv", Ukraine), ammonium sulfate, $(NH_4)_2SO_4$ (grade A, chemically pure, Private Joint Stock Company "SUMYKHIMPROM", Ukraine).

Animals, Plants and Biological Materials

The experiment was conducted in the production conditions of the basic farm of the Institute of Animal Science of the NAAS in the Kharkiv region, using purebred bulls of Black-and-White, Red Steppe, and Angler dairy breeds and Simmental, Lebedyn, and Gray Ukrainian dairy breeds of combined productivity. Three groups of 25 bulls each were formed and kept untethered in group sections of the same facility until they were 4 months old. After that, they were tethered until the end of the intensive growing period.

Instruments

The amino acid content was analyzed using ion-exchange liquid column chromatography **[26]**, **[27]**, **[28]** on an automatic amino acid analyzer T-339 M manufactured by Microtechna (Czech Republic) in 100 µL of hydrolyzate with the following sequence of phosphate-buffered amino acid eluates from the column: asparagine, threonine, serine, glutamine, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine.

Laboratory Methods

The selected samples were examined in the production conditions of the laboratory for assessing the quality of feed and products of animal origin, part of the Testing Center of the Institute of Animal Husbandry of the National Academy of Agrarian Sciences of Ukraine certified for technical competence by the National Accreditation Agency of Ukraine, according to the requirements DSTU ISO/IEC 17025:2006 та DSTU EN ISO/IEC 17025:2019 as a basic organisation of the metrological service of the Ministry of Agrarian Policy and Food of Ukraine. The content of amino acids was determined by ion-exchange liquid column chromatography. The amino acid score, calculated according to the H. Mitchell and R. Block method, was considered an indicator of the biological value of beef protein. It shows the ratio of the content of an essential amino acid in the tested protein to its amount in the "ideal" protein **[29]**. The amino acid composition of chicken egg proteins (ideal protein) was taken as the standard for the biological value of protein. Additionally, methods generally accepted in experimental work and laboratory practice were used.

Description of the Experiment

Sample preparation: Samples of the longest back muscle were taken after primary processing of the carcasses, before the start of the cooling process. Samples from each carcass were cut out using a special boning knife. After that, they were freed from the connective tissue, placed in a sterile plastic film bag with a Zip-Lock fastener to prevent air access, and labelled. The samples were delivered to the laboratory in a cooler bag at a temperature of +6 ±2 °C. Protein hydrolysates were prepared in the laboratory to determine the amino acid content. The percentage of essential and nonessential amino acids is the sum of the latter's content; the concentration of each was determined by adding an alcohol solution of ninhydrin (pH 5.8) at 98 °C for 24 hours, followed by photometry of the stained samples. The acids cysteine and tryptophan content were not recorded in the absence of the corresponding standard solutions required for the construction of the calibration curve. The amino acid content was calculated as a percentage of the mass fraction of protein content in samples of the longest back muscle of the studied breeds. The mass fraction of protein was calculated using a conversion factor of 6.25 multiplied by the mass fraction of total nitrogen determined with Nessler's reagent after wet ashing the material in sulfuric acid by the methods specified in GOST 766-85 and expressed as a percentage of the mass fraction of the longest back muscle samples of bulls. Ammonium sulfate was used as a standard solution to prepare the calibration curve to determine total nitrogen content. The ratio of essential and nonessential amino acids is calculated by the amino acid index.

Number of samples analyzed: A total of 126 samples of the longest back muscle have been examined.

Number of repeated analyses: The experiments were repeated on six breeds, with the experimental data subjected to mathematical statistical analysis.

Number of experiment replication: The experiments were carried out in 7 age periods of bulls' growth with three samples taken from each breed, resulting in 21 repeated analyses.

Design of the experiment: A scientific and economic experiment was conducted in the first stage. The animals were provided with the feed of our production. Then we performed a controlled slaughter of bulls from six cattle breeds at the age of 3, 6, 9, 12, 15, 18, and 21 months, during which three samples of the longest back

muscle were taken from each carcass of the corresponding breed and age at the level of 9-12 ribs. Subsequently, the samples were delivered to the laboratory to determine the amino acid content. The final stage involved summarising the results, subjecting them to statistical analysis, and testing the validity of the hypothesis.

Statistical Analysis

The obtained results within the studied breeds and relevant age periods were processed by methods of variation statistics using the software package for analysis of variance (ANOVA) StatPlus 5 (6.7.0.3) (AnalystSoft Inc., USA). Results are presented as mean ± standard error (x ±SE). Tukey's test was used to compare the difference in mean values between groups, where differences were considered statistically significant at $p < 0.05$ for all data.

RESULTS AND DISCUSSION

Most scientific publications have convincingly demonstrated the significant advantage of using animal protein in the human diet, which is characterised by a high balance of essential and non-essential amino acids closest to human amino acid composition **[30]**. Beef contains a wide range of amino acids **[31]**, which influence its taste **[32]**, play a key role in its quality, depend directly on the breed factor **[33]** and are determined by the age of the animals **[34]**. They are one of the main components of meat **[35]**. However, Besung et al. **[36]** found no significant differences when comparing Bali and Wagyu beef. The picture is somewhat different for Japanese black cattle **[37]**. In this respect, the work of Nogi et al. **[38]** is interesting. The contradictions between the differences in amino acid content between breeds and the scientific justification for the time of slaughter became the basis for separate studies.

It is worth noting that the general pattern for all breeds is a slight age-related increase in the amino acid score of beef proteins from 3 to 6 months of age for lysine from 0.75 to 0.89% (+0.14%), methionine – from 0.36 to 0.47% (+0.11%), threonine – from 0.63 to 0.73% (+0.16%), isoleucine – from 0.39 to 0.45% (+0. 06%), leucine – from 0.53 to 0.58% (+0.05%), phenylalanine – from 0.51 to 0.59% (+0.08%), arginine – from 0.53 to 0.58% (+0.05%), histidine – from 1.06 to 1.22% (+0. 16%), valine – from 0.42 to 0.49% (0.07%) against the background of an increase in the total score – from 5.18 to 5.99% (+0.81%) and amino acid index – from 0.58 to 0.67% (0.09%). The results of the experiment are shown in Table 1.

Breed and age differences in beef in most amino acid scores were not statistically significant. Nevertheless, the difference between Lebedyn and Angler, Gray Ukrainian, Simmental, and Black-and-White breeds at 3 months of age became significant at $p < 0.05$, as well as the difference between Gray Ukrainian, Angler and Simmental breeds at $p < 0.05$. With the increase in the age of bulls to 6 months, the most significant changes were noted in the threonine amino acid between the Black-and-White and Red Steppe, Angler, Lebedyn, and Simmental breeds ($p < 0.01$). The highest statistical significance ($p < 0.001$) was achieved for the essential amino acid histidine rate between the Black-and-White, Simmental, Angler, and Lebedyn breeds. The data obtained extend the results regarding the impact of breed factor on beef quality **[39]**.

A comparison of the average data for beef samples with the standard indicates an increase in the total amount of amino acids, with a statistically significant difference between the Angler and Black-and-White, Simmental breeds ($p < 0.05$) and between the Black-and-White and Simmental breeds ($p < 0.01$). The amino acid index values calculated for samples taken from bulls at 3 and 6 months did not reveal any significant differences between the breeds.

The formation of the fullness of proteins of the pulp of bull carcasses in the period from 9 to 12 months of age was accompanied by an increase in the scores for methionine from 0.50 to 0.65% (+0.15%), isoleucine - from 0.46 to 0.61% (+0.15%), leucine – from 0.62 to 0.67% (+0.05%), phenylalanine – from 0.61 to 70% (+0.09%), arginine – from 0.75 to 1.03% (+0.28%), histidine – from 1.54 to 1.74% (+0.20%), valine – from 0.51 to 0.59% (0.08%) against the background of a decrease in the average values of amino acid scores for lysine – from 1.05 to 1.04% (-0.01%) and threonine – from 0.97 to 0.61% (-0.36%). Nevertheless, the total sum of the scores of all essential amino acids increased by 0.80% during this age period, which cannot be reliably stated about the amino acid index, which increased by only 0.09%.

At the same time, the difference in the value of the histidine amino acid in the beef of bulls of 9 months of age between the Lebedyn and Black-and-White, Simmental, Angler, Gray Ukrainian breeds was the most prominent and statistically significant ($p < 0.01$) than the rest of the parameters of its evaluation, where it was $p < 0.05$ for the amino acid lysine between Lebedyn and Angler breeds, $p < 0.05$ for isoleucine between Gray Ukrainian and Simmental, Black-and-White breeds, $p < 0.05$ for the leucine between Gray Ukrainian and Simmental, Black-and-White breeds.

Table 1 Formation of the fullness of carcass pulp proteins in ontogeny chicken egg protein, %.

Chicken egg protein (standard)	Breed						On average by breeds
	Lebedyn	Black-and-White	Red Steppe	Simmental	Angler	Gray Ukrainian	
3 months							
Lysine – 6.9	0.72 ±0.01^{a}	0.75 ±0.02^{a}	0.73 ±0.01^{a}	0.79 ±0.03^{a}	0.78 ±0.02^{a}	0.73 ±0.01^{a}	0.75 ±0.02
Methionine – 3.3	0.35 ±0.04^{a}	0.30 ±0.03^{a}	0.38 ±0.05^{a}	0.31 ±0.03^{a}	0.44 ±0.07^{a}	0.35 ±0.04^{a}	0.36 ±0.04
Threonine – 5.0	0.72 ±0.02^{a}	0.55 ±0.01^{b}	0.68 ±0.02^{ab}	0.57 ±0.03^{b}	0.64 ±0.02^{b}	0.64 ±0.02^{b}	0.63 ±0.02
Isoleucine – 6.9	0.41 ±0.01^{a}	0.37 ±0.02^{a}	0.40 ±0.01^{a}	0.36 ±0.03^{a}	0.45 ±0.03^{a}	0.35 ±0.02^{a}	0.39 ±0.02
Leucine – 9.4	0.55 ±0.03^{a}	0.49 ±0.02^{a}	0.53 ±0.03^{a}	0.49 ±0.02^{a}	0.58 ±0.04^{a}	0.51 ±0.03^{a}	0.53 ±0.03
Phenylalanine – 5.8	0.49 ±0.02^{a}	0.52 ±0.03^{a}	0.52 ±0.02^{a}	0.48 ±0.01^{a}	0.55 ±0.03^{a}	0.50 ±0.01^{a}	0.51 ±0.02
Arginine – 6.7	0.50 ±0.03^{a}	0.54 ±0.04^{a}	0.52 ±0.04^{a}	0.54 ±0.02^{a}	0.54 ±0.03^{a}	0.55 ±0.04^{a}	0.53 ±0.03
Histidine – 2.4	1.10 ±0.06^{ab}	1.08 ±0.05^{ab}	1.16 ±0.02^{ab}	0.83 ±0.01^{a}	0.97 ±0.02^{a}	1.23 ±0.07^{b}	1.06 ±0.04
Valine – 7.4	0.41 ±0.02^{a}	0.42 ±0.01^{a}	0.43 ±0.03^{a}	0.40 ±0.04^{a}	0.43 ±0.06^{a}	0.43 ±0.04^{a}	0.42 ±0.03
Total amount of amino acids – 9.0	5.25 ±0.07^{a}	5.02 ±0.05^{a}	5.35 ±0.06^{a}	4.77 ±0.02^{a}	5.38 ±0.08^{a}	5.29 ±0.04^{a}	5.18 ±0.05
Amino acid index – 1.0	0.58 ±0.01^{a}	0.56 ±0.02^{a}	0.59 ±0.02^{a}	0.53 ±0.03^{a}	0.60 ±0.04^{a}	0.59 ±0.03^{a}	0.58 ±0.03
6 months							
Lysine – 6.9	0.92 ±0.03^{a}	0.88 ±0.01^{a}	0.92 ±0.03^{a}	0.85 ±0.02^{a}	0.91 ±0.03^{a}	0.84 ±0.02^{a}	0.89 ±0.02
Methionine – 3.3	0.43 ±0.06^{a}	0.54 ±0.01^{a}	0.51 ±0.09^{a}	0.44 ±0.07^{a}	0.42 ±0.06^{a}	0.48 ±0.08^{a}	0.47 ±0.06
Threonine – 5.0	0.69 ±0.03^{a}	0.85 ±0.02^{b}	0.70 ±0.01^{a}	0.66 ±0.04^{a}	0.70 ±0.02^{a}	0.80 ±0.03^{ab}	0.73 ±0.02
Isoleucine – 6.9	0.45 ±0.03^{a}	0.41 ±0.01^{a}	0.45 ±0.03^{a}	0.44 ±0.02^{a}	0.45 ±0.03^{a}	0.51 ±0.04^{a}	0.45 ±0.03
Leucine – 9.4	0.58 ±0.02^{a}	0.56 ±0.03^{a}	0.58 ±0.03^{a}	0.58 ±0.03^{a}	0.56 ±0.02^{a}	0.61 ±0.05^{a}	0.58 ±0.03
Phenylalanine – 5.8	0.57 ±0.04^{a}	0.60 ±0.03^{a}	0.55 ±0.02^{a}	0.55 ±0.02^{a}	0.69 ±0.05^{a}	0.59 ±0.03^{a}	0.59 ±0.03
Arginine – 6.7	0.61 ±0.01^{a}	0.59 ±0.01^{a}	0.55 ±0.03^{a}	0.55 ±0.03^{a}	0.57 ±0.02^{a}	0.60 ±0.03^{a}	0.58 ±0.02
Histidine – 2.4	1.14 ±0.02^{a}	1.49 ±0.01^{b}	1.11 ±0.02^{ab}	1.34 ±0.03^{a}	1.24 ±0.02^{a}	0.98 ±0.04^{ab}	1.22 ±0.02
Valine – 7.4	0.45 ±0.02^{a}	0.54 ±0.01^{a}	0.46 ±0.05^{a}	0.45 ±0.03^{a}	0.48 ±0.04^{a}	0.54 ±0.06^{a}	0.49 ±0.04
Total amount of amino acids – 9.0	5.84 ±0.05^{abc}	6.46 ±0.07^{a}	5.83 ±0.04^{abc}	5.86 ±0.05^{b}	6.02 ±0.04^{c}	5.95 ±0.07^{adc}	5.99 ±0.05
Amino acid index – 1.0	0.65 ±0.03^{a}	0.72 ±0.04^{a}	0.65 ±0.03^{a}	0.65 ±0.03^{a}	0.67 ±0.02^{a}	0.66 ±0.02^{a}	0.67 ±0.03
9 months							
Lysine – 6.9	1.15 ±0.06^{a}	1.14 ±0.05^{ab}	1.01 ±0.03^{ab}	0.99 ±0.04^{ab}	0.94 ±0.02^{b}	1.07 ±0.04^{ab}	1.05 ±0.04
Methionine – 3.3	0.60 ±0.05^{a}	0.52 ±0.04^{a}	0.51 ±0.05^{a}	0.39 ±0.07^{a}	0.39 ±0.07^{a}	0.57 ±0.02^{a}	0.50 ±0.05
Threonine – 5.0	1.00 ±0.02^{a}	1.02 ±0.02^{a}	1.02 ±0.02^{a}	0.91 ±0.03^{a}	0.96 ±0.04^{a}	0.92 ±0.03^{a}	0.97 ±0.03
Isoleucine – 6.9	0.46 ±0.02^{ab}	0.35 ±0.01^{a}	0.34 ±0.01^{ab}	0.47 ±0.02^{a}	0.54 ±0.03^{ab}	0.60 ±0.04^{b}	0.46 ±0.02
Leucine – 9.4	0.70 ±0.04^{ab}	0.52 ±0.03^{ac}	0.61 ±0.04^{a}	0.55 ±0.02^{b}	0.59 ±0.03^{a}	0.75 ±0.05^{a}	0.62 ±0.04
Phenylalanine – 5.8	0.55 ±0.02^{a}	0.62 ±0.04^{a}	0.65 ±0.03^{a}	0.59 ±0.04^{a}	0.59 ±0.03^{a}	0.64 ±0.03^{a}	0.61 ±0.03
Arginine – 6.7	0.99 ±0.02^{a}	1.01 ±0.04^{a}	0.99 ±0.02^{a}	0.79 ±0.03^{a}	0.82 ±0.03	0.91 ±0.02	0.75 ±0.03
Histidine – 2.4	1.78 ±0.04^{a}	1.63 ±0.03^{b}	1.67 ±0.03^{ab}	1.43 ±0.02^{b}	1,31 ±0.01^{b}	1.44 ±0.02^{b}	1.54 ±0.03
Valine – 7.4	0.53 ±0.01^{a}	0.53 ±0.01^{a}	0.53 ±0.01^{a}	0.47 ±0.02^{a}	0.48 ±0.02^{a}	0.49 ±0.02^{a}	0.51 ±0.02
Total amount of amino acids – 9.0	7.76 ±0.02^{a}	7.34 ±0.03^{bc}	7.33 ±0.03^{b}	6.59 ±0.04^{b}	6.62 ±0.05^{b}	7.39 ±0.02^{b}	7.17 ±0.03
Amino acid index – 1.0	0.86 ±0.02^{a}	0.82 ±0.03^{ab}	0.81 ±0.02^{ab}	0.73 ±0.03^{b}	0.74 ±0.03^{b}	0.82 ±0.03^{ab}	0.80 ±0.03
12 months							
Lysine – 6.9	1.06 ±0.04^{ab}	0.98 ±0.03^{b}	0.91 ±0.02^{b}	1.17 ±0.03^{ac}	1.14 ±0.03^{c}	1.00 ±0.02^{b}	1.04 ±0.03
Methionine – 3.3	0.68 ±0.06^{a}	0.60 ±0.05^{a}	0.61 ±0.04^{a}	0.64 ±0.03^{a}	0.64 ±0.03^{a}	0.74 ±0.08^{a}	0.65 ±0.05
Threonine – 5.0	0.92 ±0.03^{ab}	0.90 ±0.03^{b}	1.02 ±0.04^{ab}	1.04 ±0.04^{a}	0.98 ±0.03^{ab}	0.81 ±0.02^{b}	0.61 ±0.03
Isoleucine – 6.9	0.63 ±0.03^{a}	0.65 ±0.02^{a}	0.54 ±0.01^{a}	0.64 ±0.03^{a}	0.59 ±0.02^{a}	0.61 ±0.02^{a}	0.61 ±0.02
Leucine – 9.4	0.63 ±0.03^{a}	0.62 ±0.03^{a}	0.69 ±0.04^{a}	0.67 ±0.02^{a}	0.63 ±0.03^{a}	0.75 ±0.04^{a}	0.67 ±0.03
Phenylalanine – 5.8	0.72 ±0.03^{a}	0.67 ±0.02^{a}	0.66 ±0.02^{a}	0.72 ±0.04^{a}	0.77 ±0.05^{a}	0.64 ±0.02^{a}	0.70 ±0.03
Arginine – 6.7	1.02 ±0.04^{a}	0.98 ±0.03^{a}	0.94±0.02^{a}	1.11 ±0.05^{a}	1.07 ±0.04^{a}	1.05 ±0.03^{a}	1.03 ±0.04
Histidine – 2.4	1.74 ±0.04^{ab}	1.85 ±0.05^{ab}	1.45 ±0.02^{b}	1.83 ±0.03^{a}	1.71 ±0.02^{b}	1.85 ±0.04^{ab}	1.74 ±0.03
Valine – 7.4	0.60 ±0.02^{a}	0.60 ±0.02^{a}	0.54 ±0.03^{a}	0.60 ±0.04^{a}	0.62 ±0.04^{a}	0.60 ±0.04^{a}	0.59 ±0.03
Total amount of amino acids – 9.0	8.00 ±0.05^{a}	7.81 ±0.04^{a}	7.36 ±0.02^{a}	8.42 ±0.03^{b}	8.15 ±0.02^{c}	8.05 ±0.04^{ac}	7.97 ±0.03
Amino acid index – 1.0	0.89 ±0.01^{abc}	0.87 ±0.01^{a}	0.82 ±0.02^{a}	0.94 ±0.03^{a}	0.91 ±0.01^{bc}	0.89 ±0.02^{abc}	0.89 ±0.02

A distinctive feature of beef samples by the total sum of amino acids scores is a statistically significant increase in its values between Lebedyn and Gray Ukrainian, Black-and-White, Red Steppe, Angler, and Simmental breeds at p <0.001 in all cases of comparison and the constancy of the amino acid index values between Lebedyn and Angler breeds (p <0.05), Simmental breed (p <0.05). A similar pattern of changes has been reported **[40]**.

Table 1 Cont.

Chicken egg protein (standard)	Breed						On average by breeds
	Lebedyn	Black-and-White	Red Steppe	Simmental	Angler	Gray Ukrainian	
15 months							
Lysine – 6.9	0.94 ±0.02^{a}	0.95 ±0.01^{a}	0.87 ±0.03^{ab}	0.83 ±0.03^{b}	0.87 ±0.03^{ab}	0.72 ±0.04^{b}	0.86 ±0.03
Methionine – 3.3	0.51 ±0.03^{a}	0.65 ±0.04^{b}	0.48 ±0.02^{a}	0.51 ±0.03^{a}	0.47 ±0.01^{a}	0.38 ±0.04^{a}	0.50 ±0.04
Threonine – 5.0	0.81 ±0.04^{ab}	0.85 ±0.04^{a}	0.71 ±0.03^{b}	0.73 ±0.02^{ab}	0.71 ±0.02^{ab}	0.58 ±0.01^{b}	0.73 ±0.03
Isoleucine – 6.9	0.45 ±0.02^{a}	0.41 ±0.02^{a}	0.45 ±0.02^{a}	0.51±0.04^{a}	0.41 ±0.02^{a}	0.41 ±0.02^{a}	0.44 ±0.02
Leucine – 9.4	0.54 ±0.02^{a}	0.53 ±0.03^{a}	0.55 ±0.02^{a}	0.56 ±0.04^{a}	0.53 ±0.03^{a}	0.48 ±0.02^{a}	0.53 ±0.03
Phenylalanine – 5.8	0.61 ±0.03^{a}	0.59 ±0.01^{a}	0.43 ±0.02^{b}	0.60 ±0.03^{a}	0.58 ±0.01^{ab}	0.49 ±0.02^{b}	0.55 ±0.02
Arginine – 6.7	0.86 ±0.02^{a}	0.84 ±0.02^{a}	0.82 ±0.01^{a}	0.82 ±0.01^{a}	0.80 ±0.02^{a}	0.66 ±0.03^{a}	0.80 ±0.02
Histidine – 2.4	1.30 ±0.02^{ab}	1.27 ±0.02^{ab}	1.17 ±0.01^{b}	1.37 ±0.03^{a}	1.16 ±0.01^{b}	1.05 ±0.01^{b}	1.22 ±0.02
Valine – 7.4	0.50 ±0.01^{abc}	0.55 ±0.02^{ac}	0.47 ±0.01^{bc}	0.47 ±0.01^{bc}	0.41 ±0.01^{c}	0.39 ±0.02^{ab}	0.47 ±0.01
Total amount of amino acids – 9.0	6.52 ±0.03^{c}	6,64 ±0.02^{ac}	5.95 ±0.02^{b}	6.40 ±0.01^{bc}	5.94 ±0.04^{bc}	5.16 ±0.03^{b}	6.10 ±0.02
Amino acid index – 1.0	0.72 ±0.02^{a}	0.74 ±0.03^{ab}	0.66 ±0.01^{a}	0.71 ±0.03^{ab}	0.66 ±0.01^{ab}	0.57 ±0.01^{b}	0.68 ±0.02
18 months							
Lysine – 6.9	0.89 ±0.02^{ab}	0.86±0.03^{a}	0.84 ±0.01^{ac}	0.94 ±0.04^{ab}	0.92 ±0.03^{ab}	0.98 ±0.01^{b}	0.91 ±0.02
Methionine – 3.3	0.60 ±0.03^{a}	0.54 ±0.04^{a}	0.52 ±0.01^{a}	0.52 ±0.01^{a}	0.59 ±0.03^{a}	0.60 ±0.03^{a}	0.56 ±0.03
Threonine – 5.0	0.80 ±0.03^{ab}	0.75 ±0.02^{a}	0.72 ±0.02^{a}	0.82 ±0.04^{ab}	0.77 ±0.02^{ab}	0.91 ±0.05^{b}	0.80 ±0.03
Isoleucine – 6.9	0.52 ±0.01^{a}	0.50 ±0.02^{a}	0.46 ±0.03^{a}	0.50 ±0.01^{a}	0.51 ±0.01^{a}	0.56 ±0.03^{a}	0.51 ±0.02
Leucine – 9.4	0.57 ±0.02^{a}	0.54 ±0.01^{a}	0.53 ±0.01^{a}	0.58 ±0.02^{ab}	0.59 ±0.02^{ab}	0.68 ±0.03^{b}	0.58 ±0.02
Phenylalanine – 5.8	0.51 ±0.02^{ab}	0.47 ±0.03^{a}	0.50 ±0.01^{a}	0.55 ±0.02^{ab}	0.55 ±0.02^{ab}	0.60 ±0.03^{b}	0.53 ±0.02
Arginine – 6.7	0.69 ±0.01^{a}	0.60 ±0.01^{a}	0.78 ±0.02^{ab}	0.64 ±0.03^{a}	0.77 ±0.02^{ab}	0.83 ±0.04^{b}	0.72 ±0.02
Histidine – 2.4	1.45 ±0.02^{a}	1.40 ±0.02^{a}	1.33 ±0.01^{b}	1.50 ±0.03^{a}	1.46 ±0.02^{a}	1.50 ±0.03^{a}	1.44 ±0.02
Valine – 7.4	0.45 ±0.01^{a}	0.43 ±0.01^{a}	0.43 ±0.01^{a}	0.46 ±0.02^{a}	0.45 ±0.02^{a}	0.51 ±0.04^{a}	0.46 ±0.02
Total amount of amino acids – 9.0	6.46 ±0.05^{ac}	6.03 ±0.03^{a}	6.10 ±0.02^{a}	6.51 ±0.04^{c}	6.59 ±0.06^{a}	7.15 ±0.02^{b}	6.47 ±0.04
Amino acid index – 1.0	0.72 ±0.02^{ab}	0.67 ±0.01^{a}	0.68 ±0.01^{a}	0.72 ±0.02^{ab}	0.73 ±0.02^{ab}	0.80 ±0.03^{b}	0.72 ±0.02
21 months							
Lysine – 6.9	0.93 ±0.03^{a}	0.95 ±0.02^{a}	0.89 ±0.02^{a}	0.88 ±0.02^{a}	0.86 ±0.01^{a}	0.88 ±0.02^{a}	0.90 ±0.02
Methionine – 3.3	0.55 ±0.02^{abc}	0.58 ±0.03^{c}	0.59 ±0.03^{b}	0.48 ±0.02^{bc}	0.45 ±0.01^{a}	0.44 ±0.01^{ab}	0.52 ±0.02
Threonine – 5.0	0.82 ±0.01^{a}	0.83 ±0.01^{a}	0.67 ±0.03^{a}	0.99 ±0.04^{b}	0.84 ±0.01^{b}	0.85 ±0.02^{b}	0.83 ±0.02
Isoleucine – 6.9	0.44 ±0.01^{a}	0.57 ±0.02^{ab}	0.55 ±0.02^{ab}	0.50 ±0.01^{a}	0.64 ±0.03^{ab}	0.61 ±0.03^{b}	0.55 ±0.02
Leucine – 9.4	0.62 ±0.03^{ab}	0.54 ±0.04^{a}	0.56 ±0.04^{a}	0.62 ±0.03^{ab}	0.60 ±0.02^{ab}	0.69 ±0.02^{b}	0.61 ±0.03
Phenylalanine – 5.8	0.63 ±0.02^{ab}	0.68 ±0.02^{a}	0.64 ±0.01^{ab}	0.62 ±0.02^{ab}	0.57 ±0.02^{b}	0.57 ±0.02^{b}	0.62 ±0.02
Arginine – 6.7	0.70 ±0.01^{a}	0.68 ±0.02^{a}	0.71 ±0.01^{a}	0.73 ±0.03^{a}	0.72 ±0.03^{a}	0.71 ±0.01^{a}	0.71 ±0.02
Histidine – 2.4	1.29 ±0.02^{a}	1.53 ±0.04^{ab}	1.58 ±0.03^{b}	1.42 ±0.02^{a}	1.52 ±0.03^{ab}	1.53 ±0.02^{b}	1.48 ±0.03
Valine – 7.4	0.46 ±0.02^{a}	0.46 ±0.02^{a}	0.50 ±0.03^{a}	0.51 ±0.03^{a}	0.44 ±0.02^{a}	0.44 ±0.02^{a}	0.47 ±0.02
Total amount of amino acids – 9.0	7.16 ±0.03^{b}	7.58 ±0.04^{a}	7.43 ±0.03^{b}	7.50 ±0.02^{a}	7.38 ±0.03^{abc}	7.47 ±0.02^{abc}	7.42 ±0.03
Amino acid index – 1.0	0.80 ±0.02^{a}	0.84 ±0.02^{a}	0.83 ±0.01^{a}	0.83 ±0.01^{a}	0.82 ±0.02^{a}	0.83 ±0.02^{a}	0.83 ±0.02

Note: Values are means ±SE; n = 3, different letters indicate significant differences between groups within each row by Tukey's test.

Increase in the amino acid scores for lysine in beef samples from bulls aged 12 months of Simmental and Gray Ukrainian breeds (p <0.01), Black-and-White (p <0.01), Red Steppe (p <0.01), Angler and Gray Ukrainian (p <0.05), Black-and-White (p <0.05), Red Steppe (p <0.01); for threonine – Simmental and Black-and-White breeds (p <0.05), Gray Ukrainian (p <0.01); for histidine – Simmental and Angler (p <0.05), Red Steppe (p <0.001), resulted in a significant increase in the total amount of scores: between Simmental and Angler (p <0.01), Gray Ukrainian (p <0.01), Lebedyn (p <0.01), Black-and-White (p <0.01), Red Steppe (p <0.001), between Angler and Lebedyn (p <0.05), Red Steppe (p <0.001), Black-and-White (p <0.01) and between Simmental and Red Steppe (p <0.05) breeds increased as a result of higher intensity and efficiency of protein synthesis in the muscle tissue of bulls of these breeds.

In turn, the quantitative indicators of amino acid scores in the period from 15 to 18 months of age were also determined by the breed of bulls and varied with age: increased for lysine from 0.86 to 0.91% (+0.05%), methionine – from 0.50 to 0.56% (+0. 06%), threonine – from 0.73 to 0.80% (+0.07), isoleucine – from 0.44 to 0.51% (+0.07%), histidine from 1.22 to 1.44% (+0.22%) and, conversely, decreased for leucine – from 0. 53 to 0.58% (-0.05%), valine – from 0.47 to 0.46% (-0.01%), phenylalanine – from 0.55 to 0.53% (-0.02%); arginine – from 0.80 to 0.72% (-0.08%). The research is consistent with **[41]**.

The breed of bulls also had a decisive influence on the differences in the values of the scores of individual amino acids in the carcass flesh in ontogeny. So far, at 15 months of age, a statistically significant difference in the rate of the amino acid lysine has been proven between the Black-and-White and Simmental breeds (p <0.05), between the Lebedyn and Simmental (p <0.05), and the Gray Ukrainian breeds (p <0.01); amino acid methionine – between Black-and-White and Simmental (p <0.05), Lebedyn (p <0.05), Red Steppe (p <0.05), Angler (p <0.05), Gray Ukrainian (p <0.01); threonine – between Black-and-White and Red Steppe (p <0.05), Gray Ukrainian (p <0.01); phenylalanine – between Simmental and Gray Ukrainian (p <0.05), Red Steppe (p <0.01), Black-and-White and Gray Ukrainian (p <0.05), Red Steppe (p <0.01), between Lebedyn and Red Steppe, Gray Ukrainian (p <0.01); histidine – between Simmental and Red Steppe, Angler, Gray Ukrainian (p <0.01), Lebedyn and Red Steppe, Gray Ukrainian (p <0.01); valine – between Black-and-White and Red Steppe, Simmental (p <0.05), Angler and Gray Ukrainian (p <0.01).

Notably, the main trend in the formation of the sum of the majority of amino acid scores related to the breed of the experimental groups was saved, and some of them reached high statistical significance. In particular, between Black-and-White and Simmental, Red Steppe, Angler, and Gray Ukrainian (p <0.001), between Lebedinsky and Red Steppe, Gray Ukrainian (p <0.001). Despite this, the highest values of the amino acid index were inherent in samples taken from bulls of the Black-and-White breed, which exceeded the Gray Ukrainian breed at the level of p <0.01. Lebedyn and Red Steppe breeds dominated the Gray Ukrainian breed by this indicator, at p <0.01 in both cases of comparison. The identified differences between breeds and age periods of bulls complement the materials obtained in **[42]**.

It is worth noting that the maximum amplitude of fluctuations in the values of amino acid scores for lysine in the studied samples of beef from 18-month-old bulls allowed to prove a significant difference between Gray Ukrainian and Black-and-White (p <0.05), Red Steppe breeds (p <0.001); threonine - between Gray Ukrainian and Black-and-White (p <0.05), Red Steppe breeds (p <0.05); leucine - between Gray Ukrainian and Black-and-White (p <0.05), Red Steppe (p <0.05), between Gray Ukrainian and Lebedyn (p <0.05), Black-and-White (p <0.05), Red Steppe breeds (p <0.05); phenylalanine - between Gray Ukrainian and Black-and-White (p <0.05), Red Steppe breeds (p <0.05); arginine - between Gray Ukrainian and Lebedyn (p <0.05), Simmental (p <0.05), Black-and-White breeds (p <0.01); histidine – between Gray Ukrainian and Red Steppe (p <0.01), Simmental and Red Steppe (p <0.01), Angler and Red Steppe (p <0.01), Lebedyn and Red Steppe (p <0.01), Black-and-White and Red Steppe (p <0.01).

The breed of bulls largely determined the increase in the total amount of amino acid scores between the Ukrainian Grey and Angler (p <0.001), Simmental (p <0.001), Lebedyn (p <0.001), Red Steppe (p <0.001), Black-and-White (p <0.001) breeds, as well as between Simmental and Red Steppe (p <0.001), Black-and-White (p <0.001) breeds. Despite the statistically significant differences between breeds for the corresponding indicator, the highest biological value is inherent in the samples of Gray Ukrainian beef, which in terms of amino acid index prevailed over both Red Steppe and Black-and-White breeds at an identical level of significance p <0.05. The dependence of the amino acid composition of beef on the breed factor has been established **[43]**.

Regarding the difference in the values of amino acid scores in the period from 15 to 21 months of age, an increase in lysine from 0.86 to 0.90% (+0.04%), methionine from 0.50 to 0.52% (+0.02%), threonine from 0.73 to 0.83% (+0.10%), isoleucine – from 0.44 to 0.55% (+0.11%), leucine – from 0.53 to 0.61% (+0.08%), phenylalanine – from 0.55 to 0.62% (+0.07%), histidine – from 1.22 to 1.48% (+0.26%) with a simultaneous decrease in arginine – from 0.80 to 0.71% (-0.09%) is observed. Beef contained the same score of the amino acid valine compared to chicken egg protein.

A characteristic interbreed difference in the rate of the amino acid methionine is inherent in the beef samples from 21-month-old bulls. However, the results were not significant or stable, reaching the highest level of significance between the Red Steppe and Simmental breeds up to p <0.05, Angler – p <0.05, Gray Ukrainian – p <0.01, as well as between Black-and-White and Angler – p <0.05, and Gray Ukrainian – p <0.05. The leading position in the threonine amino acid score was occupied by Simmental beef samples, where its values were higher than those of Ukrainian Grey, Angler, Black-and-White, and Lebedyn breeds at the same level of significance (p <0.05), and the same indicator in Simmental samples was also better than in samples of the Red Steppe breed (p <0.01). In addition, by the values of the amino acid isoleucine, a significant difference was found only between the Gray Ukrainian and Simmental (p <0.05), Lebedyn (p <0.01) breeds; by the amino acid leucine - between the Gray Ukrainian and Red Steppe (p <0.05), Black-and-White (p <0.05); in terms of the amino acid phenylalanine – between Black-and-White, Angler and Ukrainian Grey breeds (p <0.05); in terms of the amino acid histidine – between Red Steppe and Simmental (p <0.05), Lebedyn breeds (p <0.01), Ukrainian Grey and Simmental (p <0.05), Lebedyn breeds (p <0.01). The identified features ultimately determined the changes in the value of the total sum of all amino acid scores. Comparing the biological value of beef scores with chicken egg protein, it can be argued that in the final period of the experiment, according to the total amount of points obtained, samples

of the Black-and-White breed significantly exceeded samples of the Red Steppe (p <0.05), Angler (p <0.05), Lebedyn (p <0.01), Simmental and Angler (p <0.05), and Lebedyn (p <0.001) breeds. A higher total score of samples of the Gray Ukrainian breed caused significant differences in beef samples from Lebedyn bulls (p <0.01). The synchronous pattern of increasing the total protein complex of short-chain amino acids in the samples of Angler breed beef was also observed concerning the samples of the Lebedyn (p <0.05). However, it should be emphasised that there were no statistical differences between the values of the amino acid index in any of the breeds. Our results are consistent with those of the research **[44]**.

Beef obtained from bulls of 21 months of age was characterised by a rather high biological value compared to 3 months of age due to an increase in the rates of amino acids by methionine by 0.16%, isoleucine by 0.16%, histidine by 0.42%, arginine – by 0.18%, threonine – by 0.20%, valine – by 0.05%, leucine – by 0.08%, phenylalanine – by 0.11% and lysine – by 0.15%, which, in turn, ensures its potential ability to be sold in the retail network without restrictions of higher quality.

The revealed nonlinear trend in changes in the beef protein completeness index and the total amount of amino acids is determined by the peculiarities of the formation of the fractional composition of proteins, and muscle and connective tissue at different stages of ontogenesis.

Thus, based on the results obtained, the rational slaughter age for bulls of the breeds studied can be considered to be 18-21 months, while for veal production it is 12 months, but the data obtained should first be consistent with the regulated norm of live weight of animals for the corresponding age period.

The dependence of the biological value of meat on the age of animals is consistent with the findings of Kim et al. **[13]** and Kodani et al. **[14]**. In addition to age, the biological value of beef is determined by the content of certain amino acids in different anatomical parts of bull carcasses. In particular, Cho et al. **[45]**, when analysing samples taken from different parts of the carcasses of 10 Hanwoo bulls at 24 months of age, the highest content of glutamine and alanine was found, and slightly lower content of arginine, phenylalanine, and lysine. This indicates the high biological value of beef regardless of the sampling location. Additional examples of meat quality formation depending on the animal breed can be found in work Bischof et al. **[15]**. A similar result was obtained in a study Vopálenský et al. **[46]** conducted on eight groups of beef cattle breeds of the same age, where scientists demonstrated a number of interbreed differences between the values of non-essential and essential amino acids in the longest back muscle. Among the essential amino acids, lysine has the highest content, while methionine has the lowest. Among the non-essential amino acids, glutamic acid predominated, with serine being the least abundant. The amino acid composition of beef also depends on different parts of the carcass. A comparison of different parts of beef within the same breed **[47]** showed that the most common amino acids in their composition (in descending order) were glutamic acid, aspartic acid, lysine, leucine, cysteine, arginine, glycine, and phenylalanine. However, histidine and methionine were detected in much smaller amounts and their quantitative values were almost the same regardless of the part of the carcass.

Other scientists confirmed our conclusion in their studies **[16]**. A similar issue is studied in the paper Hollo et al. **[17]**. When studying the amino acid composition of beef, Christensen et al. **[33]** also proved the breed dependence of the content of certain amino acids in black cattle concerning Hanwoo and Wagyu breeds.

As part of the solution to the problem of providing the population with high-quality food products that meet medical standards and developing the concept of managing the production of safe products for consumption, further research should be conducted in the area of developing a general methodology for assessing the fatty acid composition of beef, taking into account the breed and age characteristics of animals, milk quality depending on the technological conditions of productive animals. A key component in solving this problem is the technology of keeping and feeding animals, feed quality **[48]**, **[49]**, **[50]** as well as veterinary welfare **[51]**, **[52]**, **[53]**. The availability of systematic research on the combined assessment of the biological value of proteins and the lipid composition of beef will allow the development of technologies for its intensive production based on individual breeds, thereby improving product quality while reducing economic energy and feed costs.

CONCLUSION

The balance of beef protein in terms of amino acid composition is crucial for predicting the protein completeness of new products. The obtained breed and age characteristics of the formation of the biological value of beef protein made it possible to determine its compliance with the human body's physiological needs, which is a prospect for expanding the range of food products with a balanced amino acid composition. The age of bulls is a limiting factor in the formation of the biological value of beef. With an increase in age from 3 to 21 months of intensively reared bulls of dairy and combined breeds, there is a gradual increase in the biological value of proteins, which is confirmed by the values of the amino acid index, but its formation is not straightforward. The presence of the first peak increase in the average values of the amino acid index in beef from bulls at 12 months of age (089%), followed by a steady decrease to 15 months (0.68%) and a repeated increase in the

values in animals at 21 months (0.83%) was noted. Beef obtained from bulls of 21 months of age was characterised by a fairly high biological value compared to 3 months of age due to an increase in the rates of amino acid values for methionine by 0.16%, isoleucine – by 0. 16%, histidine – by 0.42%, arginine – by 0.18%, threonine – by 0.20%, valine – by 0.05%, leucine – by 0.08%, phenylalanine – by 0.11% and lysine – by 0.15%. This, in turn, ensures its potential ability to be sold in the retail network without restrictions on higher grades. The bull breed also had a significant influence on the differences in the values of the scores of individual amino acids in carcass flesh during ontogeny. However, the most significant differences between the breeds studied were manifested within a single age period. Since there is still no clear justification for the optimal slaughter age of young cattle of different productivity directions bred in Ukrainian farms, the results suggest that the rational age for slaughtering bulls of the studied breeds is 18-21 months. In comparison, for veal production it is 12 months. However, the obtained data should first be consistent with the regulated norm of the live weight of animals for the corresponding age period.

REFERENCES

1. Paliy, A., Nanka, A., Marchenko, M., Bredykhin, V., Paliy, A., Negreba, J., Lazorenko, L., Panasenko, A., Rybachuk, Z., & Musiienko, O. (2020). Establishing changes in the technical parameters of nipple rubber for milking machines and their impact on operational characteristics. In Eastern-European Journal of Enterprise Technologies (Vol. 2, Issue 1 (104), pp. 78–87). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2020.200635
2. Korkh, I. V., Boiko, N. V., Pomitun, I. A., Paliy, A. P., Pavlichenko, O. V., Negreba, Y. V., Rysovanyi, V. I., & Siabro, A. S. (2023). The impact of environmental temperature on ewe reproduction, adaptive responses during insemination, and productive characteristics of the lambs obtained from them. In Regulatory Mechanisms in Biosystems (Vol. 14, Issue 3, pp. 358–364). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/10.15421/022353
3. Rodionova, K., Khimych, M., & Paliy, A. (2021). Veterinary and sanitary assessment and disinfection of refrigerator chambers of meat processing enterprises. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 616–626). HACCP Consulting. https://doi.org/10.5219/1628
4. Paredi, G., Sentandreu, M.-A., Mozzarelli, A., Fadda, S., Hollung, K., & de Almeida, A. M. (2013). Muscle and meat: New horizons and applications for proteomics on a farm to fork perspective. In Journal of Proteomics (Vol. 88, pp. 58–82). Elsevier BV. https://doi.org/10.1016/j.jprot.2013.01.029
5. Zhang, W., Xiao, S., Samaraweera, H., Lee, E. J., & Ahn, D. U. (2010). Improving functional value of meat products. In Meat Science (Vol. 86, Issue 1, pp. 15–31). Elsevier BV. https://doi.org/10.1016/j.meatsci.2010.04.018
6. Wyness, L. (n.d.). Nutritional aspects of red meat in the diet. In J. Wood (Ed.), Nutrition and Climate Change: Major issues confronting the meat industry (pp. 1–22). Nottingham University Press. https://doi.org/10.7313/upo9781908062413.002
7. Cobos, Á., & Díaz, O. (2015). Chemical Composition of Meat and Meat Products. In Handbook of Food Chemistry (pp. 471–510). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-36605-5_6
8. Sá, A. G. A., Moreno, Y. M. F., & Carciofi, B. A. M. (2019). Food processing for the improvement of plant proteins digestibility. In Critical Reviews in Food Science and Nutrition (Vol. 60, Issue 20, pp. 3367–3386). Informa UK Limited. https://doi.org/10.1080/10408398.2019.1688249
9. Simon Sarkadi, L. (2019). Amino acids and biogenic amines as food quality factors. In Pure and Applied Chemistry (Vol. 91, Issue 2, pp. 289–300). Walter de Gruyter GmbH. https://doi.org/10.1515/pac-2018-0709
10. Scollan, N. D., Costa, P., Hallett, K. G., Nute, G. R., Wood, J. D., & Richardson, R. I. (2017). The fatty acid composition of muscle fat and relationships to meat quality in Charolais steers: influence of level of red clover in the diet. In Proceedings of the British Society of Animal Science (Vol. 2006, pp. 23). Elsevier BV. https://doi.org/10.1017/S1752756200017002
11. Yaremchuk, O. S., Razanova, O. P., Skoromna, O. I., Chudak, R. A., Holubenko, T. L., & Kravchenko, O. O. (2022). Post-slaughter indicators of meat productivity and chemical composition of the muscular tissues of bulls receiving corrective diet with protein-vitamin premix. In Regulatory Mechanisms in Biosystems (Vol. 13, Issue 3, pp. 219–224). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/022228
12. Farionik, T. V., Yaremchuk, O. S., Razanova, O. P., Ohorodnichuk, G. M., Holubenko, T. L., & Glavatchuk, V. A. (2023). Effects of mineral supplementation on qualitative beef parameters. In Regulatory Mechanisms in Biosystems (Vol. 14, Issue 1, pp. 64–69). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/022310

13. Kim, Y. H. B., Kemp, R., & Samuelsson, L. M. (2016). Effects of dry-aging on meat quality attributes and metabolite profiles of beef loins. In Meat Science (Vol. 111, pp. 168–176). Elsevier BV. https://doi.org/10.1016/j.meatsci.2015.09.008
14. Kodani, Y., Miyakawa, T., Komatsu, T., & Tanokura, M. (2017). NMR-based metabolomics for simultaneously evaluating multiple determinants of primary beef quality in Japanese Black cattle. In Scientific Reports (Vol. 7, Issue 1, pp. 1297–1310). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-017-01272-8
15. Bischof, G., Witte, F., Terjung, N., Januschewski, E., Heinz, V., Juadjur, A., & Gibis, M. (2022). Effect of sampling position in fresh, dry-aged and wet-aged beef from M. Longissimus dorsi of Simmental cattle analyzed by 1H NMR spectroscopy. In Food Research International (Vol. 156, Issue 4, p. 111334). Elsevier BV. https://doi.org/10.1016/j.foodres.2022.111334
16. Razanova, O. P., Holubenko, T. L., Bernyk, I. M., Novgorodska, N. M., & Solomon, A. M. (2023). Biological value of veal obtained from bulls of different breed origin and grown using dairy and meat breeding technology. In Bulletin of Sumy National Agrarian University. The Series: Livestock, (Vol. 3, pp. 55–62). Publishing House Helvetica (Publications). https://doi.org/10.32782/bsnau.lvst.2023.3.8
17. Hollo, G., Nuernberg, K., Hollo, I., Csapo, J. Seregi, J., Repa, I., & Ender, K. (2007). Effect of feeding on the composition of longissmus muscle of Hungarian Grey and Holstein Friesian bulls. III. Amino acid composition and mineral content. In Archiv fur Tierzucht (Vol. 50, Isssue 6, pp. 575–586). Copernicus GmbH. https://doi.org/10.5194/aab-50-575-2007
18. Šubrt, J., Kráčmar, S., & Diviš, V. (2002). The profile of amino acids in intramuscular protein of bulls of milked and beef commercial types. In Czech Journal of Animal Science (Vol. 47, Issue 1, pp. 21–29). Czech Academy of Agricultural Sciences.
19. Picard, B., Jurie, C., Bauchart, D., Dransfield, E., Ouali, A., Martin, J. F., Jailler, R., Lepetit, J., & Culioli, J. (2007). Muscle and meat characteristics from the main beef breeds of the Massif Central. In Sciences Des Aliments (Vol. 27, Issue 2, pp. 168–180). Lavoisier. https://doi.org/10.3166/sda.27.168-180
20. Lidder, P., & Sonnino, A. (2012). Biotechnologies for the management of genetic resources for food and agriculture. In Advances in Genetics (Vol. 78, pp. 1–167). Elsevier BV. https://doi.org/10.1016/B978-0-12-394394-1.00001-8
21. Li, L. Q., Tian, W. Q., & Zan, K. S. (2011). Effects of age on quality of beef from Qinchuan Cattle Carcass. In Agricultural Sciences in China (Vol. 10, Issue 11, pp. 1765–1771). Elsevier BV. https://doi.org/10.1016/S1671-2927(11)60176-4
22. Marinangeli, C. P., & House, J. D. (2017). Potential impact of the digestible indispensable amino acid score as a measure of protein quality on dietary regulations and health. In Nutrition Reviews (Vol. 75, Issue 8, pp. 658–667). Oxford University Press (OUP). https://doi.org/10.1093/nutrit/nux025
23. Nalyvayko, L., Rodionova, K., Pankova, S., Shomina, N., Katerynych, O., & Khimych, M. (2021). Comparative characteristics of eggs of chickens of domestic and foreign selection in their diverse age. Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 245–253). HACCP Consulting. https://doi.org/10.5219/1501
24. Caire-Juvera, G., Vázquez-Ortiz, F. A., & Grijalva-Haro, M. I. (2013). Amino acid composition, score and in vitro protein digestibility of foods commonly consumed in norhwest Mexico. In Nutricion Hospitalaria (Vol. 28, Issue 2, pp. 365–371). Arán Ediciones, S. L. https://doi.org/10.3305/nh.2013.28.2.6219
25. DSTU 4673:2006. Cattle for slaughter. General specifications. Quality management systems – Requirements.
26. Csapó, J., Albert, Cs., Lóki, K., & Csapó-Kiss, Zs. (2008). Separation and determination of the amino acids by ion exchange column chromatography applying post-column derivatization. In Acta Universitasis Sapientae (Vol. 1, pp. 5–29). Sapientia Hungarian University of Transylvania.
27. Karlsson, E., & Hirsh, I. (2011). Ion exchange chromatography. In Methods Biochem Anal (Vol. 54, pp. 93–133). Wiley. https://doi.org/10.1002/9780470939932.ch4
28. Cummins, P. M., Rochfort, K. D., & O'Connor, B. F. (2016). Ion-Exchange Chromatography: Basic Principles and Application. In Methods in Molecular Biology (pp. 209–223). Springer New York. https://doi.org/10.1007/978-1-4939-6412-3_11
29. Holubenko, T. L. (2018). Nutritional value of veal used in baby food production. In Ukrainian Journal of Ecology (Vol. 8, Issue 1, pp. 637–643). Alex Matsyura Publishing.
30. Bal-Prylypko, L., Yancheva, M., Paska, M., Ryabovol, M., Nikolaenko, M., Israelian, V., Pylypchuk, O., Tverezovska, N., Kushnir, Y., & Nazarenko, M. (2022). The study of the intensification of technological parameters of the sausage production process. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 27–41). HACCP Consulting. https://doi.org/10.5219/1712

31. Sengor, G. F., Gun, H., & Kalatoglu, H. (2008). Determination of the amino acid and chemical composition of canned smoked mussels (Mytilus galloprovincialis, L.). In Turkish Journal of Veterinary & Animal Sciences (Vol. 32, Issue 1, pp. 1–5). TUBITAK.
32. Genchev, A., Mihaylova, G., Ribarski, S., Pavlov, A., & Kabakchiev, M. (2008). Meat quality and composition in japanese quails. In Trakia Journal of Sciences (Vol. 6, Issue 4, pp. 72–82). Trakia University Press.
33. Christensen, M., Ertbjerg, P., Failla, S., Sañudo, C., Richardson, R. I., Nute, G. R., Olleta, Panea, J. L. B., Albertí, P., Juárez, M., Hocquette, J. F., & Williams, J. L. (2011). Relationship between collagen characteristics, lipid content and raw and cooked texture of meat from young bulls of fifteen European breeds. In Meat Science (Vol. 87, Issue 1, pp. 61–65). Elsevier BV. https://doi.org/10.1016/j.meatsci.2010.09.003
34. Watanabe, A., Ueda, Y., & Higuchi, M. (2004). Effects of slaughter age on the levels of free amino acids and dipeptides in fattening cattle. In Animal Science Journal (Vol. 75, Issue 4, pp. 361–367). Wiley. https://doi.org/10.1111/j.1740-0929.2004.00198.x
35. Cuvelier, C., Clinquart, A., Hocquette, J. F., Cabaraux, J. F., Dufrasne, I., Istasse, L., & Hornick, J. L. (2006). Comparison of composition and quality traits of meat from young finishing bulls from Belgian Blue, Limousin and Aberdeen Angus breeds. In Meat Science (Vol. 74, Issue 3, pp. 522–531). Elsevier BV. https://doi.org/10.1016/j.meatsci.2006.04.032
36. Besung, I. N. K., Rasdianah, I., Suardana, W., & Suwiti, N. K. (2019). Amino acids on Bali cattle and Wagyu beef based on different function of muscle. In Jurnal Veteriner (Vol. 20, Issue 2, pp. 228–233). Universitas Udayana.
37. Inoue, K., Kobayashi, M., Shoji, N., & Kato, K. (2011). Genetic param-eters for fatty acid composition and feed efficiency traits in Japanese Black cattle. In Animal (Vol. 5, Issue. 7, pp. 987–999). Elsevier BV. https://doi.org/10.1017/S1751731111000012
38. Nogi, T., Honda, T., Mukai, F., Okagaki, T., & Oyama, K. (2011). Heritabilities and genetic correlations of fatty acid compositions inlongissimus muscle lipid with carcass traits in Japanese Black cattle. In Journal of Animal Science (Vol. 89, Issue 3, pp. 615–621). Oxford University Press (OUP). https://doi.org/10.2527/jas.2009-2300
39. Hollo, G., Csapo, J., Szues, E., Tozser, J., Repa, I., & Hollo, I. (2001). Influence of breed, slaughter weight and gender on chemical composition of beef. Part 1. Aminoacid profile and biological value of proteins. In Asian-Australasian Journal of Animal Sciences (Vol. 14, Issue 11, pp. 1555–1559). Asian Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.2001.1555
40. Lee, S. H., Kim, C. N., Ko, K. B., Park, S. P., Kim, H. K., Kim, J. M., & Ryu, Y. C. (2019). Comparisons of beef fatty acid and amino acid characteristics between Jeju Black cattle, Hanwoo, and Wagyu breeds. In Food Science of Animal Resources (Vol. 39, Issue 3, pp. 402–409). Korean Society for Food Science of Animal Resources. https://doi.org/10.5851/kosfa.2019.e33
41. Jayasena, D. D., Ahn, D. U., Nam, K. C., & Jo, C. (2013). Factors affecting cooked chicken meat flavour: A review. In World's Poultry Science Journal (Vol. 69, Issue 3, pp. 515–526). Informa UK Limited. https://doi.org/10.1017/S0043933913000548
42. Koutsidis, G., Elmore, J. S., Oruna-Concha, M. J., Campo, M. M., Wood, J. D., & Mottram, D. S. (2008). Water-soluble precursors of beef flavour: I. Effect of diet and breed. In Meat Science (Vol. 79, Issue 1, pp. 124–130). Elsevier BV. https://doi.org/10.1016/j.meatsci.2007.08.008
43. Cho, S., Seol, K., Kang, S., Kim, Y., Seo, H., Lee, W., Kim, J., & Van Ba, H. (2020). Comparison of tastes-related components and eating quality between hanwoo steer and cow longissimus thoracis muscles. In Food Science of Animal Resource (Vol. 40, Issue 6, pp. 908–923). Korean Society for Food Science of Animal Resources. https://doi.org/10.5851/kosfa.2020.e58
44. Jeong, D., Oh, M. R., Seong, P. N., Cho, S., Kang, G. H., Kim, J. H., Jeong, S. G., Lee, J. S, & Park, B. Y. (2012). Comparison of meat quality traits, free amino acid and fatty acid on longissimus lumborum muscles from Hanwoo, Holstein and Angus steers, fattened in Korea. In Korean Journal for Food Science of Animal Resources (Vol. 32, Issue 5, pp. 591–597). Korean Society for Food Science of Animal Resources. https://doi.org/10.5851/kosfa.2012.32.5.591
45. Cho, S. H., Kim, J. H., Seong, P. N., Choi, Y. H., Park, B. Y., Lee, Y. J., In, T. S., Chun, S. Y., & Kim, Y. K. (2007). Cholesterol, free amino acid, nucleotide-related compounds, and fatty acid composition of Korean Hanwoo bull beef. In Korean Journal for Food Science of Animal Resources (Vol. 27, Issue 4, pp. 440–449). Korean Society for Food Science of Animal Resources. https://doi.org/10.5851/kosfa.2007.27.4.440
46. Vopálenský, J., Straková, E., Suchý, P., & Šimek, F. (2017). Amino acid levels in muscle tissue of eight meat cattle breeds. In Czech Journal of Animal Science (Vol. 62, Issue 8, pp. 339–346). Czech Academy of Agricultural Sciences. https://doi.org/10.17221/96/2016-CJAS

47. Zainal Samicho, Z., Siti Roha Ab Mutalib, S. R., & Abdullah, N. (2013). Amino acid composition of droughtmaster beef at various beef cuts. In Agricultural Sciences (Vol. 4, Issue 5B, pp. 61–64). Scientific Research Publishing, Inc. https://doi.org/10.4236/as.2013.45B012
48. Masebo, N. T., Marliani, G., Cavallini, D., Accorsi, P. A., Di Pietro, M., Beltrame, A., Gentile, A., & Jacinto, J. G. P. (2023). Health and welfare assessment of beef cattle during the adaptation period in a specialized commercial fattening unit. In Research in Veterinary Science (Vol. 158, pp. 50–55). Elsevier BV. https://doi.org/10.1016/j.rvsc.2023.03.008
49. Orobchenko, O., Kurbatska, O., Paliy, A., & Palii, A. (2023). Toxicological evaluation of feed contaminated with mycotoxins using a luminescent microorganism: Photobacterium phosphoreum. In Veterinarska Stanica (Vol. 54, Issue 2, pp. 147–163). Croatian Veterinary Institute, Zagreb. https://doi.org/10.46419/vs.54.2.7
50. Nanka, O., Shigimaga, V., Paliy, A., Sementsov, V., & Paliy, A. (2018). Development of the system to control milk acidity in the milk pipeline of a milking robot. In Eastern-European Journal of Enterprise Technologies (Vol. 3/9 (93), pp. 27–33). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2018.133159
51. Cooke, A. S., Mullan, S., Morten, C., Hockenhull, J., Le Grice, P., Le Cocq, K., Lee, M. R. F., Cardenas, L. M., & Rivero, M. J. (2023). Comparison of the welfare of beef cattle in housed and grazing systems: hormones, health, and behaviour. In Journal of Agricultural Science (Vol. 161, Issue 3, pp. 450–463). Cambridge University Press (CUP).
52. https://doi.org/10.1017/S0021859623000357
53. Zavgorodnii, A. I., Pozmogova, S. A., Kalashnyk, M. V., Paliy, A. P., Plyuta, L. V., & Palii, A. P. (2021). Etiological factors in triggering non-specific allergic reactions to tuberculin in cattle. In Regulatory Mechanisms in Biosystems (Vol. 12, Issue 2, pp. 228–233). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/022131
54. Nalon, E., Contiero, B., Gottardo, F., & Cozzi, G. (2021). The Welfare of Beef Cattle in the Scientific Literature From 1990 to 2019: A Text Mining Approach. In Frontiers in Veterinary Science (Vol. 7). Frontiers Media SA. https://doi.org/10.3389/fvets.2020.588749
55. Simmonds, R. C. (2017). Bioethics and Animal Use in Programs of Research, Teaching, and Testing. In Management of Animal Care and Use Programs in Research, Education, and Testing (pp. 35–62). CRC Press. https://doi.org/10.1201/9781315152189-4

Funds:

This research received no external funding.

Acknowledgments:

The research was financed by the National Academy of Agrarian Sciences of Ukraine as part of the comprehensive scientific and technical program "Development of innovative production technologies with minimal impact on the environment (Environmentally safe technologies in animal husbandry)", No. 29 (2020-2024).

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

The experiments conducted on animals do not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 "On the Protection of Animals from Cruelty") as amended as of 04.08.2017, and the "General Ethical Principles for Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001) and international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1985) (Simmonds, 2017) **[54]**. The research program was reviewed and approved by the Bioethics Committee of the Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine in accordance with the current procedure, No. 1 (15.01.2020).

Contact Address:

Anatoliy Paliy, National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine", Laboratory of Veterinary Sanitation and Parasitology, Hryhoriia Skovorody St., 83, 61023, Kharkiv, Ukraine,
Tel.: 0662253434
E-mail: paliy.dok@gmail.com
ORCID: http://orcid.org/0000-0002-9193-3548

Stepan Michalchenko, Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, Laboratory for feeding, physiology of feeding of agricultural animals and fodder production, Livestock St., 1 A, 61026, Kharkiv, Ukraine,
Tel.: 0974302560
E-mail: stepanadamovichm@gmail.com
ORCID: https://orcid.org/0009-0007-61/76-3537

Igor Korkh, Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, Laboratory of breeding and technological research in small animal husbandry and horse breeding, Livestock St., 1 A, 61026, Kharkiv, Ukraine,
Tel.: 0954250958
E-mail: dr.fox2011@ukr.net
ORCID: https://orcid.org/0000-0002-7010-1574

***Kateryna Rodionova**, Odesa State Agrarian University, Faculty of Veterinary madicine, Department of infectious pathology, biosafety and veterinary-sanitary inspection named after professor V.Ya. Atamas, Panteleimonovskaya Str., 13, 65012, Odesa, Ukraine,
Tel. 0662486856
E-mail: katerina.rodionova@ukr.net
ORCID: https://orcid.org/0000-0002-7245-4525

Svetlana Tkachuk, National University of Life and Environmental Sciences of Ukraine, Department of Veterinary hygiene named after professor A. K. Skorokhodko, Vystavkova Str., 16, 03041, Kyiv, Ukraine,
Tel.: 0675920900
E-mail: ohdin@ukr.net
ORCID: https://orcid.org/0000-0002-6923-1793

Mariia Khimych, Odesa State Agrarian University, Faculty of Veterinary madicine, Department of infectious pathology, biosafety and veterinary-sanitary inspection named after professor V.Ya. Atamas, Panteleimonovskaya Str., 13, 65012, Odesa, Ukraine,
Tel.: 067-799-21-13
E-mail: khimichms@gmail.com
ORCID: https://orcid.org/0000-0003-2646-3196

Nina Dankevych, Odesa State Agrarian University, Faculty of Veterinary madicine, Department of Surgery, Obstetrics and Diseases of Small Animals, Panteleimonovskaya Str., 13, 65012, Odesa, Ukraine,
Tel.: 066-898-78-31
E-mail: dankevych82@gmail.com
ORCID: https://orcid.org/0000-0001-8927–5219

Nataliia Boiko, Institute of Animal Science of the National Academy of Agrarian Sciences of Ukraine, Laboratory of breeding and technological research in small animal husbandry and horse breeding, Livestock St., 1 A, 61026, Kharkiv,
Ukraine. Tel.: 0661570700
E-mail: nbojko775@gmail.com
ORCID: https://orcid.org/0000-0001-6742-8456

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 18.11.2023
Revised: 2.2.2024
Accepted: 27.9.2024
Published: 7.10.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 847-859
https://doi.org/10.5219/1940
ISSN: 1337-0960 online
www.potravinarstvo.com

Effect of soy flour and flour improvers on nutritional value, texture, colour and sensory characteristics of wafer biscuits

Nazieh Alkhalaileh, Ghadeer Frehat

ABSTRACT

Biscuits are a popular and desirable snack food by consumers. Still, the quality of these products decreases during the storage process, so this study focuses on improving the wafer's quality and nutritional value and reducing their fragility by partially replacing wheat flour with soybean flour in the following proportions: 5% (T1), 10% (T2), 15% (T3), 20% (T4), and adding ammonium carbonate in different quantities .he sensory evaluation results showed that the best wafer treatment was supported with 15% soy flour and 150 g of ammonium carbonate. At the same time, the mean for sensory evaluation is 8.80. Also, the results of the chemical analysis (protein, moisture, fat, carbohydrate, ash, fibre)texture and colour after direct manufacturing and after three months of storage. The result showed a significant increase in the percentage of protein and fat from 6.56±0.30 to 12.19 ± 0.35, 11.56±0.1 to 19.64±0.55, respectively the moisture content was higher in the control was 3.88±0.02 for wafer sample 2.47±0. while fibre was 1.93±0.15, the carbohydrates in the control sample were higher than in other treatments. Texture and colour were improved compared with the control. Conclusions: Commercial-batter biscuits with high nutritional value content and high-quality properties can be prepared by replacing wheat flour with 15% soy flour and adding 150 g of ammonium carbonate.

Keywords: wafer, plant protein, flour, soy flour, wheat

INTRODUCTION

Wafers are snack foods with a sweet taste, crispy texture, and delicate structure that make them acceptable to consumers **[1]**. They are made by mixing wheat flour, fat, sugar, salt, and sodium bicarbonate with water, letting it sit at room temperature for 25 minutes, heating it to 160 and 190 °C, then cooling it **[2]**. Maintaining the appropriate level of crispness of wafer biscuits during the shelf life is one of the challenges faced by wafer biscuit manufacturers **[3]**. The quality of the wafer, its shelf life, its sensory properties (flavour, colour, taste, and texture), and the volume changes that cause the crispiness of the wafer is affected by the properties and qualities. The raw materials used in the manufacture of wafer biscuits **[4]** are represented by the absorption of moisture by the main components used in the manufacture of wafer biscuits, which occurs during the transportation of the product from the factory to the consumer or storage **[5]**. The partial replacement of wheat flour with soy flour and the addition of flour improvers is a method to improve the quality of wafer biscuits, the hydration rate, and the crumb structure **[6]**. It also improves the health benefits as it contains beta-carotene pigment, which acts as an antioxidant **[7]** and improves the nutritional value, as soybeans are a source of high nutritional value protein, fibre, vitamins and minerals **[8]**. Soybeans are rich in polyunsaturated and monounsaturated linoleic and linolenic, essential fatty acids for human health **[9]**. Soybean flour has higher levels of antioxidants than wheat flour, such as ascorbic acid, daidzein, Geniestein, Carnosol, romantic acid, and catechin **[10]**. They contain β-carotene pigment, which increases the batter wafer's ability to absorb water **[11]** improves product elasticity **[12]** and the crust colour of the final product **[13]**, reduces the level of wafer crispness and preserves sensory quality during the shelf life **[14]**

Additionally, roasted soy flour enhances the flavour and texture of the wafer making it softer and low crisper **[15]**. Also, it indicates that adding 7% soybean flour to wafer biscuits improved the colour and reduced viscosity **[16]**.

The researchers noticed a highly significant link between batter viscosity and the water level ($p < 0.05$). Increasing the water level reduces viscosity **[17]**. When the amount of water added to the wafer batter is approximately less than 150% of the flour weight, the completeness of the sheet decreases, resulting in thicker sheets. Using less water (140%) significantly increased the viscosity, causing issues such as problematic batter deposits and incomplete sheets. The addition of gluten when water is less than 150% appears to affect viscosity **[18]**.

The water's temperature and the batter's viscosity affect the sheet's quality. The water temperature should be around 20°C to prevent gluten strand formation. The batter's viscosity in warm conditions drops if the flour has a high α-amylase activity. Using the batter within 10-30 min could minimise the unfavourable effect on viscosity. So, the moisture barrier property of the packaging is designed to protect the contents from potential damage caused by humidity, liquid spills, or other moisture **[19]**.

The importance of research is to overcome the main defect of wafers, which is their high fragility. They are very soft in texture and cannot retain their sensory qualities or the desired level of brittleness during their shelf life. This requires. Find a formula for a wafer biscuit that helps overcome the defects of offers, such as fragility, poor texture, colour, and decreased nutrition value. This requires determining the best percentage of soybean flour and ammonium carbonate to add to the wafer biscuit formulation, besides investigating the effect of adding different ingredients on the sensory, physical, and chemical properties and nutritional values of wafer biscuits.

Scientific Hypothesis

Adding ratios and quantities of soy flour and ammonium carbonate can extend the shelf life of the products and enhance their quality parameters. It can also improve the nutritional value, colour, texture, and sensory properties and reduce the crispness and fragility of the wafer.

MATERIAL AND METHODOLOGY

Wafer ingredients:

Wheat flour were obtained from Gulf Industrial Development, Southern Amman Mills, Jordan. Toasted soy flour and lecithin were purchased from (Sonic Biochem Extraction Pvt Ltd, Madhya Pradesh, India). Sodium chloride and potassium iodate were obtained from Amra Salt Factory, Sahab, Jordan. Non-hydrogenated palm oil was bought from Johor, Malaysia; sodium bicarbonate and AC was bought from China; potato starch was purchased from Nowamyl S.A., Poland; protease and xylanase enzymes were obtained from Orba Biokimya Simbiyotek, Turkey, table 1 shows the weight of the ingredients used in the control treatment.

Table 1 The weight of the ingredients used in the wafer dough preparation as a control treatment.

Ingredient	Ammount
White Flour	60 Kg
Water	0.89L
Salt	100 g
Sodium bicarbonate	200 g
Palm oil	1 L
Lecithin powder	10 g
Lemon salt	10 g
Potato Starch	100g
Yeast extract	50g
Enzyme	30g

Chemicals

Chemical reagents in the experimental design were of analytical grade quality. They were purchased from Sigma-Aldrich, Japan, Leica Biosystem, USA, and Sulfuric acid (brand A, chemically pure, Khimlaborreaktiv LLC, Ukraine). Phenolphthalein solution (NaOH,) (Novokhim), Kharkiv, Ukraine). Sodium hydroxide.

Instruments

Texture analyser (6700 TVT; Perten, Sweden), Kjeldahl (VAP 450; Gergardet, Germany), Gerber (Funke Gerber, Denmark), Oven (TR 240; Nabertherm, Germany), muffle furnace (Nat 30/65; Nabertherm, Germany), and pH meter (S400; Mettler Toledo, Switzerland) spectrophotometer (X-rite VS-450, UK). On Colour software

for Colourimeters (CyberSoft, UK) calculated the CIE Lab values viscometer Fungilab Expert (Fungilab Leading Viscosity Techno Lab, Spain). Analyzer (Sartorius MA45, Goettingen, Germany), mixer (YILMAZ REDUKTOR, Turkey).

Laboratory Methods

The test was carried out on wafer biscuits immediately after manufacturing and after three months of storage as Figure 1.

Viscosity Measurement: The viscosity of the batter was measured using the Fungilab Expert viscometer (Fungilab Leading Viscosity Techno Lab, Spain). The viscosity should be 8 CP and not more than 18 CP. It is judged by adjusting the viscosity based on the data from batter mixers and ovens **[20]**.

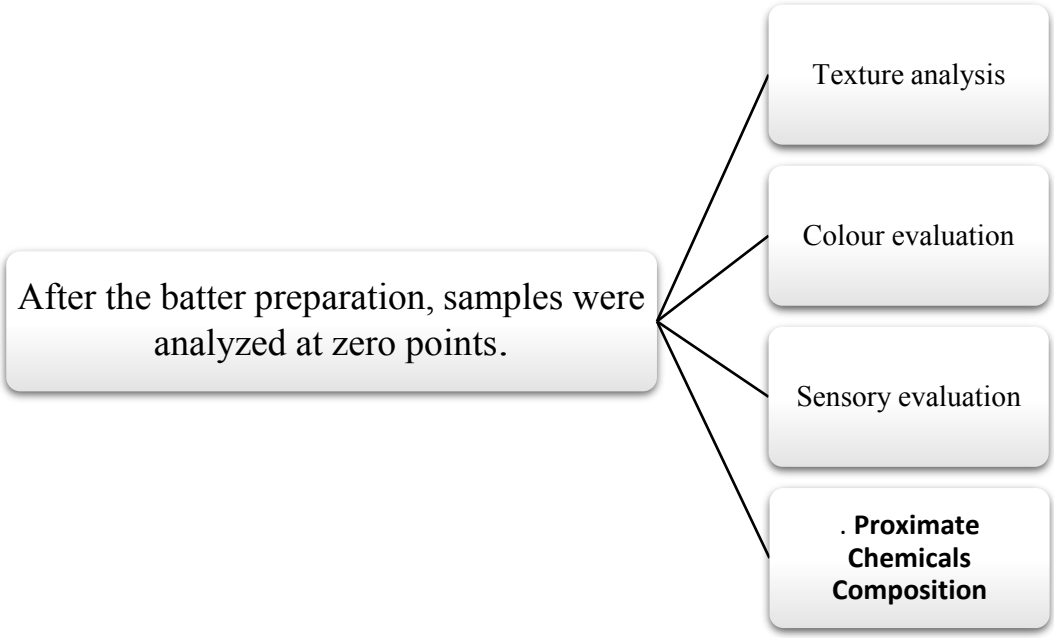


Figure 1 The test was carried out on wafer biscuits.

Chemical Analysis:

The wafer composition of moisture, fat, protein, fibre, and ash was determined by Standard AOAC methods **[21]**. Total carbohydrates were calculated as: 100 − (protein + fat + moisture + ash + fibre).

Sensory Evaluation of Wafer Biscuits:

The sensory properties, appearance, texture, colour, toughness, taste, and overall quality of all the samples of the wafers were evaluated by the 15-member panel of experts according to the hedonic scale (9 points) and rated as follows: 1 point= dislike very much; 9 points = like very much **[22]**.

Texture analysis:

Texture profile analysis (TPA) assesses hardness, a prevalent test that measures several texture attributes simultaneously. The TPA curve (force-time or force-displacement) measures texture parameters. The texture analyser (TVT6700, Perten, Sweden) was equipped with a 15 kg load cell, and a stainless-steel cylinder probe (673040) was used. The probe height and diameter are 45 and 40 mm, respectively **[23]**, **[24]**.

Measurement of the colour of wafer biscuits:

The colour intensity of the top and bottom surfaces of the biscuit colour measuring System (Chroma Meter CR-450, Minolta LTD Japan) was used to calculate the CIE lab system. The colour system, i.e., L*, a with the CIE LAB system, where the results are expressed as follows: (L*) a luminance or lightness component [L = 0 (black), L = 100 (white), (a* values) component for greenness (−a) to redness (+a), and the (b*) component from blueness (−b) to yellowness (+b). A light trap calibrated the colourimeter, and a white colourimeter CR-300 (Ramsey, NJ, USA) was used **[25]**.

Shock test:

The shock Test exposes the wafer to various conditions, such as heat and cold. The sample was exposed to heat in the oven at 40–45 °C for a whole day. On the second day, it was exposed to cooling to 0 °C for a whole day, and the procedure was repeated. Subsequently, the taste, colour, texture, and quality were checked.

The samples that have been shocked tested are kept for some time to check if there is a change in the characteristics and quality of the wafer sheet. The shock test results were assessed by a hedonic scale at point 9 and rated as follows: 1 point= dislike very much; 9 points = like very much **[22]**.

Description of the Experiment

This experiment used a factorial design with three factors: the amount of soy flour, AC, and storage time at the beginning. Through sensory evaluation, the researchers determined the best percentage replacement of white flour with soy flour and the amount of AC added to the batter wafer. After that, the quality of the products was measured.

Sample preparation:

Preparing wafer dough fortified with soybean flour:

The wafer biscuits were produced by weighing all ingredients found in (Table 1) with partial replacement of wheat flour with soy flour 5%, 10%, 15%, 20 %, then mixing all ingredients using a blender for two minutes) (dough temperature 18 – 20 °C, humidity 17%), then left at room temperature for 25 minutes.

Preparing wafer dough According to the following transactions:

- T1: In the control treatment, 3 kg of Soybean flour (which represents 5% of wheat flour) and 57 kg of wheat flour were used.
- T2: 6 kg of Soybean flour (10% of wheat flour) and 54 kg of wheat flour (90% of wheat flour in the control).
- T3: 9 kg of Soybean flour represents 15% of wheat flour and 51 kg of wheat flour (85% of wheat flour in the control).
- T4: 12 kg of Soybean flour represents 20% of wheat flour, and 48 kg of wheat flour represents 80% of wheat flour in control.

Preparing wafer dough fortified with ammonium carbonate (AC):

Weighing every compound in Table 1 with an addition of 50, 100,150, and 200 g of ammonium carbonate (AC) and the mixture for two minutes at a temperature of approximately 18 – 20 °C and a humidity of approximately 17%. Then, let it sit at room temperature for 25 minutes.

Prepared the following treatment:

- T5: It consists of formula treatment T3, adding 50g of (AC) Instead of sodium carbonate.
- T6: It consists of formula treatment T3 with the addition of 100g of (AC) Instead of sodium carbonate
- T7: It consists of formula treatment T3 with 150g of (AC)Instead of sodium carbonate.
- T8: It consists of formula treatment T 3 with 200g of (AC)Instead of sodium carbonate.

Treatment is chosen according to sensory evaluation:

- T9: It consists of formula T3 with the addition of 150g ammonium carbonate Instead of sodium carbonate.
- T10: It consists of formula treatment T0 with adding 150gm AC (without soy flour)
- T11: It consists of formula treatment T7 without AC
- T0: Control treatment:

The manufacture of wafer biscuits is as follows:

Wafer dough was put in the flat wafer baking system's oven. The oven was adjusted to 119/220°C, and the wafer batters were baked for 2 minutes until done. Then, they were removed.

The wafer sheets from the baking machine were cooled to room temperature (25 °C). The cream is then put inside the crispy wafer and put in the cooler (10° C).

Number of samples analysed: 72 samples.

Number of repeated analyses: All measurements of instrument readings were performed three times.

Number of experiment replications: The number of repetitions of each experiment to determine one value was three.

Statistical Analysis

1. First, data analysis was done with SPSS version 28.
2. Data were analysed using IBM software, and a p-value of less than 0.05 was considered statistically significant.
3. The mean and standard deviation were used to express the scale data.
4. The ideal percentage to add soy flour and AC was examined using a one-way ANOVA.
5. Repeated action to analyse the chemical compositions of different flours used in wafer batters was examined by MANOVA.

RESULTS AND DISCUSSION

The result of the sensory evaluation shows (Table 2) that the best amount of soy flour that was added instead of wheat flour to the same ingredients used in treatment T0 (control) is 9 kg of soy flour (representing 15% of wheat flour)It received the highest sensory rating which was 8.80 ± 0.41 While replacing wheat flour in percentage (5.0 % or 20.0%) with soy flour, it got the lowest averages of 4.33 ± 0.97 and 4. ±0.51, respectively researcher that when adding a percentage higher than 15% or less than this, it was less accepted by the panellist.

Table 2 shows the sensory evaluation results for the control sample and wafer biscuits prepared by partially replacing wheat flour with soy soybean flour and adding ammonium carbonate.

The wafer was prepared by Replacing wheat flour with a different % of soy flour	Mean score	Control- Mean score	The wafer was prepared by Replacing wheat flour with a different % of soy flour and adding ammonium carbonate	Mean score
The wafer was prepared by replacing wheat flour with 5 % of soy flour.	4.33 +0.97	8.87±0.35a	The wafer was manufactured by replacing 15 % wheat flour with of soy flour and adding 50 g ammonium carbonate	4.20+0.41
The wafer was prepared by replacing wheat flour with 10 % of soy flour	7.27 ±0.94	8.87±0.35a	The wafer was manufactured by replacing 15 % wheat flour with soy flour and adding 100 g ammonium carbonate	7.20 0.33
The wafer was prepared by replacing wheat flour with 15 % of soy flour	8.80± 0.41	8.87±0.35a	The wafer was manufactured by replacing 15 % wheat flour with soy flour and adding 150 g of ammonium carbonate	8.93± 0.26
The wafer was prepared by replacing wheat flour with 20 % of soy flour	4.40± 0.51	8.87±0.35a	The wafer was manufactured by replacing 15 % wheat flour with soy flour and adding 200 g ammonium carbonate	4,20+0.46

Note: Different superscript letters indicate significant differences ($p \leq 0.05$) in the same row; all, values are expressed as the mean ±SD (standard deviation).

The fortified wafer biscuits with ammonium carbonate (AC)

Ammonium carbonate (AC) is a common leavening agent in the biscuit and crackers industry. AC improves the texture and volume of wafers **[23]**. The sensory evaluation of wafer biscuits fortified with four amounts of ammonium carbonate is 50.0g, 100.0g, -150g, and 200.0g, as explained (Table 2). The results showed that the Wafers from a formula fortified with more or less 150 g of ammonium carbonate obtained a lower acceptability rating than wafer biscuits fortified with 150 gm of ammonium carbonate.

This result may be due to ammonium, which reduces colour contrast and enhances wafer bulk and texture **[26]**. If used at the correct rate, a percentage would be less than 01% of the mixture weight **[27]**. Ammonium also improves texture, and this is due to the decomposition of AC at low temperatures **[28]**, the generation of carbon dioxide, and the lower pH value **[29]**.

Effect of partial replacement wheat flour with soy flour on sensory acceptance of wafer biscuits:

Table 3 shows the results of the sensory evaluation of wafer biscuits prepared by Partial replacement for wheat flour with soy soybean flour and the addition of ammonium carbonate

The results showed no significant differences between the treatments T9 and the control sample after manufacturing immediately ($p > 0.05$). The mean score was 8.87±0.35 and 8.17±0.35, respectively. The researchers noted from Table 3 that the sensory evaluation score of the control sample decreased as storage continued. After the first month, it decreased to 6.33±0.95 and 4.67±0.62 after the third month of storage, the lowest degree of sensory evaluation.

These results mean that the problem of fragility can be overcome by fortifying the dough of Wafer biscuits with specific proportions of soy flour and AC.

Table 3 Sensory evaluation of wafer biscuits prepared with partial replacement of wheat flour with soy soybean flour and ammonium carbonate.

Time	T9	T0	Sample number
zero time	8.87±0.35a	8.17±0.35a	3
1month	8.73±0.59a	6.33±0.95b	3
2months	8.80±0.56a	6.73±0.96b	3
3months	8.53±1.03a	4.67±0.62b	3

Note: Different superscript letters indicate significant differences ($p \leq 0.05$) in the same row; all values are expressed as the mean ±SD (standard deviation).

The chemical composition of the wafer biscuit prepared of partial replacement of wheat flour with soy soybean flour and ammonium carbonate

The raw materials, such as the type of flour and its content of gluten, carbohydrates, fibre, and (fermentation time), had an impact on the viscosity of the batter, which affected its properties and nutritional value of the final product **[4]**. Table 4 shows the chemical composition of the wafer biscuits manufactured by replacing wheat flour with 15% soy flour, with A C or without AC, and the control sample. (T9, T10, T0)The researchers noted from Table 4 that the fortified wafer with soy flour had lower moisture content than the control sample, where the moisture content was 3.88±0.02 for the control sample and 2 .47±0.13 for the fortified wafer; this can be attributed to the increase in the proportion of soy flour that is added to the sample reduces the moisture content in the supplemented biscuit **[30]** because soybean flour contains β-carotene pigment which increases the ability of the wafer ' to absorb water and retention of moisture in the crumb of the baked products **[31]**. Also, soy flour contains a greater amount of total dry solid with high emulsifying properties compared to wheat flour and has low moisture. This is in agreement with the findings of **[32]** that an increase in the proportion of soy flour reduces the moisture content of biscuits. Meanwhile, **[33]** published that the non-enzymatic browning rate increases when water activity is high in food. This is considered one of the major problems that occurs during the processing and storage of dehydrated and semi-moist foods. This reaction leads to a darkening of colour and protein insolubility when reducing sugars, proteins, or free amines. This finding does not agree with the finding **[34]** that recommended moisture levels should be between 6% and 11% for obtaining an acceptable crispy wafer. This range does not apply to wafers, according to **[35]**, as for the protein and fat content. There was a significant increase in the percentage of protein t in-fortified wafers with soy flour, which increased from 6.56±0.30 to 12.47±0.29 to T9; the baked products that are wheat-based have content low in the quantity and quality of proteins, and it's deficient in some essential amino acids. Therefore, fortifying wheat-based baked products with protein-rich materials such as soybeans could be the approach to overcoming malnutrition. **[36]** while fat increased from 12.47±0.47 to 20.26±0.29. This is due to soy flour containing more protein and fat than wheat flour. Our results agree with those who reported that soy flour contains 20–24% fat, while wheat flour contains 0.9–1.1%, most of which is unsaturated **[37]**. Also, as for the ash, the results revealed no statistically significant differences between the samples of soy flour and AC or with soy flour without AC and the control sample at zero time and after three months of production ($p < 0.001$), which expresses the amount of mineral salts in food. But the values of carbohydrate content (65.17±0.59a, 64.25±0.55a, 77.78±0.62b) in the fortified samples were lower than that in control, at zero time and after three months of production ($p < 0.001$). As for the fibre content in fortified wafer biscuits with soy flour (with or without AC) and control were 1.93±0.15, 1.70±0.0, and 2.06±0.15 and these results indicate that there were no statistically significant differences between the samples of their fibre content, respectively, at zero time and after three months of production.

It has been reported that a high dietary fibre intake helps to prevent and reduce the risk of several diseases, including diabetes, obesity, cardiovascular diseases, and hypertension **[38]**. Also, soy flour contains more fat than wheat flour **[39]**. These results are in agreement with **[38]**. Soybeans are rich in polyunsaturated and monounsaturated fatty acids, including linoleic and Linolenic, which are essential for human health **[39]**.

Table 4 Chemical composition for standard formulations of wafer.

The chemical composition of various flours used in wafer batters	Times of measurement	T9	T10	T0 Control
Fat (M±SD)	Time 0	19.75±0.06a	20.26±0.29a	12.47±0.29b
Fat (M±SD)	after 3 month	19.75±0.06a	20.26±0.29a	12.47±0.29b
Carbohydrates (M±SD)	Time 0	64.25±0.55a	65.17±0.59a	75.78±0.62b
	After 3months	65.09±0.44a	65.10±0.55a	75.79±0.32b
Fiber (M±SD)	Time 0	1.93±0.15a	1.70±0.0a	2.06±0.15b
	After 3months	2.10±0.26a	2.070±0.0a	1.90±0.10b
Protein	Time 0	11.77±0.77a	12.19±0.35a	6.56±0.30b
	After 3months	11.77±0.10a	12.28±0.22a	6.95±0.37b
Ash (M±SD)	Time 0	1.56±0.05a	1.47±0.06a	1.29±0.03b
	After 3months	1.48±0.08a	1.40±0.05a	1.28±0.04b
Moisture (M±SD)	Time 0	2.47±0.13ab	2.40±0.10a	3.88±0.02b
	After 3months	2.53±0.10ab	2.41±0.04a	3.95±0.09b

Note: Different superscript letters indicate significant differences ($p \leq 0.05$) in the same row; all values are expressed as the mean ±SD (standard deviation).

The effect of partial replacement wheat flour with soy flour on texture wafer biscuits

The results in Table 5 showed that the wafer biscuits made with partial replacement of wheat flour with soy flour had a hardness value of 3138.0, higher than the control wafer's 2556.4. Perhaps the increased hardness is due to the increased protein content in soybean flour. This result agrees with other studies that reported increased protein content causes higher hardness **[40]**. Some studies report that higher fibre content is generally linked to an increase in the wafer's hardness **[41]**. Moisture level affects the texture of the wafers by softening the starch and protein matrix, which alters the strength of the wafer sheets **[42]**. The raw materials, such as the type of flour and its content of gluten, carbohydrates, fibre, and time after mixing (fermentation time), had an impact on the viscosity of the batter **[43]**, which affects texture, properties, and nutritional value. of the final product **[44]**. Low starch levels cause low gelatinisation, which might increase the hardness of wafer biscuits **[45]**. The hardness level of the samples during the storage period decreased, especially the control sample.

Table 5 Investigating the effect of adding different ingredients on the texture of the wafer batters.

times	wafer		
	T9 Texture hardness g	Texture hardness g	T0 Texture hardness g
zero time (M±SD)	3138.0a	2878.37	2556.43a
One month(M±SD)	2878.03	2735.6	1438.82
Two months (M±SD)	3075.07	2805.18	15789.2
Three months(M±SD)	3413.9	3162.1	2096.93
Test values	F=2.544, p=0.252	F= 1.61. P= 0,612	F=1.030, p=0.417

Note: All values are expressed as the mean ±SD (standard deviation
T9“Wafer with 15% soy flour plus ammonium carbonate Force (g)(Hardness)
T10 Wafer with 15% soy flour Force (g)(Hardness

The effect of soy flour and flour improvers on the colour of wafer biscuits

Colour is one of the most important components of food quality, indicating a product's quality. Colour measured based on CIE L*, a*, b* colour. L scale: Light vs dark where a low number (0 – 50) indicates dark and a light number (51 – 100) b* yellowness index scale: Yellow vs blue where a positive number indicates yellow and a negative number indicates blue **[46]**. The results of the data analysis show (Table 6) that there are no significant differences between all treatments immediately after manufacturing and after three months of storage; this means that the addition of soy flour did not affect colour (L* brightness) colour and the readings ranged between 70.93±64 to 74.02±0.02, greater than 50, an indicator of light a* redness index). For commercial batter wafer colour evaluation at different points in time, the samples remained unchanged after 3 months of storage.

The results of data analysis show that (a* redness index) for AC + soy four was 3.29±0.29 and commercial batter control was 3.91±0.36, which is the lowest compared with AC + wheat flour was 5.77±0.24, which is the highest at zero time. All numbers are positive. The data analysis results show no significant differences between all treatments immediately after manufacturing and after 3 months of storage. In contrast, a higher b* value led to a higher yellow-ness.

Variations in the batter formulations cause differences in the wafer sheet's colour, which is affected by moisture content, the amount of reducing carbohydrates, and free amino acids) and baking conditions (time and temperature) **[47]**.

Table 6 The effect of storage duration on the colour of wafer biscuits.

storage duration	Treatments			
	T 10	**T9**	**T11**	**Control**
		(L* brightness)		
Time zero (M±SD)	72.51±0.85a	74.02±0.02a	72.24±1.75a	74.69±1.71 a
First month(M±SD)	73.73±1.45a	73.34±0.31a	71.34±0.36a	72.77±1.37 a
Two months (M±SD)	73.47±0.47a	72.33±0.29a	74.45±0.33a	75.45±0.40 a
Three months(M±SD)	71.70±0.61a	70.93±64a	73.19±0.74a	74.69±1.71a
		a* redness index)		
Time zero (M±SD)	5.77±0.24a	3.29±0.29b	4.47±0.48 c	3.91±0.36 d b c
First month(M±SD)	4.80±0.90 a	2.53±0.29 b	3.09±0.13 c b	3.36±0.47 d b c
Two months (M±SD)	5.61±0.47 a	2.78±0.20 b	2.43±0.09 c b	5.35±0.49 a
Three months(M±SD)	5.12±0.12 a	2.59±0.26 b	4.13±0.24 c	3.99±0.42 d c
		b* yellowness		
Time zero (M±SD)	27.47±0.56a	26.66±0.81a	27.63±0.19a	28.07±1.71a
First month(M±SD)	26.68±0.33a	25.08±0.39 a b	26.41±0.44a	27.58±1.0 a c
Two months (M±SD)	28.89±0.79a	25.90±0.23b c	24.31±0.31c	31.72±1.18d
Three months(M±SD)	31.33±0.80a	22.56±0.44b	27.63±0.32c	28.07±1.61d

Note: difference letters indicate significant differences ($p \leq 0.05$) in the same row; all values are expressed as the mean ±SD (standard deviation).

Shock Test:

The wafer was repeatedly exposed to various conditions (heat and cold) to check for a change in the characteristics of the wafer, and the panellist rated their sensory score. The results in Figure 2 have revealed that at time zero (production time), there were no statistically significant differences in the sensory mean scores between the four groups ($p > 0.05$ for all). In contrast, after four days of wafers' batter processing of the shock test, results exhibited that T9, T10, and T11 significantly had a higher mean sensory score than the control group ($p = 0.005$, respectively). The AC with soy flour had a higher mean sensory score than the control group ($p \leq 0.001$. No statistically significant mean differences were noted between the three manipulated groups ($p > 0.05$ for all Samples).

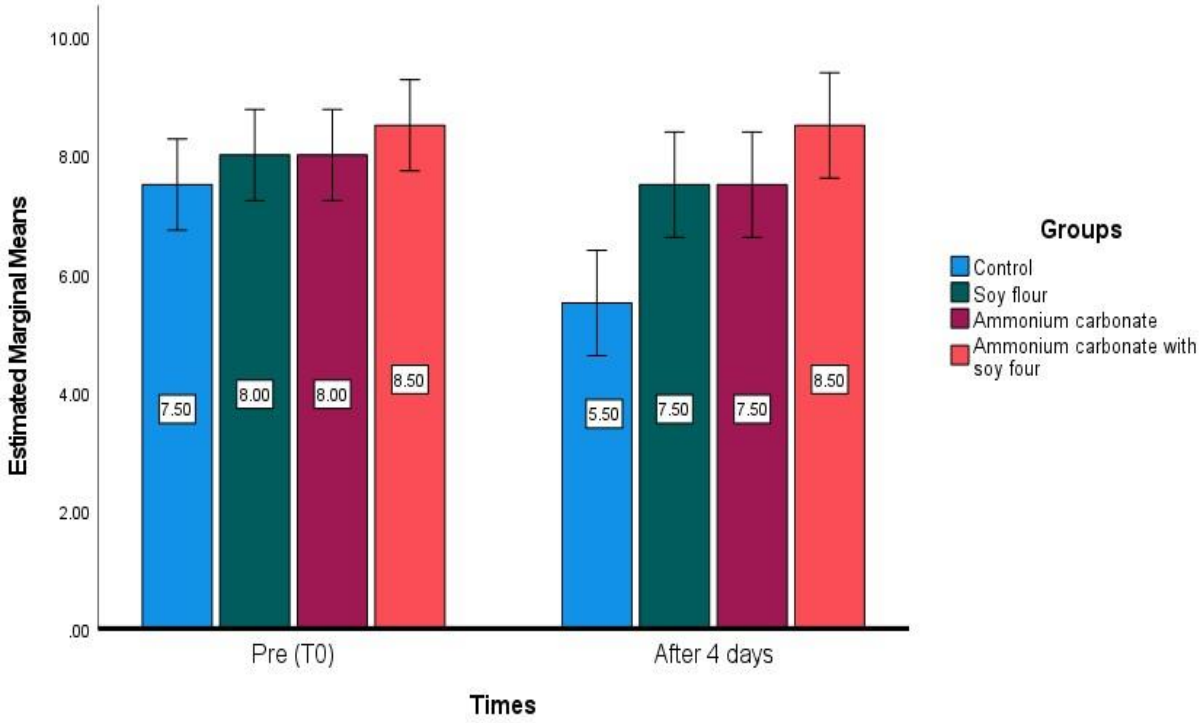


Figure 2 Shock test sensory evaluation after exposure to various conditions (heat and cold).

Figure 3 Produced wafer biscuit without soybean flour and ammonium carbonate.

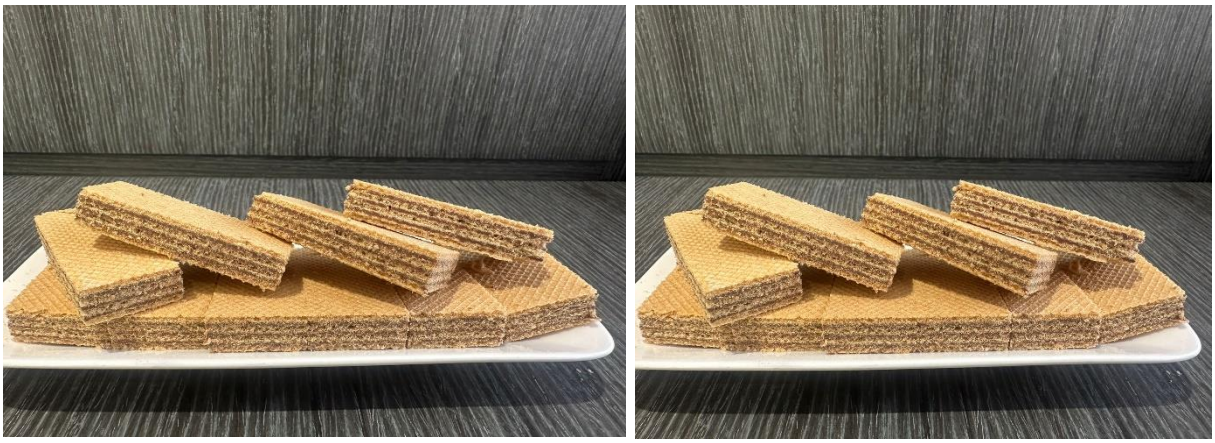


Figure 4 Produced wafer biscuit with say bean flour and ammonium carbonate.

CONCLUSION

The sensory evaluation showed that the wafer biscuit was manufactured by replacing 15% wheat flour with soy flour (9 kg soy flour and 51 kg wheat flour.). With the addition of 150 g, the highest sensory evaluation scores.was 8.80 ± 0.41. The results show that adding ratios and quantities specific to soy flour and ammonium carbonate can extend the product's shelf life and enhance its quality parameters. It can also improve the nutritional value, colour, texture, and sensory properties and reduce the crispness and fragility of the wafer. It was also noted that the wafer was made with partial replacement of wheat flour with soy flour and was characterised by increased hardness compared to the control group. Meanwhile, the control wheat flour had the lowest value of 2556.43. It has been noted that adding 15% soy flour to the biscuits caused an increase in the nutritional compositions (proteins, ash, and fibre content). The result showed a significant increase in the percentage of protein and fat from 6.56±0.30 to 12.19± 0.35 and from 11.56±0.l to 19.64±0.55, respectively. Moisture content was higher in the control group. The carbohydrates in the control sample were higher than those in other treatments. Texture and colour were improved compared with the control. Wafer biscuits with high nutritional value content and high-quality properties can be prepared by replacing wheat flour with 15% soy flour and adding 150 g of ammonium carbonate.

REFERENCES

1. Canali, G., Balestra, F., Glicerina, V., Pasini, F., Caboni, M. F., & Romani, S. (2020). Influence of different baking powders on physico-chemical, sensory and volatile compounds in biscuits and their impact on textural modifications during soaking. In Journal of Food Science and Technology (Vol. 57, Issue 10, pp. 3864–3873). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-020-04418-1
2. Nasabi, M., Naderi, B., Akbari, M., Aktar, T., Kieliszek, M., & Amini, M. (2021). Physical, structural and sensory properties of wafer batter and wafer sheets influenced by various sources of grains. In LWT (Vol. 149, p. 111826). Elsevier BV. https://doi.org/10.1016/j.lwt.2021.111826
3. Chen, K.-I., Erh, M.-H., Su, N.-W., Liu, W.-H., Chou, C.-C., & Cheng, K.-C. (2012). Soyfoods and soybean products: from traditional use to modern applications. In Applied Microbiology and Biotechnology (Vol. 96, Issue 1, pp. 9–22). Springer Science and Business Media LLC. https://doi.org/10.1007/s00253-012-4330-7
4. Bennett, J. O., Krishnan, A. H., Wiebold, W. J., & Krishnan, H. B. (2003). Positional Effect on Protein and Oil Content and Composition of Soybeans. In Journal of Agricultural and Food Chemistry (Vol. 51, Issue 23, pp. 6882–6886). American Chemical Society (ACS). https://doi.org/10.1021/jf0343711
5. Romani, S., Rocculi, P., Tappi, S., & Dalla Rosa, M. (2016). Moisture adsorption behaviour of biscuit during storage investigated by using a new Dynamic Dewpoint method. In Food Chemistry (Vol. 195, pp. 97–103). Elsevier BV. https://doi.org/10.1016/j.foodchem.2015.06.114
6. Kong, F., & Singh, R. P. (2016). Chemical Deterioration and Physical Instability of Foods and Beverages. In The Stability and Shelf Life of Food (pp. 43–76). Elsevier. https://doi.org/10.1016/b978-0-08-100435-7.00002-2
7. Banureka, V., & Mahendran, T. (2011). Formulation of Wheat-Soybean Biscuits and their Quality Characteristics. In Tropical Agricultural Research and Extension (Vol. 12, Issue 2, p. 62). Sri Lanka Journals Online (JOL). https://doi.org/10.4038/tare.v12i2.2791
8. Jegadeesan, S., & Yu, K. (2020). Food Grade Soybean Breeding, Current Status and Future Directions. In Legume Crops [Working Title]. IntechOpen. https://doi.org/10.5772/intechopen.92069
9. Hartman, G. L., West, E. D., & Herman, T. K. (2011). Crops that feed the World 2. Soybean—worldwide production, use, and constraints caused by pathogens and pests. In Food Security (Vol. 3, Issue 1, pp. 5–17). Springer Science and Business Media LLC. https://doi.org/10.1007/s12571-010-0108-x
10. Amit, S. K., Uddin, Md. M., Rahman, R., Islam, S. M. R., & Khan, M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. In Agriculture & Food Security (Vol. 6, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s40066-017-0130-8
11. Tiefenbacher, K. F. (2019). Glossary of Terms in Wafers, Waffles and Adjuncts. In The Technology of Wafers and Waffles II (pp. 325–411). Elsevier. https://doi.org/10.1016/b978-0-12-809437-2.00010-1
12. AOAC. (2000). Official Methods of Analysis, in Association of Official AnalyticalChemists International .5
13. Mihafu, F. D., Issa, J. Y., & Kamiyango, M. W. (2020). Implication of Sensory Evaluation and Quality Assessment in Food Product Development: A Review. In Current Research in Nutrition and Food Science Journal (Vol. 8, Issue 3, pp. 690–702). Enviro Research Publishers. https://doi.org/10.12944/crnfsj.8.3.03

14. Nurdin, N. M., Navratilova, H. F., Ekawidyani, K. R., Pratiwi, D., & Kurniawan, M. Y. (2020). Soy Flour-Based Snack Bar as Potential Snack Alternative for Diabetes Mellitus. In Jurnal Gizi dan Pangan (Vol. 15, Issue 3, pp. 125–132). Jurnal Gizi dan Pangan/Journal of Nutrition and Food. https://doi.org/10.25182/jgp.2020.15.3.125-132
15. Lu, R. (2013). Principles of solid food texture analysis *. In Instrumental Assessment of Food Sensory Quality (pp. 103–128). Elsevier. https://doi.org/10.1533/9780857098856.1.103
16. León, K., Mery, D., Pedreschi, F., & León, J. (2006). Colour measurement in L∗a∗b∗ units from RGB digital images. In Food Research International (Vol. 39, Issue 10, pp. 1084–1091). Elsevier BV. https://doi.org/10.1016/j.foodres.2006.03.006
17. Kudełka, W., Kowalska, M., & Popis, M. (2021). Quality of Soybean Products in Terms of Essential Amino Acids Composition. In Molecules (Vol. 26, Issue 16, p. 5071). MDPI AG. https://doi.org/10.3390/molecules26165071
18. Cappelli, A., Oliva, N., & Cini, E. (2020). A Systematic Review of Gluten-Free Dough and Bread: Dough Rheology, Bread Characteristics, and Improvement Strategies. In Applied Sciences (Vol. 10, Issue 18, p. 6559). MDPI AG. https://doi.org/10.3390/app10186559
19. Gulkirpik, E., Toc, M., Atuna, R. A., Amagloh, F. K., & Andrade Laborde, J. E. (2021). Evaluation of Oxidative Stability of Full Fat Soybean Flour in Storage and Sensory Quality of Tuo Zaafi-Enriched with Soy Flour as Influenced by Traditional Processing Methods. In Foods (Vol. 10, Issue 9, p. 2192). MDPI AG. https://doi.org/10.3390/foods10092192
20. Hertzler, S. R., Lieblein-Boff, J. C., Weiler, M., & Allgeier, C. (2020). Plant Proteins: Assessing Their Nutritional Quality and Effects on Health and Physical Function. In Nutrients (Vol. 12, Issue 12, p. 3704). MDPI AG. https://doi.org/10.3390/nu12123704
21. Qin, P., Wang, T., & Luo, Y. (2022). A review on plant-based proteins from soybean: Health benefits and soy product development. In Journal of Agriculture and Food Research (Vol. 7, p. 100265). Elsevier BV. https://doi.org/10.1016/j.jafr.2021.100265
22. Amit, S. K., Uddin, Md. M., Rahman, R., Islam, S. M. R., & Khan, M. S. (2017). A review on mechanisms and commercial aspects of food preservation and processing. In Agriculture & Food Security (Vol. 6, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s40066-017-0130-8
23. Mironeasa, S. (2022). Current Approaches in Using Plant Ingredients to Diversify Range of Bakery and Pasta Products. In Applied Sciences (Vol. 12, Issue 6, p. 2794). MDPI AG. https://doi.org/10.3390/app12062794
24. Päivärinta, E., Itkonen, S., Pellinen, T., Lehtovirta, M., Erkkola, M., & Pajari, A.-M. (2020). Replacing Animal-Based Proteins with Plant-Based Proteins Changes the Composition of a Whole Nordic Diet—A Randomised Clinical Trial in Healthy Finnish Adults. In Nutrients (Vol. 12, Issue 4, p. 943). MDPI AG. https://doi.org/10.3390/nu12040943
25. O'Bryan, C. A., Kushwaha, K., Babu, D., Crandall, P. G., Davis, M. L., Chen, P., Lee, S.-O., & Ricke, S. C. (2014). Soybean Seed Coats: A Source of Ingredients for Potential Human Health Benefits-A Review of the Literature. In Journal of Food Research (Vol. 3, Issue 6, p. 188). Canadian Center of Science and Education. https://doi.org/10.5539/jfr.v3n6p188
26. Tiefenbacher, K. F. (2017). Technology of Main Ingredients—Sweeteners and Lipids. In Wafer and Waffle (pp. 123–225). Elsevier. https://doi.org/10.1016/b978-0-12-809438-9.00003-x
27. Gökmen, V., Açar, Ö. Ç., Serpen, A., & Morales, F. J. (2007). Effect of leavening agents and sugars on the formation of hydroxymethylfurfural in cookies during baking. In European Food Research and Technology (Vol. 226, Issue 5, pp. 1031–1037). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-007-0628-6
28. Iglesias, O., Ind, N., & Alfaro, M. (2013). The organic view of the brand: A brand value co-creation model. In Journal of Brand Management (Vol. 20, Issue 8, pp. 670–688). Springer Science and Business Media LLC. https://doi.org/10.1057/bm.2013.8
29. Manley, D. (2000). Classification of biscuits. In Technology of Biscuits, Crackers and Cookies (pp. 221–228). Elsevier. https://doi.org/10.1533/9781855736597.3.221
30. Serrem, C. A., de Kock, H. L., & Taylor, J. R. N. (2010). Nutritional quality, sensory quality and consumer acceptability of sorghum and bread wheat biscuits fortified with defatted soy flour. In International Journal of Food Science & Technology (Vol. 46, Issue 1, pp. 74–83). Wiley. https://doi.org/10.1111/j.1365-2621.2010.02451.x
31. Dogan, I. S. (2006). Factors affecting wafer sheet quality. In International Journal of Food Science & Technology (Vol. 41, Issue 5, pp. 569–576). Wiley. https://doi.org/10.1111/j.1365-2621.2005.01117.x
32. Mohd Zain, N., Abd. Ghani, M., & Mohd Kasim, Z. (2023). Effects of Water Addition Level on Physical Properties, Rheological Profile and Sensory Evaluation of Gluten-Free Bread: A Preliminary Approach. In

Sains Malaysiana (Vol. 52, Issue 2, pp. 487–499). Penerbit Universiti Kebangsaan Malaysia (UKM Press). https://doi.org/10.17576/jsm-2023-5202-13

33. Sahin, A. W., Wiertz, J., & Arendt, E. K. (2020). Evaluation of a new method to determine the water addition level in gluten-free bread systems. In Journal of Cereal Science (Vol. 93, p. 102971). Elsevier BV. https://doi.org/10.1016/j.jcs.2020.102971
34. HOUGH, G., BUERA, M. D. P., CHIRIFE, J., & MORO, O. (2001). SENSORY TEXTURE OF COMMERCIAL BISCUITS AS A FUNCTION OF WATER ACTIVITY. In Journal of Texture Studies (Vol. 32, Issue 1, pp. 57–74). Wiley. https://doi.org/10.1111/j.1745-4603.2001.tb01034.x
35. Martínez-Navarrete, N., Moraga, G., Talens, P., & Chiralt, A. (2004). Water sorption and the plasticization effect in wafers. In International Journal of Food Science & Technology (Vol. 39, Issue 5, pp. 555–562). Wiley. https://doi.org/10.1111/j.1365-2621.2004.00815.x
36. Anderson, J. W., Baird, P., Davis Jr, R. H., Ferreri, S., Knudtson, M., Koraym, A., Waters, V., & Williams, C. L. (2009). Health benefits of dietary fiber. In Nutrition Reviews (Vol. 67, Issue 4, pp. 188–205). Oxford University Press (OUP). https://doi.org/10.1111/j.1753-4887.2009.00189.x
37. Akubor, P., and Ukwuru,M.,(2005). Function al properties and biscuit making the potential of soy bean and cassava flour blends. Plant Foods for Human Nutrition (Vol. 58 , issue 3, pp. 1- 12) https://doi.org/10.1023/B:QUAL.0000040344.93438.df
38. Friedman, M., & Brandon, D. L. (2001). Nutritional and Health Benefits of Soy Proteins. In Journal of Agricultural and Food Chemistry (Vol. 49, Issue 3, pp. 1069–1086). American Chemical Society (ACS). https://doi.org/10.1021/jf0009246
39. Dias, J. S. (2012). Nutritional Quality and Health Benefits of Vegetables: A Review. In Food and Nutrition Sciences (Vol. 03, Issue 10, pp. 1354–1374). Scientific Research Publishing, Inc. https://doi.org/10.4236/fns.2012.310179
40. Dogan, I. S., Yildiz, O., & Meral, R. (2015). Optimization of corn, rice and buckwheat formulations for gluten-free wafer production. In Food Science and Technology International (Vol. 22, Issue 5, pp. 410–419). SAGE Publications. https://doi.org/10.1177/1082013215610981
41. Shiriki, D., Igyor, M. A., & Gernah, D. I. (2015). Nutritional Evaluation of Complementary Food Formulations from Maize, Soybean and Peanut Fortified with Moringa oleifera Leaf Powder. In Food and Nutrition Sciences (Vol. 06, Issue 05, pp. 494–500). Scientific Research Publishing, Inc. https://doi.org/10.4236/fns.2015.65051
42. Nurdin, N. M., Navratilova, H. F., Ekawidyani, K. R., Pratiwi, D., & Kurniawan, M.Y. (2020). Soy Flour-Based Snack Bar as Potential Snack Alternative for DiabetesMellitus. Journal Gizi Dan Pangan (Vol . 8, Issue 3, pp. 125-32). https://doi.org/10.25182/jgp.2020.15.3.125-132
43. Qin, P., Wang, T., & Luo, Y. (2022). A review on plant-based proteins from soybean: Health benefits and soy product development. Journal of Agriculture and Food Research, (Vol . 7, pp. 226 -235) https://doi.org/10.1016/j.jafr.2021.100265
44. Taghdir, M., Mazloomi, S. M., Honar, N., Sepandi, M., Ashourpour, M., & Salehi, M. (2017). Effect of soy flour on nutritional, physicochemical, and sensory characteristics of gluten-free bread. Food Science and Nutrition (Vol. 5 , Issue 3, pp. 439-445). https://doi.org/10.1002/fsn3.411
45. Katz, E. E., & Labuza, T. P.(1981).Effect of water activity on the sensory crispness and mechanical deformation of snack food products. Journal of FoodScience (Vol. 46, pp .403-409). https://doi.org/10.1111/j.1365-2621.1981.tb04871.x
46. Majlinda Sanaabdyl Sinani (2018).The Optimal Quantities Usage Of Soy Flour In The Qualitative Bread Evaluation. Journal of Applied Biological Sciences(vol.12 ,Issue 44 ,pp. 51-54). https://dergipark.org.tr/en/download/article-file/538619.c
47. Mihafu, F. D., Issa, J. Y., & Kamiyango, M. W. (2020). Implication of Sensory Evaluation and Quality Assessment in Food Product Development: A Review. In Current Research in Nutrition and Food Science Journal (Vol. 8, Issue 3, pp. 690–702). Enviro Research Publishers. https://doi.org/10.12944/crnfsj.8.3.03

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Mutah University and Canary Sweets Factory in the capital of Amman

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article contains no studies requiring an ethical statement or Ethical Consideration. Experiments were carried out with the approval of the Canary factory in Amman, Jordan.

Contact Address:

***Nazieh** I. **Alkhalaileh**, Mutah University, Faculty of Agriculture, Department of Nutrition and Food Science, Jordan P.O. Box 61710,
Tel 00962786615393
E-mail: nazieh@mutah.edu.jo
ORCID: https://orcid.org/0000-0001-6081-0468

Ghadeer Frehat, Mutah University, Faculty of Agriculture, Department of Nutrition and Food Science, Jordan P.O. Box 61710,
Tel:00962798176521
E- mail: ghadeerahmadkhozai@gmail.com
ORCID: https://orcid.org/0009-0005-0493-1384

potravinárstvo®
OPEN ACCESS
Received: 8.9.2024
Revised: 30.9.2024
Accepted: 7.10.2024
Published: 7.10.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 860-873
https://doi.org/10.5219/2020
ISSN: 1337-0960 online
www.potravinarstvo.com

The influence of lingonberry and sea buckthorn powder-aqua solutions on the nutritional value of sander roe

Zhanna Kazangeldina, Raushangul Uazhanova, Gulshat Zhaksylykova, Sholpan Abzhanova, Lyazzat Baibolova, Kamilya Tyutebayeva, Gaukhar Kuzembayeva, Yerkin Yerzhigitov

ABSTRACT

Fish roe is a rich source of polyunsaturated fatty acids, essential amino acids and several vitamins. The paper presents the possibility of using fish caviar treatment with berry solutions instead of traditional preservatives. The purpose of this study is to determine the effect of berry solutions from lingonberry powder (Vaccinium vitis-idea) and buckthorn (*Hippophae rhamnoides*) on the nutritional, biological value and safety of walleye caviar through the use of wild plants as preservatives. Samples of the caviar of the common walleye Sander (*Sander lucioperca*) were selected as research objects. The results showed that experimental caviar samples treated with berry solutions showed differences ($p>0.05$) compared to the control sample with saline solution, this proves an increase in the nutritional value and safety of fish caviar. It is also shown that the use of berry solutions from cranberries (LS) and sea buckthorn (SBS) combined with salt is 35% more effective compared to the control sample containing only salt (S). This means that using berry solutions significantly improves the results compared to the traditional canning method. This effect may be due to the additional beneficial properties of berry extracts, such as antioxidant and antimicrobial activity, which contribute to better preservation of caviar quality and safety. It was found that using a berry solution from cranberry powder revealed the best result. Also, it was shown that the greatest value and composition was possessed by the LS sample, which more satisfied the daily requirement of vitamins A, E and B group in comparison with other samples ($p>0.05$), the content of vitamin A (0.016±0.014); vitamin B (0.24±0.16); vitamin E (2.89±0.04); vitamin PP (2.1±0.05). This is because treating pikeperch caviar with berry solutions increases the amount of water-soluble and fat-soluble vitamins.

Keywords: common lingonberry (*Vaccinium vitis-idaea*), pikeperch roe, sea buckthorn (*Hippophae rhamnoides*), food safety, amino acids, vitamins

INTRODUCTION

Caviar, a symbol of luxury today, has come a long way since it was mainly distributed in the regions around the Caspian Sea. In the early 1700s, caviar was popular in these parts, but during the 18th century, it became a subject of import to Europe. Here, caviar quickly gained popularity among elite society and became a symbol of status and sophistication. By the end of the 20th century, the increased demand for caviar on the international market led to serious environmental consequences. Producers began overfishing wild sturgeon to meet this demand and maximise profits, which led to a sharp decline in their population. The mass production of caviar for export threatened not only wild sturgeon populations but also the sustainability of the business itself. These events

have led to stricter regulations and the development of alternative production methods, such as aquaculture, to conserve sturgeon and ensure sustainable caviar production **[1]**.

Caviar is a semi-canned fish product, and certain storage and packaging conditions are important to ensure its safety. Storing caviar at a low temperature, around 0 °C, slows the growth of microorganisms and preserves its freshness. Packaging caviar under anaerobic conditions, i.e., without access to air, is also critical, as it helps prevent oxidation and bacterial growth that can cause product spoilage. These measures preserve the quality of caviar and extend its shelf life while ensuring consumer safety **[2]**. Caviar is a valuable food product due to its unique chemical composition, which includes complete proteins, highly unsaturated fatty acids, vitamins, and macro- and microelements. Caviar products from various fish species, such as sturgeon, salmon, cod, herring, and sea urchins, have a high nutritional value.

Pikeperch caviar is especially popular. It combines valuable nutritional properties with a relatively low cost, making it available to a wide range of people with different levels of financial well-being. Pikeperch caviar is rich in easily digestible proteins, fats, and vitamins of groups A, D, E, and B.

Caviar has a low lipid content, but it contains all important water-soluble vitamins. Its chemical composition includes the following vitamins:

- Thiamine (vitamin B1): 50-95%
- Riboflavin (vitamin B2): 110-830%
- Folic acid (vitamin B9): 10-12%
- Cyanocobalamin (vitamin B12): 0,7-1,4%
- Niacin (vitamin B3): 1 000-3 200%
- Pantothenic acid (vitamin B5): 50-200%.

These vitamins play an important role in metabolism and maintaining the body's health, making caviar tasty and healthy food **[3]**.

One of the priority tasks in caviar production is ensuring the high quality of finished products, which includes controlling the permissible level of bacterial contamination. To achieve these goals, producers use preservatives. Preservatives help prevent the growth and multiplication of pathogenic microorganisms that can cause the product to spoil and threaten consumers' health.

Preservatives in caviar perform several important functions:

Stabilisation of the product, preventing its oxidation and change in taste.

Increasing the shelf life of caviar while preserving its nutritional value.

Reducing the risk of dangerous bacteria such as Clostridium botulinum, which can cause botulism.

Currently, in response to customers' increasing needs and requirements for the quality of food products, there is a steady tendency to expand the range of products. One key direction is the search and introduction of natural food additives, which can increase the nutritional value of products while having a preserving effect. This allows for the reduction of the dose or the elimination of the use of chemical preservatives.

Natural food additives such as herbal extracts, spices, essential oils, organic acids, and antioxidants are becoming increasingly popular in the food industry. They improve the taste and flavour of products and help extend shelf life by inhibiting the growth of pathogens.

This approach aligns with the current trends towards healthy eating and environmental safety and meets consumer demand for products with minimal artificial additives. As a result, natural preservatives and food additives are becoming an important element of innovation in the food industry, contributing to safer, healthier, and higher-quality food products.

However, to ensure the preservation of caviar quality without preservatives, it is necessary to increase its salinity, which leads to the deterioration of its organoleptic properties and reduces the product's usefulness. In recent years, products with a reduced sodium chloride content have been produced to prevent violations of salt metabolism in the human body and cardiovascular and other diseases. At the same time, preserving caviar only with sodium chloride and storing it at sub-zero temperatures does not provide the necessary microbiological level of safety during long-term storage.

Preservatives—sodium benzoic acid and potassium sorbate— traditionally preserve fish products. However, with modern tendencies to reduce chemical preservatives, natural preservatives and spice oil extracts from natural plant components are offered to replace them.

To those mentioned above, the development of caviar preparation technology with vegetable additives that have a positive effect not only on the organoleptic characteristics of products but also on the storability, providing the realisation of the direction of expanding the range of caviar products, is relevant. One of the main tasks of caviar product technologies is to produce products safe for consumers, have pleasant organoleptic characteristics without adding preservatives that inhibit bacterial spoilage of the product, and have a long shelf life **[4]**.

Based on the above, natural substances that will ensure finished products' microbiological and food safety must be searched for. Wild fruits can be an alternative to existing preservatives. Two of the most popular wild fruits are cowberries and sea buckthorn.

Lingonberries (*Vaccinium vitis-idaea*) grow in the north of Kazakhstan. Lingonberries contain many biologically important substances - sugars from 8 to 10% (glucose - up to 3.6%, fructose - up to 4.6%, sucrose - up to 0.6%), organic acids (2.0-3.5 %), vitamins B - 0.03 mg %, E - 1.0 mg %, provitamin A (carotene) - 0.05-0.10 mg%, minerals (0.26-0.35 %), tannins (100-400 mg). The organic acids in cranberries contain citric, malic, tartaric, salicylic, boric and benzoic acids. Benzoic acid is an antiseptic and a strong natural antioxidant which significantly affects the strengthening of cell membranes in the body **[5]**, **[6]**, **[7]**.

Figure 1 Common lingonberry (*Vaccinium vitis-idaea*).

Sea buckthorn (*Hippophae rhamnoides*) belongs to the genus sea buckthorn (*Hippophaë*), family Elaeagnaceae. Sea buckthorn fruits contain carbohydrates (8.0-8.5%), including pectin substances (0.4-0.5%), organic acids (2.0-2.5%), tannins, vitamins C (200 mg%), E (5 mg%), group B (2.32 mg%), various macro- and microelements (0.3-0.7%), and sea buckthorn oil (1.7-8.0%), rich in unsaturated fatty acids (linoleic and linolenic) **[8]**, **[9]**, **[10]**.

Figure 2 Buckthorn (*Hippophae rhamnoides*).

The purpose of this study is to determine the influence of berry solutions of lingonberry (*Vaccinium vitis-idaea*) and sea buckthorn (*Hippophae rhamnoides*) powder on the nutritional, biological value, and safety of pikeperch caviar due to the use of wild fruits as preservatives.

We selected samples of fresh pikeperch roe aged in berry solutions as objects of study. Fresh caviar – Lightly salted, requires refrigeration, short shelf life To prepare the berry solution, we used powders of common cowberry and sea buckthorn.

Scientific Hypothesis

This paper presents the results of a study of pikeperch (*Sander lucioperca*) caviar using berry solutions prepared from lingonberry (*Vaccinium vitis-idaea*) and sea buckthorn (*Hippophae rhamnoides*) powder. Treatment of caviar with lingonberry powder solution has a positive effect on its nutritional value, microbiological safety and organoleptic properties due to the antimicrobial and antioxidant properties of lingonberries, which improves the quality of the product and increases its shelf life compared to traditional methods of preservation.

MATERIAL AND METHODOLOGY

Samples

Pikeperch roe *Sander lucioperca*, belonging to the perch family (Percidae), was obtained from Bolashak Bastau Company, Republic of Kazakhstan. Fruit of lingonberry (*Vaccinium vitis-idaea*) and sea buckthorn (*Hippophae rhamnoides*) were received from Synthite Industries (Almaty, Kazakhstan).

Chemicals

Barium hydroxide $Ba(OH)_2$, sulfuric acid H_2SO_4, barium sulfate $BaSO_4$, hexane C_6H_{14} were purchased from Sigma-Aldrich, Inc. (Merck KGaA, Darmstadt, Germany), which guarantees high quality and reliability of the chemicals used in the experiments.

Instruments

Capillary electrophoresis: “Droplet 105A” (Agilent Technologies (USA)).

Gas chromatograph: “Crystallux 4000M” (Meta-Chrome, Russia)

Laboratory Methods

The study of the chemical composition of caviar consisted in determining the content of protein, fat, moisture, ash, vitamins, and minerals according to ISO 12875:2011, Traceability of finfish products — Specification on the information to be recorded in captured finfish distribution chains.

Toxic metals were determined according to GOST 30178-96 **[11]**.

The out mass fraction of amino acids was measured by capillary electrophoresis using the capillary electrophoresis system "Kapel 105 A" **[12]**. A 0.1000 g weight of the product under study was placed in a glass vial, 5 cm^3 of barium hydroxide solution was added. The vial is placed in the desiccator, which is placed in a desiccator for 14 - 16 hours at a temperature of 110 °C. At the end of hydrolysis, hot hydrolysate from the vial was quantitatively transferred to a measuring flask with a capacity of 100 cm^3, in which previously placed 40 - 50 cm^3 of distilled water was, add 1 - 3 drops of methyl red indicator and neutralise the solution by first adding 3.5 cm^3 of the sulfuric acid solution, and then add sulfuric acid solution drop by drop until the colour changes from yellow to pink. Then, bring the volume of the solution to the mark with distilled water, stir, and leave for 10 - 15 minutes until the solution is clear above the barium sulfate precipitate. 1.0 cm^3 of the obtained solution is placed in an Eppendorf tube and centrifuged for 2-3 min at 6000 vol/min, after which 0.5 cm^3 of the solution is transferred into a dry Eppendorf tube and used for analysis.

The study of fatty acids was carried out on the gas chromatograph "Crystallux 4000 M". It is equipped with a flame ionisation detector and quartz capillary column SP – 2560 100 m×0.25 mm ID, 0.2 µm fixed phase F. The Supelco ® 37 FAME Mix standard was used as an identification mix. The following chromatographic separation parameters were used: nitrogen was used as a gas carrier, T1 temperature for column 65-70 °C (retention period 5 min), T2 at a speed of 4G/min 240 °C, evaporator temperature 230 °C, detector 260 °C, volume of the introduced sample 1 µL. To control the modes of analysis and to process the chromatogram and the data obtained, the program "NetChrom 2" was used. We showed the latest results in percentages obtained from total fatty acids. The fatty phase was separated for the preparation of methyl esters. A sample of oil taken for the test was thoroughly mixed. Then, the oil (2-3 drops) was taken with a glass test tube and dissolved in 1.9 cm^3 of hexane. After stirring the reaction mixture vigorously (2 min), it was allowed to stand for 5 min and filtered. Then, the mixture ready for analysis was sent to the gas chromatograph **[13]**.

The methods of sampling and sample preparation for microbiological analyses were determined according to ISO 6579-1:2017/Amd 1:2020. Meat-peptone agar was used for all microbiological analyses (BioMedia, Russia).

Description of the Experiment

Sample preparation:

The berries were distributed evenly on mesh baking trays and dried convectively in cabinet-type dryers at 30 - 60 °C to a residual humidity of 5 - 8.5 %, obtaining dried berries. Then, they were cooled and ground to a particle size of no more than 50 µm, obtaining berry powder.

The whole 200-400 grams oyster was carefully immersed and kept in different solutions: in salt solution at the rate of 3% salt per 100 ml of water; in berry solution (with lingonberries) at the rate of 3% salt, 1% lingonberry powder per 100 ml of water; in berry solution (with sea buckthorn) at the rate of 3% sea buckthorn powder per 100 ml of water, 1% salt, at the temperature of 10-15 °C, for 20 min.

The research was conducted at the «Research Laboratory for Assessing the Quality and Safety of Food Products», Almaty Technological University.

Number of samples analyzed: we analysed 24 samples.

Number of repeated analyses: 3

Number of experiment replication: 3

Design of the experiment: The physicochemical parameters of caviar were carried out according to standard methods. These methods include assessing caviar appearance, colour, consistency, taste, and aroma. Standard methods ensure accuracy and comparability of results, which allows objective assessment of the quality of caviar and its compliance with regulatory requirements.

Statistical Analysis

The statistical analysis was done using the SPSS program (IBM SPSS STATISTICS 20, SPS Tc.). The Tukey HSD test was selected to compare the roe's quality parameters. The analysis of the methods was carried out using a single-factor analysis of variance (ANOVA). This means that the comparison was performed at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Changes in the physicochemical composition of experimental samples aged in solutions are presented in Table 1.

The analysis of Table 1 showed that the protein content of the samples did not differ significantly from each other. The amount of protein are samples S (18.34±0.21), LS (18.35±0.21) and SBS (18.35±0.33), respectively. There was a slight decrease in fat in the experimental sample LS (0.19±0.2). In sample S (79.04±1.05), the moisture content is higher; this rather depends on the characteristics of the fish, the stage of maturity of the eggs and the method of processing. The sample S (1.51±0.03) shows an increase in ash content, while the LS (1.52±0.01) and SBS (1.58±0.01) samples show a decrease.

Table 1 Physicochemical parameters of the roe of Sander fish of the studied samples

Component	S	LS	SBS
Protein, %	18.34±0.21[a]	18.35±0.21[a]	18.35±0.33[a]
Fat, %	0.20±0.1[a]	0.21±0.1[a]	1.21±0.2[b]
Water, %	78.14±1.87[a]	76.94±1.62[a]	76.48±2.62[a]
Ash, %	1.45±0.03[a]	1.52±0.01[b]	1.58±0.01[c]

The study of vitamin composition showed (Table 2) that LS and SBS significantly increased in the experimental samples. The LS sample has the highest value and composition, which more satisfied the daily requirement of vitamins A, E and B group compared to other samples, ($p>0.05$), the content of vitamin A (0.016±0.014); vitamin B (0.24±0.16); vitamin E (2.89±0.04); vitamin PP (2.1±0.05). This is because treatment of pikeperch caviar with berry solutions increases the amount of water-soluble and fat-soluble vitamins.

Table 2 Results of studies on vitamin content in tested specimens.

Specimens/Vitamins, mg/100 g	S	LS	SBS
Vitamin A	0.014±0.01[a]	0.016±0.014[a]	0.015±0.010[a]
Vitamin B	0.20±0.12[a]	0.24±0.16[a]	0.21±0.11[a]
Vitamin E	2.84±0.01[a]	2.89±0.02[б]	2.89±0.03[б]
Vitamin PP	2.07±0.06[a]	2.3±0.05[c]	2.18±0.04[b]

A comparison of the results obtained from the mineral composition of the samples (Table 3) indicates that an increase in the content of macro- and microelements is observed in experimental samples of LS and SBS. In the sample LS, potassium (294.36±0.93), calcium (44.51±0.50), sodium (42.524±0.12), iron (1.25±0.05), phosphorus (240.14±0.14), higher than in other samples, in the sample SBS, potassium (283.36±0.65), calcium (44.64±0.35), sodium (41.84±0.34), magnesium (25.45±0.31), iron (1.17±0.04), phosphorus (236.71±0.12), which indicates that caviar extract in berry solutions significantly increases the mineral content.

Table 3 The content of minerals compositions in the studied roe samples.

Mineral elements, mg/g	KS	LS	SBS
Potassium	271.21±0.22[a]	294.36±0.93[c]	283.36±0.65[b]
Calcium	40.75±0.21[a]	44.51±0.50[b]	44.64±0.35[b]
Sodium	38.50±0.18[a]	42.524±0.12[c]	41.84±0.34[b]
Magnesium	24.01±0.15[a]	25.44±0.55[b]	25.45±0.31[b]
Iron	1.11±0.02[a]	1.25±0.05[a]	1.17±0.04[a]
Phosphorus	233.12±0.93[a]	240.14±0.14[c]	236.71±0.12[b]

Table 4 summarises the results of the samples for toxic elements. As can be seen from the data, in sample KS, arsenic (< 0.25) was detected; in sample LS and SBS, cadmium (< 0.25),) in sample SBS, lead (< 1.0), while no toxic elements were detected in sample LS.

Table 4 The content of toxic elements in the studied roe samples.

Toxic elements, mg/g	KS	LS	SBS
Pb	not found	not found	< 1.0
Cd	< 0.25	not found	< 0.25
As	not found	not found	not found

In samples KS, LS and SBS studied comparison of the amino acid composition of caviar aged in different solutions (Table 5), showing the presence of all essential amino acids, the content of which is from 0.18 to 1.90 g, which is more than 46.9 % of the total amount of essential amino acids. The analysis revealed a rather high content of leucine (0.94-0.96 %), valine (0.88-1.05 %), lysine (0.62-1.63 %), phenylalanine (0.18-0.23 %), threonine (0.76-0.85 %) and tryptophan (1.86-1.90 %).

Table 5 The amino acid composition in the studied roe samples.

Amino acid	KS	LS	SBS
Valine	0.98±0.25[a]	1.05±0.54[a]	0.98±0.35[a]
Leucine + Isoleucine	0.94±0.38[a]	0.96±0.42[a]	0.95±0.29[a]
Lysine	1.63±0.26[b]	1.65±0.59[b]	1.63±0.21[a]
Methionine	0.54±0.22[a]	0.55±0.25[a]	0.55±0.14[a]
Threonine	0.80±0.31[a]	0.85±0.38[a]	0.81±0.25[a]
Tryptophan	1.86±0.16[a]	1.90±0.12[a]	1.87±0.08[a]
Phenylalanine	0.18±0.01[a]	0.23±0.02[b]	0.22±0.02[b]
Arginine	1.04±0.36[a]	1.08±0.31[a]	1.05±0.51[a]
Tyrosine	0.50±0.42[a]	0.52±0.41[a]	0.50±0.15[a]
Histidine	0.40±0.14[a]	0.50±0.37[a]	0.48±0.24[a]
Proline	1.13±0.26[a]	1.14±0.70[a]	1.15±0.31[a]
Serine	0.57±0.39[a]	0.59±0.35[a]	0.58±0.25[a]
Alanine	1.31±0.41[a]	1.33±0.25[a]	1.32±0.21[a]
Glycine	1.02±0.72[a]	1.04±0.14[a]	1.03±0.28[a]

The microbiological index of caviar aged in different solutions was compared in CW, LC and SBS samples (Table 6). The analysis showed that in the experimental sample LS, SBS, there was a slight decrease in the number of mesophilic aerobic and facultatively anaerobic microorganisms. The obtained results indicate that using sea buckthorn and lingonberry hulls not only increases the nutritional value of finished products, but also extends the shelf life of finished products. After treating the cans with a solution of lingonberry and sea buckthorn powder, the shelf life is up to 6 months.

Table 6 Results of microbiological parameters in the studied caviar samples.

Indicators	S	LS	SBS
Number of mesophilic aerobic and facultatively anaerobic microorganisms	$8 \cdot 10^3$	$2 \cdot 10^3$	$5 \cdot 10^3$
Coliforms	undetected	undetected	undetected
Sulphite-reducing clostridia	undetected	undetected	undetected
Staphylococcus aureus S.aureus	undetected	undetected	undetected
Salmonella	undetected	undetected	undetected
Moulds	undetected	undetected	2
Yeasts	15	9	12

The fatty acid composition shows (Figure 1) that the sum of saturated fatty acids in sample KS is 10.97%, in sample LS 15.16%, in sample SBS 15.52% and is mainly represented by palmitic and stearic acids. The proportion of monounsaturated fatty acids in LS and SBS samples is 33.10% and 34.66%, respectively, 1.62% and 3.18% more than in sample KS. Among the monounsaturated acids, oleic acid dominates with 21.48% (S), 21.96% (LS) and 22.94% (SBS), followed by palmitoleic acid with 6.75% (SBS), 6.95% (S) and 8.12% (LS), respectively. High polyunsaturated acid content (49.80-66.32%) was observed in LS and SBS samples, of which docosahexaenoic acid (22:6 wt.%) dominated with 30.20% and eicosapentaenoic acid (20:5 wt.%) with 6.01%. While sample S was dominated by linolenic acid (18:2 om6) -12.67%.

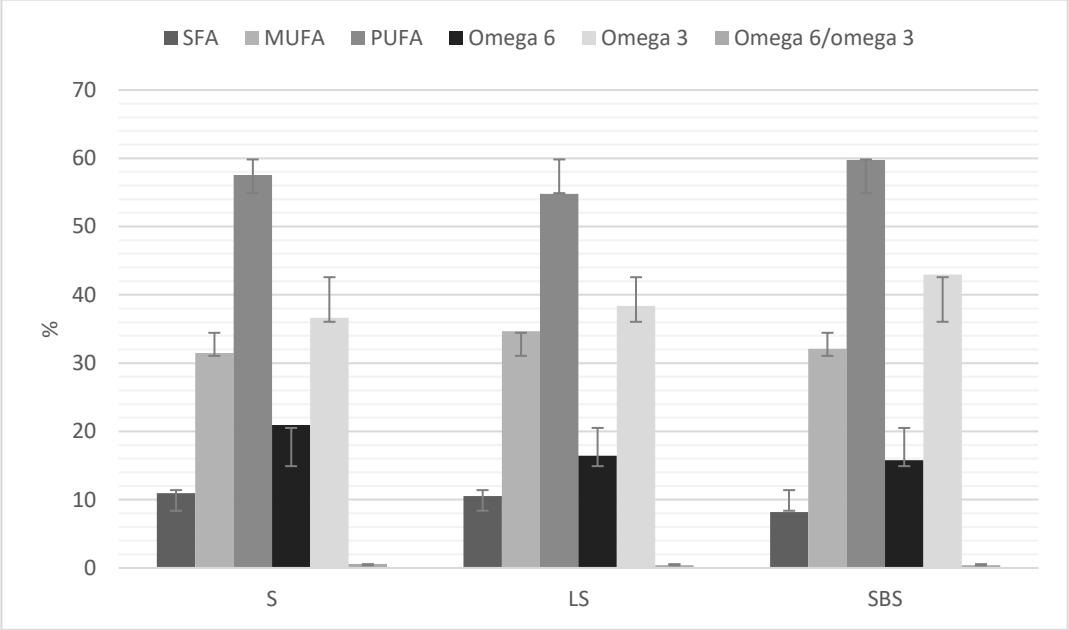


Figure 3 Comparison of fatty acid amounts in roes fat.

Studies show that using sea buckthorn in feed products improves the quality of end products, including poultry, pork, and fish **[14]**. Thus, using sea buckthorn in feed products can improve the nutritional value and quality of end products such as poultry, pork, and fish **[15]**.

Preservatives are used to obtain safe finished products in producing salted caviar from different fish species. The production method greatly influences the quality of ready-salted caviar **[16]**.

It should be noted that the technology for processing fish eggs and caviar is quite labour-intensive and has many stages. Therefore, strict observance of all parameters of the technological process, processing, and preservation of caviar grains is required. Kopylenko L.R. conducted scientific substantiation and developed technology for preserving sturgeon and salmon fish caviar **[17]**.

The results of the sample study showed significant differences ($p<0.05$) in terms of humidity: S, LS and SBS samples; ash: LS and SBS samples; fats: s and LS samples; proteins: LS and SBS samples, which indicates that different treatments affected the original composition of caviar, which draws attention to the high ash content for the S sample due to the use of NaCl without berry powder. Compared to raw materials, the humidity indicator decreased for all samples due to the immersion of caviar in brine and the use in the NaCl recipe. The Tuki test showed that all medium samples had significant differences in humidity ($p <0.05$); S and LS samples for Ash, LS

and SBS for oils. In this study, NaCl and Berry solution's content slightly changed the amount of proteins and moisture in the samples. The use of fruit powders and NaCl may have contributed to the reduction of moisture, which led to the highest concentration and energy intensity of S-Type ash.

The mineral element content in the samples indicates the presence and effect of berry solutions on caviar. Lingonberries and sea buckthorn berries have high concentrations of trace elements, making them potential sources of significant minerals that play an important role in nutrition and maintaining human health. Compared to sample S, the LS and SBS samples had an increase in K, Ca, Na, Mg, Fe, and P content.

The main subject of the article is pikeperch caviar. Pikeperch (*Sander lucioperca*) is the largest freshwater predator of the Perch family, including perches and ruffs. This species inhabits warm and exceptionally clean water bodies, which affects its extract and organoleptic properties of caviar.

Caviar is a food product derived from the ovaries of fish, which undergoes a complex technological process to process caviar grains. The female pikeperch lays caviar in the form of long (up to 1 m) reticulated ribbons of gelatinous substance, and accompanying males fertilise it. Pikeperch spawn is characterised by strong watering. Its water content is up to 56% or more. The diameter is 1.0-2.0 mm (sometimes 1 mm). Caviar of particulate and other fish is sold in cans and barrels, i.e., free of connective tissue of caviar eggs and caviar balls.

It should be noted that few studies of pikeperch caviar exist, both among Russian-speaking authors and in English-language sources **[18]**. In recent decades, perch fish, particularly pikeperch, have become a subject of increased interest in the fishing industry on a global scale **[19]**.

In several research papers, scientists have investigated the use of chasteberry extract in meat pates **[20]** and other meat products **[21]**, as well as the effect of sea buckthorn powder on meat products **[22]**, **[23]**, **[24]**. These studies have shown that:

1. Cranberry extract, due to its antioxidant and antimicrobial properties, can improve the flavour, shelf life, and nutritional value of meat products.
2. Sea buckthorn powder has also been studied as an additive to meat products, providing benefits in the form of increased antioxidant activity, improved organoleptic properties, and enrichment of the product with vitamins and minerals.

These results highlight the potential of using natural berry additives to improve food quality and safety, which may also apply to the caviar considered in your paper.

Researchers **[25]** used sea buckthorn extract to produce freeze-dried carp roe substitutes as an antioxidant and antimicrobial agent. Due to its natural antioxidant and antimicrobial properties, sea buckthorn extract effectively improved the product's stability and safety **[25]**. These studies highlight the potential of using plant extracts to improve fish products' quality and shelf life, which may also be useful for developing similar solutions in producing pikeperch caviar.

Other scientists have also investigated the effect of sea buckthorn and lingonberry extract on the nutritional value of fish products **[26]**. These studies have shown that berry extracts can significantly improve the nutritional value and organoleptic properties of fish products due to the following factors:

- Antioxidant properties: Sea buckthorn and cranberry extracts contain active antioxidants that help prevent fat oxidation and maintain product freshness.
- Improved vitaminization: Berries are rich in vitamins (such as vitamins C and E) that can enrich fish products, making them more nutritious.

Antimicrobial properties: These extracts can inhibit the growth and development of microbes, which helps increase the shelf life of foods.

Using sea buckthorn and cranberry extracts may help create healthier and more spoilage-resistant fish products, which is of interest for further research and application in caviar production **[27]**. Scientists have also investigated the fact that sea buckthorn has various biological properties such as anti-inflammatory, antioxidant, and anti-cancer activities. These properties can significantly contribute to the nutritional value of fish products. **[28]**.

It has been shown that fruit and berry pomace can be effectively used in pickled fish products **[29]**. Thus, using fruit and berry extracts in marinated fish products effectively improves their safety, quality, and organoleptic properties.

Studies show that adding vegetable powders, including berry powders, can increase the moisture-holding capacity of minced fish products **[30]**.

A study of vitamin composition showed that treating Sander roes with berry solutions increases the amount of fat-soluble and water-soluble vitamins and significantly increases the mineral content.

The study of vitamin composition showed that treating pikeperch eggs with berry solutions increases the amount of fat-soluble and water-soluble vitamins and significantly increases the mineral content **[31]**. Studies have shown **[32]** that fish oil can be partially replaced by vegetable oils in the diet of young pikeperch without a

negative effect on growth parameters **[33]**. However, increased lipid content in liver tissues and decreased lipid utilisation may be observed, indicating changes in metabolic processes related to lipid metabolism.

The results of toxic substance content tests determined the absence of lead and arsenic in samples S, LS and SBS, which allowed us to conclude that in the process of soaking in a solution with the addition of berry powders, toxic substances from caviar pass into solution, because under the influence of sodium chloride ions, hydration and solubility of proteins with which toxic substances are associated increases. As a result, toxic substances can pass from the roes into solution **[34]**. If the solution contains chitin or chitosan, which are sorbents, the toxic metals are not absorbed and are desorbed in the caviar grains **[35]**. This ensures that the toxic substances are removed from the caviar, thus increasing its safety and significantly improving the quality of roes products **[36]**. Also, it has been shown that coriander leaf extract can reduce heavy metal contamination in shellfish by reducing Pb, Hg, and Cu **[37]**.

The study of the composition of amino acids revealed the known changes in LS and SBS samples. This, perhaps, made it possible to obtain roe with high nutritional value when keeping roe in berry solutions, when the proteins penetrate the roe and accumulate. Since the roe did not undergo heat treatment, all biologically valuable nutrients were preserved in the product, which improved its quality.

Processing roe in berry solutions with the addition of salt will increase the shelf life of the product, as well as enrich the product with valuable nutrients **[38]**. Salt concentrations (2, 4 and 8%) affect rainbow trout caviar's physicochemical and microbiological quality parameters during 21-day storage at 4°C **[39]**. The results of the studies varied depending on the type of berry solution. Statistical analysis showed that the treatment with lingonberry solution was significantly more effective than the treatment with sea buckthorn and salt solution **[40]**.

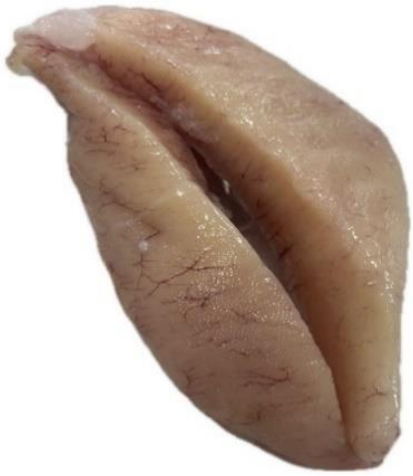


Figure 4 Pike-perch roe pike-perch caviar before processing.

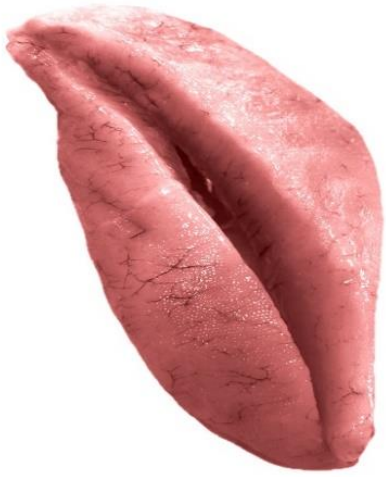


Figure 5 Pikeperch caviar pike-perch after treatment with lingonberry powder solution.

CONCLUSION

The results obtained in this study indicate differences between roe samples treated with different preservatives. The differences were particularly noticeable when all measured parameters (physical, chemical and microbiological) were combined.

The results of the sample study showed significant differences ($p<0.05$) in terms of humidity: S, LS and SBS samples; ash: LS and SBS samples; fats: s and LS samples; proteins: LS and SBS samples, which indicates that different treatments affected the original composition of caviar, which draws attention to the high ash content for the S sample due to the use of NaCl without berry powder. Compared to raw materials, the humidity indicator decreased for all samples due to the immersion of caviar in brine and the NaCl recipe. All medium samples showed significant differences in humidity ($p <0.05$); S and LS samples for Ash, LS and SBS for oils.

Also, it is shown that the sum of saturated fatty acids in sample KS is 10.97%, in sample LS is 15.16%, in sample SBS is 15.52% and is mainly represented by palmitic and stearic acids. The proportion of monounsaturated fatty acids in LS and SBS samples is 33.10% and 34.66%, respectively, which is 1.62% and 3.18% more than in sample KS. Among the monounsaturated acids, oleic acid dominates with 21.48% (S), 21.96% (LS) and 22.94% (SBS), followed by palmitoleic acid with 6.75% (SBS), 6.95% (S) and 8.12% (LS), respectively. High polyunsaturated acid content (49.80-66.32%) was observed in LS and SBS samples, of which docosahexaenoic acid (22:6 wt.%) dominated with 30.20% and eicosapentaenoic acid (20:5 wt.%) with 6.01%. While sample S was dominated by linolenic acid (18:2 om6) -12.67%.

The study found that berry solutions from lingonberry and sea buckthorn powder enrich food products with biologically active substances. Experimental work established that berry solutions improve the taste and aroma of aged roe and will exclude the addition of chemical preservatives in roe processing.

REFERENCES

1. Holembovska, N., Tyshchenko, L., Slobodyanyuk, N., Israelian, V., Kryzhova, Y., Ivaniuta, A., Pylypchuk, O., Menchynska, A., Shtonda, O., & Nosevych, D. (2021). Use of aromatic root vegetables in the technology of freshwater fish preserves. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 296–305). HACCP Consulting. https://doi.org/10.5219/1581
2. Lopez, A., Bellagamba, F., Tirloni, E., Vasconi, M., Stella, S., Bernardi, C., Pazzaglia, M., & Moretti, V. M. (2021). Evolution of Food Safety Features and Volatile Profile in White Sturgeon Caviar Treated with Different Formulations of Salt and Preservatives during a Long-Term Storage Time. In Foods (Vol. 10, Issue 4, p. 850). MDPI AG. https://doi.org/10.3390/foods10040850
3. Oeleker, K., Alter, T., Kleer, J., Pund, R.-P., Gölz, G., Hildebrandt, G., & Huehn, S. (2015). Microbiological and chemical investigation of caviar at retail. In Journal für Verbraucherschutz und Lebensmittelsicherheit (Vol. 10, Issue S1, pp. 35–37). Springer Science and Business Media LLC. https://doi.org/10.1007/s00003-015-1002-4
4. Huang, L., Song, Y., Kamal, T., Li, Y., Xia, K., Lin, Z., Qi, L., Cheng, S., Zhu, B.-W., & Tan, M. (2017). A non-invasive method based on low-field NMR to analyze the quality changes in caviar from hybrid sturgeon (Huso dauricus, Acipenser schrenckiid). In Journal of Food Processing and Preservation (Vol. 41, Issue 6, p. e13256). Hindawi Limited. https://doi.org/10.1111/jfpp.13256
5. Kowalska, K. (2021). Lingonberry (Vaccinium vitis-idaea L.) Fruit as a Source of Bioactive Compounds with Health-Promoting Effects—A Review. In International Journal of Molecular Sciences (Vol. 22, Issue 10, p. 5126). MDPI AG. https://doi.org/10.3390/ijms22105126
6. Dróżdż, P., Šėžienė, V., Wójcik, J., & Pyrzyńska, K. (2017). Evaluation of Bioactive Compounds, Minerals and Antioxidant Activity of Lingonberry (Vaccinium vitis-idaea L.) Fruits. In Molecules (Vol. 23, Issue 1, p. 53). MDPI AG. https://doi.org/10.3390/molecules23010053
7. Amundsen, M., Hykkerud, A. L., Kelanne, N., Tuominen, S., Schmidt, G., Laaksonen, O., Yang, B., Martinussen, I., Jaakola, L., & Aaby, K. (2023). Composition of Sugars, Organic Acids, Phenolic Compounds, and Volatile Organic Compounds in Lingonberries (Vaccinium vitis-idaea L.) at Five Ripening Stages. In Foods (Vol. 12, Issue 11, p. 2154). MDPI AG. https://doi.org/10.3390/foods12112154
8. Chen, Y., Cai, Y., Wang, K., & Wang, Y. (2023). Bioactive Compounds in Sea Buckthorn and their Efficacy in Preventing and Treating Metabolic Syndrome. In Foods (Vol. 12, Issue 10, p. 1985). MDPI AG. https://doi.org/10.3390/foods12101985
9. Dong, K., Binosha Fernando, W. M. A. D., Durham, R., Stockmann, R., & Jayasena, V. (2021). Nutritional Value, Health-promoting Benefits and Food Application of Sea Buckthorn. In Food Reviews International (Vol. 39, Issue 4, pp. 2122–2137). Informa UK Limited. https://doi.org/10.1080/87559129.2021.1943429

10. Vilas-Franquesa, A., Saldo, J., & Juan, B. (2020). Potential of sea buckthorn-based ingredients for the food and feed industry – a review. In Food Production, Processing and Nutrition (Vol. 2, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s43014-020-00032-y
11. Delgado-Andrade, C., Navarro, M., López, H., & López, M. C. (2003). Determination of total arsenic levels by hydride generation atomic absorption spectrometry in foods from south-east Spain: estimation of daily dietary intake. In Food Additives and Contaminants (Vol. 20, Issue 10, pp. 923–932). Informa UK Limited. https://doi.org/10.1080/02652030310001594450
12. Komarova N. V., Kamensev Ya. S. (2006). Prakticheskoe rukovodstvo po ispolzovaniu system kapillyarnogo elektroforeza. [A Practical Guide to Using Capillary Electrophoresis Systems], Veda, p. 212
13. Fodor-Csorba, K. (1992). Chromatographic methods for the determination of pesticides in foods. In Journal of Chromatography A (Vol. 624, Issues 1–2, pp. 353–367). Elsevier BV. https://doi.org/10.1016/0021-9673(92)85688-p
14. Vilas-Franquesa, A., Saldo, J., & Juan, B. (2020). Potential of sea buckthorn-based ingredients for the food and feed industry – a review. In Food Production, Processing and Nutrition (Vol. 2, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s43014-020-00032-y
15. Saracila, M., Untea, A. E., Panaite, T. D., Varzaru, I., Oancea, A.-G., Turcu, R. P., & Vlaicu, P. A. (2022). Effects of Supplementing Sea Buckthorn Leaves (Hippophae rhamnoides L.) and Chromium (III) in Broiler Diet on the Nutritional Quality and Lipid Oxidative Stability of Meat. In Antioxidants (Vol. 11, Issue 11, p. 2220). MDPI AG. https://doi.org/10.3390/antiox11112220
16. Panjaitan, F. C. A., Yeh, N., & Chai, H. (2020). Effect of different processing on quality changes of barramundi (Lates calcarifer) caviar during chilled storage (4°C). In Journal of Food Processing and Preservation (Vol. 44, Issue 10). Hindawi Limited. https://doi.org/10.1111/jfpp.14790
17. Kopylenko, L. R., & Rubtsova, T. E. (2004). Effect of Pasteurization on the Activity of Proteinases in Salmon Roe. In Applied Biochemistry and Microbiology (Vol. 40, Issue 5, pp. 441–444). Springer Science and Business Media LLC. https://doi.org/10.1023/b:abim.0000040664.96905.d9
18. Staroszczyk, H., Kusznierewicz, B., Malinowska-Pańczyk, E., Sinkiewicz, I., Gottfried, K., & Kołodziejska, I. (2020). Fish gelatin films containing aqueous extracts from phenolic-rich fruit pomace. In LWT (Vol. 117, p. 108613). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.108613
19. Jurevičiūtė, I., Keršienė, M., Bašinskienė, L., Leskauskaitė, D., & Jasutienė, I. (2022). Characterization of Berry Pomace Powders as Dietary Fiber-Rich Food Ingredients with Functional Properties. In Foods (Vol. 11, Issue 5, p. 716). MDPI AG. https://doi.org/10.3390/foods11050716
20. Bazhenova, B., Zhamsaranova, S., Zabalueva, Y., Gerasimov, A., & Zambulaeva, N. (2020). Effects of lingonberry extract on the antioxidant capacity of meat paste. In Foods and Raw Materials (Vol. 8, Issue 2, pp. 250–258). Kemerovo State University. https://doi.org/10.21603/2308-4057-2020-2-250-258
21. Bazhenova, B. A., Zhamsaranova, S. D., Zambulaeva, N. D., Zabalueva, Yu. Y., Gerasimov, A. V., & Syngeeva, E. V. (2020). Methods for improving the preservation of natural antioxidants in meat products. In Proceedings of Universities. Applied Chemistry and Biotechnology (Vol. 10, Issue 1, pp. 84–94). Irkutsk National Research Technical University. https://doi.org/10.21285/2227-2925-2020-10-1-84-94
22. Saracila, M., Untea, A. E., Panaite, T. D., Varzaru, I., Oancea, A.-G., Turcu, R. P., & Vlaicu, P. A. (2022). Effects of Supplementing Sea Buckthorn Leaves (Hippophae rhamnoides L.) and Chromium (III) in Broiler Diet on the Nutritional Quality and Lipid Oxidative Stability of Meat. In Antioxidants (Vol. 11, Issue 11, p. 2220). MDPI AG. https://doi.org/10.3390/antiox11112220
23. Qin, X., Zhang, T., Cao, Y., Deng, B., Zhang, J., & Zhao, J. (2020). Effects of dietary sea buckthorn pomace supplementation on skeletal muscle mass and meat quality in lambs. In Meat Science (Vol. 166, p. 108141). Elsevier BV. https://doi.org/10.1016/j.meatsci.2020.108141
24. Momani Shaker, M., Al-Beitawi, N. A., Bláha, J., & Mahmoud, Z. (2017). The effect of sea buckthorn (Hippophae rhamnoides L.) fruit residues on performance and egg quality of laying hens. In Journal of Applied Animal Research (Vol. 46, Issue 1, pp. 422–426). Informa UK Limited. https://doi.org/10.1080/09712119.2017.1324456
25. Binsi, P. K., Nayak, N., Sarkar, P. C., Sahu, U., Lalitha, K. V., Ninan, G., & Ravishankar, C. N. (2019). Conversion of carp roe mass to caviar substitutes: Stabilization with oregano extract. In LWT (Vol. 108, pp. 446–455). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.03.001
26. Alakomi, H.-L., Maukonen, J., Honkapää, K., Storgårds, E., Quirin, K.-W., Yang, B., & Saarela, M. (2017). Effect of Plant Antimicrobial Agents Containing Marinades on Storage Stability and Microbiological Quality of Broiler Chicken Cuts Packed with Modified Atmosphere Packaging. In Journal of Food Protection (Vol. 80, Issue 10, pp. 1689–1696). Elsevier BV. https://doi.org/10.4315/0362-028x.jfp-17-076

27. Guo, Z., Han, L., Yu, Q., & Lin, L. (2020). Effect of a sea buckthorn pomace extract-esterified potato starch film on the quality and spoilage bacteria of beef jerky sold in supermarket. In Food Chemistry (Vol. 326, p. 127001). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.127001

28. Ren, R., Li, N., Su, C., Wang, Y., Zhao, X., Yang, L., Li, Y., Zhang, B., Chen, J., & Ma, X. (2020). The bioactive components as well as the nutritional and health effects of sea buckthorn. In RSC Advances (Vol. 10, Issue 73, pp. 44654–44671). Royal Society of Chemistry (RSC). https://doi.org/10.1039/d0ra06488b

29. Roasto, M., Mäesaar, M., Püssa, T., Anton, D., Rätsep, R., Elias, T., Jortikka, S., Pärna, M., Kapp, K., Tepper, M., Kerner, K., & Meremäe, K. (2023). The Effect of Fruit and Berry Pomaces on the Growth Dynamics of Microorganisms and Sensory Properties of Marinated Rainbow Trout. In Microorganisms (Vol. 11, Issue 12, p. 2960). MDPI AG. https://doi.org/10.3390/microorganisms11122960

30. Mosharova, M. E., & Titova, I. M. (2022). Influence of vegetable powders obtained from products of processing fruit and vegetable raw materials on technological properties of minced fish. In Vestnik of Astrakhan State Technical University. Series: Fishing industry (Vol. 2022, Issue 2, pp. 115–120). Astrakhan State Technical University. https://doi.org/10.24143/2073-5529-2022-2-115-120

31. SCHULZ, C., KNAUS, U., WIRTH, M., & RENNERT, B. (2005). Effects of varying dietary fatty acid profile on growth performance, fatty acid, body and tissue composition of juvenile pike perch (Sander lucioperca). In Aquaculture Nutrition (Vol. 11, Issue 6, pp. 403–413). Hindawi Limited. https://doi.org/10.1111/j.1365-2095.2005.00369.x

32. Tsibizova, M. E., Ibragimova, A. R., & Kotelnikov, A. V. (2023). Fish culinary products for preschool children. In Vestnik of Astrakhan State Technical University. Series: Fishing industry (Vol. 2023, Issue 2, pp. 91–102). Astrakhan State Technical University. https://doi.org/10.24143/2073-5529-2023-2-91-102

33. İnanlı, A. G., Çoban, Ö. E., Yılmaz, Ö., Özpolat, E., & Kuzgun, N. K. (2019). Assessment of vitamin compositions and cholesterol levels of carp (Cyprinus carpio carpio) and rainbow trout (Oncorhynchus mykiss) caviars. In Ege Journal of Fisheries and Aquatic Sciences (Vol. 36, Issue 3, pp. 293–299). Ege University Faculty of Fisheries. https://doi.org/10.12714/egejfas.2019.36.3.10

34. Winarti, S., Pertiwi, C. N., Hanani, A. Z., Mujamil, S. I., Putra, K. A., & Herlambang, K. C. (2018). Beneficial of Coriander Leaves (Coriandrum sativumL.) to Reduce Heavy Metals Contamination in Rod Shellfish. In Journal of Physics: Conference Series (Vol. 953, p. 012237). IOP Publishing. https://doi.org/10.1088/1742-6596/953/1/012237

35. Tamagno, W. A., Alves, C., Tessaro, D., Sutorillo, N. T., Santin, W., & Barcellos, L. J. G. (2022). Deferoxamine Supplementation Abolished Iron-Related Toxicity of Ilex paraguariensis Extract: Behavioral and Biochemical Evaluation in Adult Zebrafish (Danio rerio). In Antioxidants (Vol. 11, Issue 8, p. 1507). MDPI AG. https://doi.org/10.3390/antiox11081507

36. Abdel Rahman, A. N., ElHady, M., Hassanin, M. E., & Mohamed, A. A.-R. (2019). Alleviative effects of dietary Indian lotus leaves on heavy metals-induced hepato-renal toxicity, oxidative stress, and histopathological alterations in Nile tilapia, Oreochromis niloticus (L.). In Aquaculture (Vol. 509, pp. 198–208). Elsevier BV. https://doi.org/10.1016/j.aquaculture.2019.05.030

37. Jamil Emon, F., Rohani, M. F., Sumaiya, N., Tuj Jannat, M. F., Akter, Y., Shahjahan, M., Abdul Kari, Z., Tahiluddin, A. B., & Goh, K. W. (2023). Bioaccumulation and Bioremediation of Heavy Metals in Fishes—A Review. In Toxics (Vol. 11, Issue 6, p. 510). MDPI AG. https://doi.org/10.3390/toxics11060510

38. Alak, G., Kaynar, Ö., & Atamanalp, M. (2021). The impact of salt concentrations on the physicochemical and microbiological changes of rainbow trout caviar. In Food Bioscience (Vol. 41, p. 100976). Elsevier BV. https://doi.org/10.1016/j.fbio.2021.100976

39. Gürel İnanli, A., Emir Coban, Ö., & Dartay, M. (2010). The chemical and sensorial changes in rainbow trout caviar salted in different ratios during storage. In Fisheries Science (Vol. 76, Issue 5, pp. 879–883). Springer Science and Business Media LLC. https://doi.org/10.1007/s12562-010-0279-6

40. Liato, V., Hammami, R., & Aïder, M. (2017). Influence of electro-activated solutions of weak organic acid salts on microbial quality and overall appearance of blueberries during storage. In Food Microbiology (Vol. 64, pp. 56–64). Elsevier BV. https://doi.org/10.1016/j.fm.2016.12.010

Funds:
This article was not supported by grants.

Acknowledgments: -

Conflict of Interest:
No potential conflict of interest was reported by the author(s).

Ethical Statement:
This article does not contain any studies that would require an ethical statement.

Contact Address:
Zhanna Kazangeldina, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77016530133
E-mail: zhanna.kzb@gmail.com
ORCID: https://orcid.org/0000-0003-4904-5181

Raushangul Uazhanova, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77771301741
E-mail: r.uazhanova@atu.kz
ORCID: https://orcid.org/0000-0001-7991-796X

Gulshat Zhaksylykova, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77016530133
E-mail: g.zhaksylykova@atu.kz
ORCID: https://orcid.org/0000-0003-4529-3292

***Sholpan Abzhanova**, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77753338624
E-mail: sh.abzhanova@atu.kz
ORCID: https://orcid.org/0000-0003-3209-6855

Lyazzat Baibolova, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77016530133
E-mail: l.baybolova@atu.edu.kz
ORCID: https://orcid.org/0000-0002-8118-1581

Kamilya Tyutebayeva, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77016530133
E-mail: l.baybolova@atu.edu.kz
ORCID: https://orcid.org/0000-0002-1991-8714

Gaukhar Kuzembayeva, Almaty Technological University, Department of Technology of Food products, Tole Bi Str., 100, 050000, Almaty, Republic of Kazakhstan,
Tel.: +77753338624
E-mail: zhanna.kzb@gmail.com
ORCID: https://orcid.org/0000-0003-0558-9531

Yerkin Yerzhigitov, Kazakh National Agrarian Research University, Abaya St., 8, 050010, Almaty, Republic of Kazakhstan,
Tel.: +77012661020
E-mail: sh.abzhanova@atu.kz
ORCID: https://orcid.org/0000-0003-2811-5910

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 31.3.2024
Revised: 10.10.2024
Accepted: 13.10.2024
Published: 14.10.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 874-886
https://doi.org/10.5219/1977
ISSN: 1337-0960 online
www.potravinarstvo.com

Effect of flax and hemp flour on the nutritional value of turkey-duck meat pate

Aigul Maizhanova, Kumarbek Amirkhanov, Shugyla Zhakupbekova, Gulnur Nurymkhan, Sholpan Baytukenova, Assel Dautova, Assem Spanova, Rysgul Ashakayeva

ABSTRACT

This study focused on developing a nutritionally enhanced turkey meat pate, incorporating plant-based ingredients like flaxseed and hemp flour. Two canned pate samples were produced: a control sample with turkey meat, liver, heart, fat, skin, beans, onions, and spices, and an experimental sample where 20% of turkey meat was replaced with duck meat, and beans were substituted with zucchini, flaxseed, and hemp flour. The experimental pate showed significant differences in chemical composition compared to the control. It had lower moisture and fat content but higher protein, ash, and carbohydrate content. The energy value slightly decreased from 153.01 kcal/100g to 146.9 kcal/100g. The sensory evaluation found similarities in appearance, consistency, and colour, with the experimental pate receiving a slightly higher colour rating. The amino acid profile of the experimental pate was significantly altered, with increases in methionine, isoleucine, threonine, glutamic acid, alanine, and cysteine. Vitamin and mineral content also significantly increased, particularly vitamins A, D, E, and B group vitamins, calcium, potassium, magnesium, and iron. Microscopic analysis revealed a more heterogeneous microstructure in the experimental pate due to the plant ingredients. This research demonstrates the potential of developing a healthier turkey meat pate using plant-based ingredients, catering to the growing demand for healthier food options.

Keywords: pate, duck meat, flaxseed, hemp flour, canned meat

INTRODUCTION

The turkey meat industry has witnessed significant growth globally and in Kazakhstan due to the increasing popularity of healthy nutrition and the rising demand for processed products such as turkey sausage and ham. The relevance of turkey meat processing is underscored by the popularity of turkey meat in the human diet, owing to its dietary properties **[1]**. Renowned for its rich protein content, phosphorus, B vitamins, PP vitamins, and mineral salts, turkey meat is a valuable ingredient for innovating new product varieties **[2]**, **[3]**. Its exceptional chemical composition, boasting essential minerals such as zinc, potassium, phosphorus, magnesium, and iron, along with significant B vitamin content, notably vitamin B12, and a protein content of 23 g per 100 g, all contribute to its nutritional value **[4]**, **[5]**. Moreover, its low-fat content and minimal cholesterol render it hypoallergenic and suitable for children and the elderly **[6]**.

The combination of turkey and duck meat for producing meat products offers a promising opportunity to enhance the nutritional and biological value of the final product. Introducing duck meat into the recipe with partial replacement of turkey meat can lead to a more balanced product in vitamin, mineral, and fatty acid composition **[7]**. Duck meat is characterised by a more balanced fatty acid composition and a higher content of unsaturated fatty acids, monounsaturated fatty acids, certain minerals, and vitamins than turkey meat **[8]**, **[9]**. Therefore,

combining these two meats can result in a product with enhanced nutritional benefits and unique sensory properties.

The development of functional meat products enriched with dietary fibers, vitamins, and mineral substances is gaining attention due to the growing demand for healthier food options. This trend is driven by the potential to enrich meat products with bioactive substances from raw vegetable materials, enhancing their nutritional value. Flax, for instance, is a dietary product rich in fatty acids, vitamins of various groups, antioxidants, and trace elements. The seed squeeze of flax is a source of essential micro- and macroelements for humans, including potassium, phosphorus, sodium, manganese, zinc, and nickel **[10]**, **[11]**. Moreover, flaxseed cake, which contains 30% dietary fiber, is rich in natural phenolic compounds – lignans **[12]**.

Flaxseed-derived components, such as mucilage, exhibit remarkable potential as meat binders due to their synergistic interactions with meat proteins, thermal stability, and desirable gel properties, even in high-salt environments **[13]**. Previous research has consistently demonstrated the positive impacts of incorporating flaxseed flour into meat products. Studies have shown significant improvements in organoleptic properties and functional-technological aspects of minced meat, including increased water-holding capacity and enhanced product yield **[14]**. Similarly, Sannikov & Gurinovich (2019) observed a rise in pH and water-binding capacity when adding flaxseed flour to minced meat. Their research focused on optimising the incorporation level in poultry semi-smoked sausage, ultimately establishing a 10% addition based on organoleptic evaluation **[15]**. Ayrapetyan et al. (2022) have demonstrated that including 4.4% flax meal positively affects the qualitative composition of protein, improves the fatty acid composition, and enhances the content of dietary fiber and polyphenolic compounds **[16]**.

Another promising ingredient is hemp flour, obtained from hemp cake after cold pressing of hemp seed oils. The chemical composition of hemp cake includes water (11%), crude protein (31%), protein (29.6%), fat (7.7%), fiber (24.7%), and nitrogen-free extract substances (17.7%). Notably, hemp cake is rich in phytin (calcium-magnesium salt of inositophosphoric acid), which stimulates hematopoiesis, enhances the growth and development of bone tissue, and improves the function of the nervous system in diseases associated with phosphorus deficiency in the body **[17]**, **[18]**. Vaitanis and Khodyreva (2021) developed recipes for turkey meat stuffing with hemp flour, which provided significant nutritional benefits. In a serving of 100g of stuffed turkey meat with 20% hemp flour, consumers could meet their daily requirements for dietary fiber, vitamin B, potassium, calcium, magnesium, phosphorus, iron, selenium, and zinc **[19]**. Further studies by Kerner et al. (2021) have demonstrated the feasibility of incorporating hemp seed press-cake ingredients into the production of pork meat burger patties **[20]**. These findings underscore the versatility of hemp-derived components in diversifying the formulation of meat products, offering opportunities to enhance both nutritional content and sensory attributes. Building upon these insights, this research investigates the potential utilisation of flax and hemp seed-derived ingredients in developing turkey meat pate.

Scientific hypothesis

Incorporating flaxseed and hemp flour in turkey meat pate can improve nutritional value by increasing protein, vitamin, and mineral content without deteriorating sensory properties.

MATERIAL AND METHODOLOGY

Samples

Turkey meat, duck meat, and by-products were purchased from Semey (Kazakhstan) meat pavilions. Flax flour (protein 32.4 g, fat 8.8 g, carbohydrates 6.9 g) and hemp flour (protein 30 g, fat 7.9 g, carbohydrates 24.7 g) from the company " Kompas Zdorovya" (Russia) were purchased in local supermarkets. According to the recipe, other ingredients and spices were purchased in local supermarkets.

Chemicals and biological material

Hydrochloric acid (mass fraction of hydrochloric acid (HCl), 35-38%, pure for analysis, Snabservice Astana LLP, Astana, Kazakhstan).

Formalin (mass fraction of formaldehyde 37.0%, Chemical Industrial Reagent LLP, Shymkent, Kazakhstan). Hematoxylin and eosin alcohol solution (ErgoProduction Ltd., Russia).

Instruments

Dionex Ultimate 3000 chromatograph ("Dionex", USA)

SHIMADZU LC-20 Prominence HPLC (Japan) with Supelco SUPELCOSIL LC-PAH 5 µm 4.6 x 150 mm column

microtome MS-2

Laboratory Methods

Method of determining the chemical composition

The moisture content was determined using the method described in **[21]**. After the moisture content was determined, each dried sample was used for fat determination, according to **[22]**. The samples were calcined in a muffle furnace (500°C-600°C) to measure the ash content **[23]**. The protein content was analysed according to **[24]**.

Determination of mineral content

All samples for determining the mineral content were weighed 5 g each, placed on a container and incinerated in a microwave muffle for 12 hours to a final temperature of 600°C. After microwave splitting, the samples were diluted with 10ml hydrochloric acid (HCl) in distilled water (1:1), mixed with a glass rod and passed through a paper filter. Mineral elements were determined on a spectrometer ICP-OES (Spectro, Boschstr, Germany) **[25]**.

Determination of vitamin composition

Vitamin content was determined according to GOST 55482-2013 **[26]** for water-soluble vitamins using single-substituted potassium phosphate buffer solution and GOST 32307-2013 for fat-soluble vitamins **[27]**. The analysis was performed on a Dionex Ultimate 3000 chromatograph ("Dionex", USA) with a Supelco SUPELCOSIL LC-PAH 5 µm 4.6 x 150 mm column. Eluent composition: a mixture of methanol and distilled water (92:8).

Determination of amino acid composition

Amino acids were determined using SHIMADZU LC-20 Prominence HPLC (Japan) with fluorimetric and spectrophotometric detectors. We used a chromatographic column 25 cm x 4.6 mm SUPELCO C18, with a diameter of 5 µm (USA), including a pre-column to protect the main column from foreign impurities. The HPLC analysis was based on the method. Chromatographic analysis was performed in the eluent gradient mode at a 1.2 mL/min flow rate and a column thermostat temperature of 40 °C. The measurement was carried out on a reverse phase column with fluorimetric and spectrophotometric detectors at 246 and 260 nm wavelengths using acid hydrolysis and modification of amino acids with a solution of phenylisothiocyanate in isopropanol to obtain phenylthiohydantoines. A 6.0 mm solution of CH_3COONa with a pH of 5.5 (component A), a 1% solution of isopropanol in acetonitrile (component B), and a 6.0 mM solution of CH_3COONa with a pH of 4.05 (component C) were used as the mobile phase. Samples of amino acids produced by Sigma Aldrich (Germany) were used as standards.

Sensory analysis

Sensory analysis of samples was carried out according to the Interstate standard GOST 33741-2015 **[28]**. Sensory analysis of control and experimental samples of patés was carried out using the profile method. The following indicators were considered: taste, odour, and consistency. In connection with the addition of different components, the following descriptors were selected: taste (sweet, pumpkin, carrot, salty, sharp, spicy, fatty, liver); colour (brown-grey, gray); smell (sharp liver, sharp fatty, specific, pronounced); consistency (delicate, smeary, dry, loose, stiff, watery, fibrous). The intensity of each descriptor was evaluated on a scale from 0 to 5 (if no expression of any characteristic was observed, the intensity was evaluated as zero). After statistical processing, the results were obtained, according to which the profiles were designed.

Histological Study

The minced meat samples for histological study are frozen for fixation and, without defrosting, are placed in fixing liquid -10% neutral formalin. Non-frozen minced meat samples with and without vegetable additives are taken for fixation immediately after obtaining minced meat. They are carefully placed in 10% neutral formalin to avoid destroying the block formed by minced meat. After fixation, the minced meat samples are filled with paraffin. The slices are obtained on sled microtome MS-2 with a 10-30 µm thickness. The sections are stained with hematoxylin and eosin according to the conventional technique. Hematoxylin stains cell nuclei, eosin stains cell protoplasm, and various noncellular structures to a lesser extent. The technique consists of staining the sample with Mayer's hematoxylin for 1 min, flushing with running water and rinsing with distilled water. After that, it is treated with Eosin solution (10 s) for staining in blue colour and rinsed with water. Microscopic investigation of prepared sections is carried out using an MS-100 trinocular microscope. Digital microphotographs are taken using a digital microphotography adapter with a resolution of 9 megapixels at × 4, × 10, and × 40 magnifications **[29]**.

Description of the Experiment

Sample preparation

Production of canned meat pate:

Meat pate contains turkey and duck meat, liver, heart, skin, turkey or duck fat, hemp and flax flour, onion, nutmeg, paprika, ground black pepper, sodium chloride, and broth (Table 1).

We produced two samples of turkey meat pates as canned products. The first sample (control) contains turkey meat, liver, heart, fat, turkey skin, beans, onions and spices. The second sample (experimental) additionally contains duck meat (20% of turkey meat was substituted), and zucchini, flaxseed, and hemp flour were added instead of beans.

Table 1. Recipe of control and experimental samples of meat pate

Ingredient	Control	Experimental
Turkey meat	50	30
Duck meat	-	20
Poultry liver	12	12
Poultry heart	10	10
Poultry fat	4	4
Poultry skin	3	3
Beans	10	-
Zucchini	-	4
Flax flour	-	3
Hemp flour	-	3
Onions	6	6
Cream 10%	5	-
Water for hydration	4	9
Broth	20	20
Caraway	0.2	-
Nutmeg	-	0.2
Turmeric	0,2	-
Paprika	-	0.2
Ground black pepper	0.1	0.1
Salt	1.4	1.4

The production method of the meat-vegetable pâté is carried out as follows. Turkey and duck meat are washed in running water and then separated from bones, skin, and tendons. The meat is preliminarily cut into large pieces. Next, the liver and skin of the poultry, as well as the duck fat, are blanched. The raw materials are loaded into a blancher or pot, and 4-6% of hot water is added based on the weight of the raw materials, and the mixture is blanched for 30-40 minutes. Blanching the raw materials in their juice allows broth production with the required concentration (15-20% solids).

Subsequently, all the meat raw materials are ground, first in a wolf grinder with a screen diameter of 2-3 mm, then in a cutter for 5-8 minutes until a homogeneous paste-like mass is obtained. Initially, coarser raw materials are loaded: duck and turkey meat and hearts, followed by blanched liver, skin, and poultry fat. Then, flaxseed and hemp flour, grated zucchini and onion are added. During the cutting process, table salt, paprika, nutmeg, ground black pepper, and broth are added. The prepared pâté mass is immediately transferred to the packaging.

Packaging and sealing: The pâté mass is packaged in metal cans (height 70 mm, diameter 76 mm) and sealed hermetically. The net mass of the canned food in cans No. 4 should be 250 g. Automatic doors carry out the filling of the cans. The filled cans are hermetically sealed on sealing machines. The sealing seam should be hermetic, smooth, without burrs, cuts, or wrinkles. Sealed cans, after washing, are loaded into autoclave baskets and sent for sterilisation. The time from can sealing to the start of sterilisation should not exceed 30 minutes.

Sterilisation and cooling: The sterilisation regime for canned food in cans No. 4 is as follows:

$$\frac{20 - 65 - 20}{112\ ℃} 0.08;$$

where:
20 – duration of temperature rise in the autoclave to the set value, min;
65 – duration of canned food holding time (sterilisation), min;
20 – duration of temperature decrease (cooling), min;
112 – sterilisation temperature (ºC);
0.08 – pressure in the autoclave, MPa.

Sorting: After sterilisation, the cans are unloaded and sent for sorting. The sorting is carried out visually, with the separation of cans with manufacturing defects.

Number of samples analyzed: 30 samples of canned meat pate were analysed.
Number of repeated analyses: Each study was carried out 5 times.
Number of experiment replications: The study was repeated three times, with the experimental data processed using mathematical statistics methods.

Design of the experiment: We produced 30 cans of pâté (15 control and 15 experimental) at the initial research stage. We then studied the finished pates' chemical, amino acid, vitamin, and mineral compositions and sensory and microstructural analyses.

Statistical Analysis

All data was subjected to the analysis of variance (ANOVA) using STATISTICA 13 software. When significant differences were found, the Tukey test was used to determine significant differences between individual means ($P < 0.05$). The results are presented as average values ± standard deviation.

RESULTS AND DISCUSSION

Chemical composition of canned meat pate:

The chemical composition of the control and experimental samples is presented in Table 2. The results of the chemical composition of the meat pate showed an increase in protein content by 1.77%, carbohydrates by 4.41%, ash by 1.1%, and a decrease in fat by 3.38% in the experimental samples compared to the control sample.

Table 2 Chemical composition of canned meat pate (mean±SD).

Indicator	Control	Experimental
Moisture, %	72.4±1.4	68.6±0.6
Protein, %	16.73±0.27	18.50±0.39*
Fat, %	9.48±0.14*	6.10±0.08
Ash, %	1.21±0.02	2.30±0.05*
Carbohydrate, %	0.19±0.00	4.50±0.06*
Energy value, kcal/100g	153.01	146.9
Mass fraction of sodium chloride, %	1.5	1.5

Note: *p<0.05

The experimental sample displayed notable alterations in its chemical composition compared to the control sample. The moisture content of the experimental sample decreased from 72.4% in the control to 68.6%, indicating a reduction in water content. This reduction can be attributed to incorporating additional solid ingredients, such as duck meat, zucchini, and flaxseed, which inherently contain lower moisture levels than beans. The protein content increased significantly from 16.73% to 18.50% ($p<0.05$), likely due to the high protein content of duck meat and hemp flour. This increase in protein content enhances the nutritional value of the pate, making it a more substantial source of this essential macronutrient.

Conversely, the fat content in the experimental sample (6.10%) was lower than that of the control (9.48%). This reduction in fat content could be attributed to several factors, including substituting turkey meat with leaner duck meat and incorporating zucchini, which has a lower fat content than some of the original ingredients in the control sample. Additionally, although sources of healthy fats, flaxseed and hemp flour may contribute little to the overall fat content due to their incorporation in smaller quantities.

The ash content increased from 1.21% to 2.30% ($p<0.05$), indicating a higher mineral content in the experimental sample, likely due to the mineral-rich flaxseed and hemp flour.

The carbohydrate content significantly increased from 0.19% to 4.50% ($p<0.05$), which can be attributed to the carbohydrates in zucchini, flaxseed, and hemp flour.

Despite the changes in the macronutrient composition, the pate's energy value decreased slightly from 153.01 kcal/100g to 146.9 kcal/100g. This decrease is likely due to the reduced fat content, as fat has a higher caloric value per gram than proteins and carbohydrates.

The introduction of duck meat, zucchini, flaxseed, and hemp flour in the production of turkey meat pate significantly altered its chemical composition and energy value. These changes resulted in a product with a higher protein and lower fat content, aligning with the increasing consumer demand for healthier food options.

These results are consistent with those reported by **[30]** and **[31]**, who observed a reduced moisture content and increased carbohydrates and minerals by adding flaxseed meal to beef pate. A study **[32]** achieved protein content comparable to our experimental sample, albeit with a different ingredient, eagle fern powder. Despite the distinct source, this indicates the potential of alternative additives in enhancing protein levels in meat products. It was demonstrated in the work **[33]** that poultry pate with flaxseed meal had a higher fat content than our study's results. This discrepancy could stem from variations in formulation or processing techniques, highlighting the importance of precise ingredient proportions and preparation methods. Both studies **[34]**, **[35]** corroborate our findings regarding the effects of hemp flour and hemp cake on moisture reduction and increased ash content in meat products.

Sensory analysis

Comparison of sensory characteristics between the control and experimental samples reveals overall similarities in appearance, consistency, and colour, while differences in odour and taste are noted. The experimental sample received a slightly higher rating for colour (4.6) than the control sample (4.4). This suggests that adding alternative ingredients contributed to a slightly more vibrant or appealing colour in the experimental sample (Figure 1).

Overall, adding duck meat, zucchini, flaxseed, and hemp flour in the canned turkey meat pate production resulted in minor differences in sensory characteristics compared to the control sample. While both samples were well-received in terms of appearance, consistency, and colour, the experimental sample exhibited slightly improved odour and taste, suggesting that adding alternative ingredients enhanced the overall sensory experience of the product. The integrated duck meat has positively influenced the overall appearance and colour of the product, adding richness and depth. Zucchini has contributed to a more appealing consistency, subtly altering the colour. Notably, incorporating flaxseed and hemp flour has elevated the overall aroma and taste, making the experimental sample more robust in sensory attributes. These findings highlight the potential of incorporating alternative ingredients to improve the sensory properties of processed meat products, offering opportunities for innovation and market differentiation in the food industry.

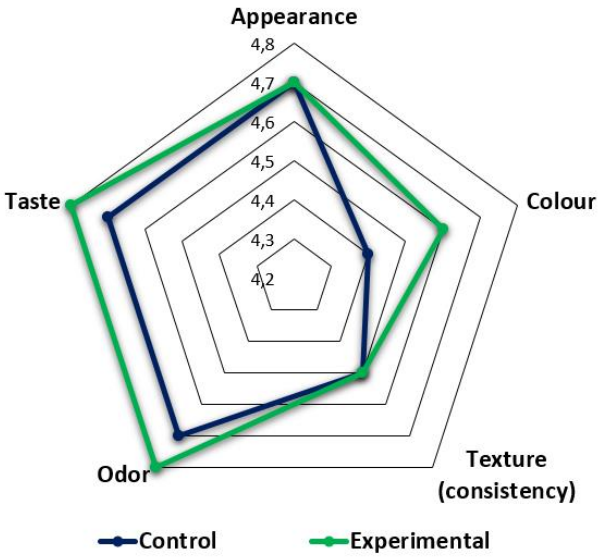


Figure 1 Sensory profile of meat pate (control and experimental samples).

Amino acid composition

The findings of the amino acid composition analysis of the experimental and control samples indicated that the experimental sample contained a higher concentration of amino acids than the control sample. Notably, the experimental sample demonstrated a significant lead in glutamic acid content by 4.7%, attributed to adding hemp flour, as it contains 5.85 g/100g. Furthermore, the experimental sample exhibited higher levels of essential amino acids, such as methionine by 0.09%, isoleucine by 0.18%, threonine by 0.58%, and tryptophan by 0.1%. This elevated concentration of these amino acids can be explained by incorporating flax and hemp flour, both plant-based protein sources.

Adding duck meat, zucchini, flaxseed, and hemp flour to the turkey meat pate significantly altered the product's amino acid composition. The most notable changes were observed in methionine, isoleucine, glutamic acid, alanine, cystine, threonine, and asparagine levels.

Methionine, another essential amino acid crucial for protein synthesis and metabolism, increased from 380 mg/100g in the control sample to 470 mg/100g in the experimental sample (Table 3). This elevation could be linked to adding flaxseed and hemp flour, rich methionine sources **[36]**, **[37]**. The higher methionine content in the experimental sample enhances its nutritional value, contributing to the overall amino acid profile.

Table 3 Amino acid composition of meat pate, g/100g of the product.

Name of amino acid	Control	Experimental
Asparagine	1.21±0.02^{a}	1.43±0.02^{b}
Glutamic	2.04±0.02^{a}	6.75±0.12^{b}
Serine	0.60±0.01^{b}	0.55±0.01^{a}
Histidine	0.33±0.00^{a}	0.35±0.00^{a}
Glycine	1.03±0.02^{b}	0.87±0.02^{a}
Proline	0.74±0.02^{b}	0.62±0.01^{a}
Arginine	0.76±0.01^{a}	0.79±0.01^{a}
Alanine	0.82±0.01^{a}	1.17±0.02^{b}
Tyrosine	0.48±0.01^{a}	0.58±0.01^{b}
cystine	0.04±0.00^{a}	0.30±0.01^{b}
Valine	0.58±0.01^{b}	0.51±0.01^{a}
Methionine	0.38±0.01^{a}	0.47±0.01^{b}
Phenylalanine	0.51±0.01^{b}	0.38±0.00^{a}
Isoleucine	0.57±0.01^{a}	0.75±0.01^{b}
Leucine	1.09±0.02^{b}	0.84±0.01^{a}
Lysine	1.25±0.03^{b}	1.01±0.01^{a}
Threonine	0.54±0.01^{a}	1.12±0.01^{b}
Tryptophan	0.21±0.00^{a}	0.19±0.00^{a}
Total	12.97	18.68

Note: $^{a-b}$ Mean ± Standard Deviation in the same row with different superscripts indicates that there are significant differences ($p < 0.01$)

Isoleucine and leucine, essential amino acids involved in protein synthesis and muscle repair, exhibited varying trends in the experimental sample compared to the control. Isoleucine content increased from 570 mg/100g to 750 mg/100g, while leucine content decreased from 1090 mg/100g to 840 mg/100g. Threonine, essential for protein synthesis and immune function **[38]**, increased from 540 mg/100g in the control sample to 1120 mg/100g in the experimental sample.

Glutamic acid content increased significantly ($p<0.001$) from 2010 mg/100g in the control sample to 6750 mg/100g in the experimental sample. This substantial increase can be attributed to the presence of duck meat, which is known to be rich in glutamic acid. Glutamic acid is a non-essential amino acid crucial in various physiological processes, including energy metabolism and neurotransmission. It also contributes to the characteristic savoury taste of meat products **[39]**, **[40]**.

Alanine content also significantly increased ($p<0.001$) from 820 mg/100g in the control sample to 1170 mg/100g in the experimental sample. Alanine is another non-essential amino acid involved in several metabolic pathways, including protein synthesis and gluconeogenesis. It contributes to meat products' overall flavour and texture **[41]**.

Cystine content dramatically increased ($p<0.001$) from 40 mg/100g in the control sample to 300 mg/100g in the experimental sample. This significant change is likely due to the addition of flaxseed and hemp flour, which are rich sources of cysteine. Cystine is a sulfur-containing amino acid vital to protein structure and function. It also involves antioxidant defence mechanisms and immune function **[42]**.

In contrast, the levels of other amino acids, including phenylalanine, leucine, lysine, tryptophan, serine, histidine, glycine, proline, and arginine, were reduced in the experimental samples. This reduction may have been influenced by changes in protein sources and the addition of alternative ingredients, which could have altered the amino acid content of the final product. These findings suggest that adding these ingredients can enhance the nutritional value of the pâté by increasing the content of certain amino acids.

The amino acid scoring analysis revealed that all essential amino acids in the control sample were limiting. In contrast, the experimental sample contained higher levels of isoleucine by 5%, lysine by 1%, methionine + cystine by 22%, threonine by 55%, and tryptophan by 10% compared to the ideal protein recommended by the FAO/WHO. These findings suggest that the experimental sample has a more favourable amino acid profile than the control sample.

Vitamin composition

The analysis of the experimental sample's vitamin composition for vitamins A, D, E, B1, B3, B5, and B6 revealed that the experimental sample contained higher levels of these vitamins than the control sample. This can be attributed to adding plant-based raw materials such as hemp and flax flour. The vitamin composition results for both the control and experimental samples are presented in Table 4. These findings suggest that incorporating hemp and flax flour may enhance the product's nutritional value by increasing its vitamin content.

Table 4 Vitamin composition.

Vitamin	Control	Experimental
A , µg/100 g	n/d	69.68±1.12
D, µg/100 g	1.04±0.01	2.25±0.04*
E, mg/100 g	0.24±0.00	0.32±0.01*
B_1 , mg/100 g	0.05±0.00	0.40±0.01*
B_3 , mg/100 g	6.72±0.14	9.36±0.20*
B_5 , mg/100 g	1.42±0.02	4.82±0.07*
B_6 , mg/100 g	0.37±0.01	0.66±0.02*

Note: n/d – not detected; *$p<0.05$

The introduction of duck meat, zucchini, flaxseed, and hemp flour to the production of canned turkey meat pâté resulted in a significant increase in the content of several vitamins in the experimental sample compared to the control sample. Specifically, the experimental sample showed a significant increase ($p<0.05$) in the content of vitamins A, D, E, B1, B3, B5, and B6. The most notable increase was observed in the content of vitamin A, which was 69.68 mcg/100g in the experimental sample, compared to not detected in the control sample.

Mineral composition

The observed increase in mineral content can be attributed to the addition of hemp and flax flour, which are rich in minerals. The mineral composition results for the control and experimental samples are presented in Table 5.

Table 5 Mineral composition.

Mineral	Control	Experimental
Calcium, mg/100g	1.42±0.03	8.47±0.15*
Potassium, mg/100g	369±4	434±6*
Magnesium, mg/100g	25.4±0.34	45.3±0.78*
Iron, mg/100g	3.47 ±0.05	4.39±0.07*

Note: *$p<0.05$

Comparing the mineral composition of the control and experimental samples reveals substantial improvements in the mineral content of the experimental sample, indicative of the positive effects of incorporating alternative ingredients.

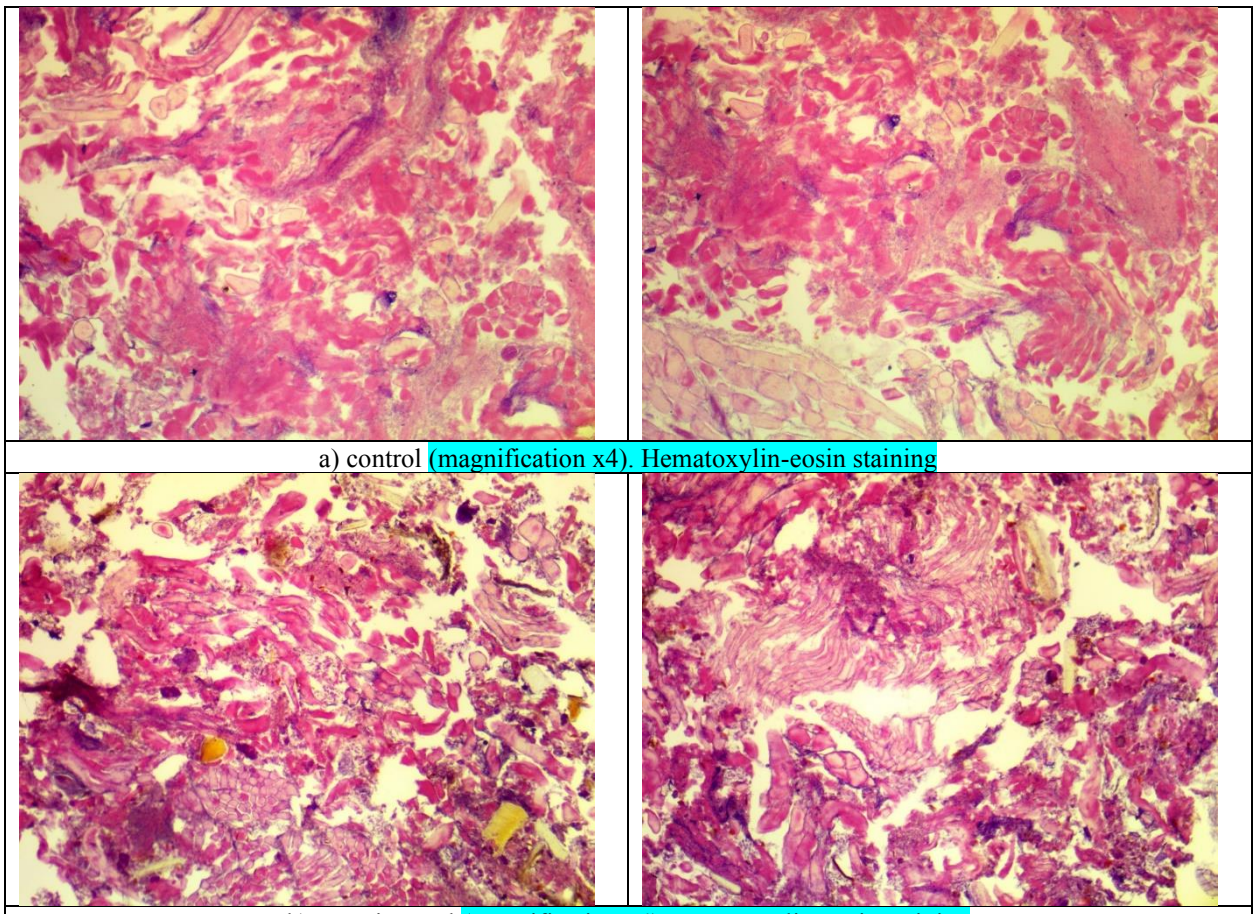
The increase in mineral content in the experimental sample is statistically significant ($p<0.05$), indicating that adding duck meat, zucchini, flaxseed, and hemp flour positively influences the mineral composition of the canned meat pate.

Calcium significantly increased from 1.42 mg/100g in the control sample to 8.47 mg/100g in the experimental sample ($p<0.05$). This notable elevation can be attributed to the inclusion of ingredients such as zucchini, flaxseed, and hemp flour, which are rich sources of calcium. Calcium is essential for bone health and muscle function **[43]**. The experimental sample showed higher levels of potassium (434 mg/100g), magnesium (45.3 mg/100g), and iron (4.39 mg/100g). The notable enhancement in the mineral content of the experimental pate is due to the inclusion of duck meat, which is typically richer in iron and potassium than turkey meat; zucchini which adds potassium and magnesium; and flaxseed and hemp flour, both of which are excellent sources of calcium, magnesium, and iron **[44]**. Additionally, Zając et al. (2019) **[34]** highlighted the enhanced mineral content with hemp flour addition, consistent with our observations.

Histologic analysis

The microscopic microstructure analysis provides valuable insights into the effects of flaxseed and hemp flour on the structure of canned meat pate. Comparing the control and experimental samples reveals distinct differences in the distribution and arrangement of tissue components, highlighting the impact of the added ingredients on the microstructure of the final product.

The control sample showed transversely and longitudinally dissected muscle fibres and fragments of connective tissue. Connective tissue fragments, characterised by fibres and fat cells, are also observed within the minced meat. The boundaries between muscle fibres are well-defined; some appear disintegrated into smaller fragments (Figure 2, a). At medium microscope magnification, preserved nuclei of muscle fibres and cells of connective tissue elements are distinguishable. Additionally, transverse striations of muscle fibres are noted, indicating the typical structure of meat tissue.



a) control (magnification x4). Hematoxylin-eosin staining

b) experimental (magnification x4). Hematoxylin-eosin staining

Figure 2 Microstructure of meat pate samples.

In contrast, the micrograph of the experimental sample reveals a more heterogeneous microstructure. Loosely arranged muscle fibres are interspersed with connective tissue fragments and large and small tissue particles of

flax and hemp flour (Figure 2, b). The nuclei of the plant supplements appear rounded and much larger than those of muscle fibres and connective tissue cells. Zachesova et al. (2018) noted that the addition of topinambur powder in the range of 8 to 10% leads to a loosening of the ground meat structure, with microvoids located between structural elements increasing on average by 1.5 times compared to the control sample **[45]**.

Furthermore, intracellular inclusions in the form of rounded pink formations and grains of pink color, stained with hematoxylin-eosin, are observed within the plant tissue particles. These inclusions indicate the presence of specific cellular structures or components within the added plant ingredients. Plant tissue nuclei are predominantly located in the centre of cells, highlighting the distinct organisation of plant-based tissues compared to animal-based tissues.

CONCLUSION

This reformulation offers a promising strategy to enhance the nutritional value of processed meat products without compromising consumer preference. The analysis of physicochemical indicators revealed that the meat pâté made from turkey and duck meat possesses high biological and nutritional value. The developed meat-based pâté enables the enrichment of the diet with minerals such as calcium, potassium, magnesium, and iron. Consumption of this meat-based pâté increases the body's resistance to negative environmental factors. This pâté is recommended for inclusion in the diet for mass catering. The results of this study suggest that incorporating flax and hemp seed-derived ingredients in turkey meat pate can provide a promising opportunity to develop functional meat products that meet the growing demand for healthier food options. Future studies can focus on optimising the formulation and sensory characteristics of the product to enhance consumer acceptance and preference.

REFERENCES

1. Johnson, R. Global turkey meat market: key findings and insights. Accessed 29 Feb 2024. https://www.thepoultrysite.com/news/2018/05/global-turkey-meat-market-key-findings-and-insights
2. Gök, V., & Bor, Y. (2016). Effect of Marination with Fruit and Vegetable Juice on the Some Quality Characteristics of Turkey Breast Meat. In Revista Brasileira de Ciência Avícola (Vol. 18, Issue 3, pp. 481–488). FapUNIFESP (SciELO). https://doi.org/10.1590/1806-9061-2016-0225
3. Amirkhanov, K., Igenbayev, A., Nurgazezov, A., Okuskhanov, E., Kassymov, S., Muslimova, N., & Yessimbekov, Z. (2017). Comparative Analysis of Red and White Turkey Meat Quality. In Pakistan Journal of Nutrition (Vol. 16, Issue 6, pp. 412–416). Science Alert. https://doi.org/10.3923/pjn.2017.412.416
4. Kambarova, A., Nurgazezova, A., Nurymkhan, G., Atambayeva, Z., Smolnikova, F., REBEZOV, M., Issayeva, K., Kazhibaeva, G., Asirzhanova, Z. & Moldabaeva, Z. (2021). Improvement of quality characteristics of turkey pâté through optimization of a protein rich ingredient: physicochemical analysis and sensory evaluation. In Food Science and Technology (Vol. 41, Issue 1, pp. 203–209). FapUNIFESP (SciELO). https://doi.org/10.1590/fst.00720
5. Wang, D., & Shahidi, F. (2018). Protein hydrolysate from turkey meat and optimization of its antioxidant potential by response surface methodology. In Poultry Science (Vol. 97, Issue 5, pp. 1824–1831). Elsevier BV. https://doi.org/10.3382/ps/pex457
6. Igenbayev, A., Okuskhanova, E., Nurgazezova, A., Rebezov, Y., Kassymov, S., Nurymkhan, G., Tazeddinova, D., Mironova, I., & Rebezov, M. (2019). Fatty Acid Composition of Female Turkey Muscles in Kazakhstan. In Journal of World's Poultry Research (Vol. 9, Issue 2, pp. 78–81). Journal of World's Poultry Research. https://doi.org/10.36380/jwpr.2019.9
7. Kokoszyński, D., Bernacki, Z., Biegniewska, M., Saleh, M., Stęczny, K., Zwierzyński, R., Kotowicz, M., Sobczak, M., Żochowska-Kujawska, J., Wasilewski, P. D., Bucek, T., & Kmiecik, M. (2020). Carcass, physicochemical and sensory characteristics of meat from genetic reserve ducks after two reproductive seasons. In South African Journal of Animal Science (Vol. 50, Issue 1, pp. 55–68). African Journals Online (AJOL). https://doi.org/10.4314/sajas.v50i1.7
8. Ghosh, S., Saha, M., Habib, M., & Sahu, N. C. (2022). Growth Performance and Meat Quality of White Pekin Ducks Reared in Backyard Farming System. In Asian Journal of Dairy and Food Research (Issue Of). Agricultural Research Communication Center. https://doi.org/10.18805/ajdfr.dr-1883
9. Wasilewski, R., Kokoszyński, D., & Włodarczyk, K. (2023). Fatty Acid Profile, Health Lipid Indices, and Sensory Properties of Meat from Pekin Ducks of Different Origins. In Animals (Vol. 13, Issue 13, p. 2066). MDPI AG. https://doi.org/10.3390/ani13132066

10. Raole, V. M., & Raole, V. V. (2022). Flaxseed and Seed Oil: Functional Food and Dietary Support for Health. In EAS Journal of Nutrition and Food Sciences (Vol. 4, Issue 2, pp. 68–77). SASPR Edu International Pvt. Ltd. https://doi.org/10.36349/easjnfs.2022.v04i02.007
11. Kaur, P., Waghmare, R., Kumar, V., Rasane, P., Kaur, S., & Gat, Y. (2018). Recent advances in utilization of flaxseed as potential source for value addition. In OCL (Vol. 25, Issue 3, p. A304). EDP Sciences. https://doi.org/10.1051/ocl/2018018
12. Novello, D., Schiessel, D. L., Santos, E. F., & Pollonio, M. A. R. (2019). The effect of golden flaxseed and by-product addition in beef patties: physicochemical properties and sensory acceptance. In International food research journal (Vol. 26. Issue 4, pp. 1237-1248). Universiti Putra Malaysia.
13. Chen, H.-H., Xu, S.-Y., & Wang, Z. (2007). Interaction between flaxseed gum and meat protein. In Journal of Food Engineering (Vol. 80, Issue 4, pp. 1051–1059). Elsevier BV. https://doi.org/10.1016/j.jfoodeng.2006.08.017
14. Tkachenko, M.N. (2019). Justification for the use of flax flour in the recipe of semi-finished meat products. In Scientific and Technical Support of the Agro-Industrial Complex in the Implementation of the State Program for the Development of Agriculture until 2020. Kurgan, April 18-19, 2019 (pp. 778-781).
15. Sannikov, P.V. & Gurinovich, G.V. (2019). Development of the technology of combined semi-smoked sausages using poultry meat and flax flour. In Innovative Convention "Kuzbass: Education, Science, Innovations" Kemerovo, December 14, 2018 (pp. 202-206).
16. Ayrapetyan, A.A., Manzhesov, V.I., & Glotova, I.A. (2022). The effect of flax flour on the consumer properties of minced meat systems. In International Research Journal 6-5 (120), 26-34.
17. Farinon, B., Molinari, R., Costantini, L., & Merendino, N. (2020). The Seed of Industrial Hemp (Cannabis sativa L.): Nutritional Quality and Potential Functionality for Human Health and Nutrition. In Nutrients (Vol. 12, Issue 7, p. 1935). MDPI AG. https://doi.org/10.3390/nu12071935
18. Alonso-Esteban, J. I., Pinela, J., Ćirić, A., Calhelha, R. C., Soković, M., Ferreira, I. C. F. R., Barros, L., Torija-Isasa, E., & Sánchez-Mata, M. de C. (2022). Chemical composition and biological activities of whole and dehulled hemp (Cannabis sativa L.) seeds. In Food Chemistry (Vol. 374, p. 131754). Elsevier BV. https://doi.org/10.1016/j.foodchem.2021.131754
19. Vaitanis, M.A., & Khodyreva, Z.R. (2021). The use of hemp flour in the production of minced meat semi-finished products. In Bulletin of the Krasnoyarsk State Agrarian University (Vol. 1, Issue 166, pp. 126-133). Krasnoyarsk State Agrarian University.
20. Kerner, K., Jõudu, I., Tänavots, A., & Venskutonis, P. R. (2021). Application of Raw and Defatted by Supercritical CO2 Hemp Seed Press-Cake and Sweet Grass Antioxidant Extract in Pork Burger Patties. In Foods (Vol. 10, Issue 8, p. 1904). MDPI AG. https://doi.org/10.3390/foods10081904
21. Antipova, L.V.; Glotova, I.A.; Rogov, I. A. (2001). Meat and meat products research methods. “KoloS” Publishing House, Moscow.
22. GOST 23042-86 Interstate standard "Meat and meat products. Methods of determination of fat".
23. GOST R 53642-2009. Meat and meat products. Method for determining the mass fraction of total ash.
24. GOST 25011-81 Interstate standard "Meat and meat products. Methods of determination of protein".
25. Nunes, A. M., de Sousa, R. A., da Silva, C. S., Peixoto, R. R. A., Vieira, M. A., Ribeiro, A. S., & Cadore, S. (2013). Fast determination of Fe, Mg, Mn, P and Zn in meat samples by inductively coupled plasma optical emission spectrometry after alkaline solubilization. In Journal of Food Composition and Analysis (Vol. 32, Issue 1, pp. 1–5). Elsevier BV. https://doi.org/10.1016/j.jfca.2013.08.004
26. GOST R 55482-2013 Meat and meat products. Method for determination of water-soluble vitamins content. - Moscow: Stand-ardinform. 2014.
27. GOST 32307-2013 Meat and meat products. Determination of fat-soluble vitamins by high performance liquid chromatography. - Moscow: Standardinform. 2014.
28. GOST 33741-2015 "Canned meat and meat-containing. Methods for determining organoleptic characteristics, net weight and mass fraction of components".
29. Idyryshev, B., Nurgazezova, A., Rebezov, M., Kassymov, S., Issayeva, K., Dautova, A., Atambayeva, Z., Ashakayeva, R., & Suychinov, A. (2022). Study of the Nutritional Value and Microstructure of Veal Cutlets with the Addition of Siberian Cedar Nut Seed Cake. In OnLine Journal of Biological Sciences (Vol. 22, Issue 3, pp. 375–387). Science Publications. https://doi.org/10.3844/ojbsci.2022.375.387
30. Elif Bilek, A., & Turhan, S. (2009). Enhancement of the nutritional status of beef patties by adding flaxseed flour. In Meat Science (Vol. 82, Issue 4, pp. 472–477). Elsevier BV. https://doi.org/10.1016/j.meatsci.2009.03.002
31. Yogesh, K., Langoo, B. A., Sharma, S. K., & Yadav, D. N. (2013). Technological, physico-chemical and sensory properties of raw and cooked meat batter incorporated with various levels of cold milled flaxseed

powder. In Journal of Food Science and Technology (Vol. 52, Issue 3, pp. 1610–1617). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-013-1185-6

32. Barkova, V.G., Velichko, N.A., & Ivanova, O.V. (2019). Development of a recipe for meat and vegetable pate from turkey meat. In Bulletin of the Krasnoyarsk State Agrarian University (Vol. 5, Issue 146, pp. 167-173).
33. Zinina, O.V., Gavrilova, K.S., & Pozdnyakova, M.A. (2018). Study of meat and vegetable pates enriched with non-traditional food ingredients. Journal of South Ural State University. Series: Food and Biotechnologies (Vol. 6, Issue 4, pp. 61-66). South Ural State University.
34. Zając, M., Guzik, P., Kulawik, P., Tkaczewska, J., Florkiewicz, A., & Migdał, W. (2019). The quality of pork loaves with the addition of hemp seeds, de-hulled hemp seeds, hemp protein and hemp flour. In LWT (Vol. 105, pp. 190–199). Elsevier BV. https://doi.org/10.1016/j.lwt.2019.02.013
35. Kotecka-Majchrzak, K., Kasałka-Czarna, N., Spychaj, A., Mikołajczak, B., & Montowska, M. (2021). The Effect of Hemp Cake (Cannabis sativa L.) on the Characteristics of Meatballs Stored in Refrigerated Conditions. In Molecules (Vol. 26, Issue 17, p. 5284). MDPI AG. https://doi.org/10.3390/molecules26175284
36. Hesse, H. (2003). Molecular aspects of methionine biosynthesis. In Trends in Plant Science (Vol. 8, Issue 6, pp. 259–262). Elsevier BV. https://doi.org/10.1016/s1360-1385(03)00107-9
37. Ye, X.-P., Xu, M.-F., Tang, Z.-X., Chen, H.-J., Wu, D.-T., Wang, Z.-Y., Songzhen, Y.-X., Hao, J., Wu, L.-M., & Shi, L.-E. (2022). Flaxseed protein: extraction, functionalities and applications. In Food Science and Technology (Vol. 42). FapUNIFESP (SciELO). https://doi.org/10.1590/fst.22021
38. Galili, G. (1995). Regulation of Lysine and Threonine Synthesis. In The Plant Cell (pp. 899–906). Oxford University Press (OUP). https://doi.org/10.1105/tpc.7.7.899
39. Walker, M. C., & van der Donk, W. A. (2016). The many roles of glutamate in metabolism. In Journal of Industrial Microbiology and Biotechnology (Vol. 43, Issues 2–3, pp. 419–430). Oxford University Press (OUP). https://doi.org/10.1007/s10295-015-1665-y
40. Hou, Y., Yin, Y., & Wu, G. (2015). Dietary essentiality of "nutritionally non-essential amino acids" for animals and humans. In Experimental Biology and Medicine (Vol. 240, Issue 8, pp. 997–1007). Frontiers Media SA. https://doi.org/10.1177/1535370215587913
41. Hatazawa, Y., Qian, K., Gong, D.-W., & Kamei, Y. (2018). PGC-1α regulates alanine metabolism in muscle cells. In M. Kanzaki (Ed.), PLOS ONE (Vol. 13, Issue 1, p. e0190904). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0190904
42. Vallée, Y., & Youssef-Saliba, S. (2021). Sulfur Amino Acids: From Prebiotic Chemistry to Biology and Vice Versa. In Synthesis (Vol. 53, Issue 16, pp. 2798–2808). Georg Thieme Verlag KG. https://doi.org/10.1055/a-1472-7914
43. Pu, F., Chen, N., & Xue, S. (2016). Calcium intake, calcium homeostasis and health. In Food Science and Human Wellness (Vol. 5, Issue 1, pp. 8–16). Tsinghua University Press. https://doi.org/10.1016/j.fshw.2016.01.001
44. Huda, N., Putra, A.A., Ahmad, R. (2011). Potential Application of Duck Meat for Development of Processed Meat Products. In Current Research in Poultry Science (Vol. 1, pp. 1-11). Science International.
45. Zachesova, I.A., Kolobov, S.V., & Pchelkina, V.A. (2018). Investigation of microstructural changes in semi-finished meat products from venison with the addition of Jerusalem artichoke powder. In Modern Science and Innovations (Vol. 3, pp. 98-103).

Funds:

This work was not supported by external funds.

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Aigul Maizhanova**, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: fquekm2710@mail.ru
ORCID: https://orcid.org/0000-0002-4845-9465

Kumarbek Amirkhanov, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: aspirant57@mail.ru
ORCID: https://orcid.org/0000-0002-7988-988X

Shugyla Zhakupbekova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: siyanie__88@mail.ru
ORCID: https://orcid.org/0000-0002-7558-9871

Gulnur Nurymkhan, Shakarim University, Department of Technological Equipment and Mechanical Engineering, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: gulnu-n@mail.ru
ORCID: https://orcid.org/ 0000-0002-0955-3520

Sholpan Baytukenova, S. Seifullin Kazakh Agro Technical Research University, Department of "Technology of food and processing industries", 010011, Astana, Kazakhstan,
Tel.: +7 707 885 5645
E-mail: baytukenovasholpan@gmail.ru
ORCID: https://orcid.org/0000-0003-0200-8455

Assel Dautova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: aska_nur@mail.ru
ORCID: https://orcid.org/0000-0002-2373-2445

Assem Spanova, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: spanoba_78@mail.ru
ORCID: https://orcid.org/0000-0003-1209-7199

Rysgul Ashakayeva, Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan,
Tel.: + 7 7222 314 602
E-mail: ryskulkamara@mail.ru
ORCID: https://orcid.org/0000-0001-8501-0600

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 13.9.2024
Revised: 28.9.2024
Accepted: 1.10.2024
Published: 15.10.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 887-898
https://doi.org/10.5219/2024
ISSN: 1337-0960 online
www.potravinarstvo.com

Summary of legal regulation of additional mandatory particulars for specific types or categories of foods according to the regulation FIC

Samuel Rybnikár, Martin Pogádl

ABSTRACT

The paper presents a summary of the legal treatment of additional mandatory particulars for specific types or categories of foods under Regulation (EU) 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. The authors of the paper analyse and interpret the relevant legislation using traditional methods of legal analysis and legal-hermeneutical methods, with an emphasis on the linguistic and systematic interpretation of those provisions that are directly related to the indication of additional mandatory particulars for foods packaged in certain gases, foods containing sweeteners, foods containing glycyrrhizinic acid or its ammonium salt, Beverages with high caffeine content or foods with added caffeine, Foods with added phytosterols, phytosterol esters, phytostanols or phytostanol esters, and frozen meat, frozen meat preparations and frozen unprocessed fishery products. The paper's authors aim to provide the reader with a comprehensive summary of the legislation on the indication of additional mandatory particulars for specific types or categories of food.

Keywords: food labeling, additional, mandatory, particular, sweetener, caffeine, date of freezing

INTRODUCTION

Mandatory food information under Regulation (EU) 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 (from now on referred to as *'the FIC Regulation'*) **[1]** may be divided into mandatory particulars as referred to in Article 9(1) of the FIC Regulation and additional mandatory particulars for specific types or categories of food. The legal basis for the existence of additional mandatory particulars is based on Article 4(2) of Council Directive 79/112/EEC of 18 December 1978 on the approximation of the laws of the Member States relating to the labelling, presentation, and advertising of foodstuffs for sale to the ultimate consumer **[2]**. This Article of that Directive did not yet contain a list of additional mandatory particulars. Still, it did allow legislation that applies to specific foods and not to foods, in general, to provide for the obligation to indicate on those foods further (additional) particulars in addition to the mandatory food particulars. Concerning the obligation to include additional mandatory particulars for specific foods, specific legislation has gradually been adopted, which has completed the legal basis for including additional mandatory particulars. These were as follows:

- Commission Directive 94/54/EC of 18 November 1994 concerning the compulsory indication on the labelling of certain foodstuffs of particulars other than those provided for in Council Directive 79/112/EEC **[3]**,
- Council Directive 96/21/EC of 29 March 1996 amending Commission Directive 94/54/EC concerning the compulsory indication on the labelling of certain foodstuffs of particulars other than those provided for in Directive 79/112/EEC **[4]**,
- Commission Directive 2002/67/EC of 18 July 2002 on the labeling of foodstuffs containing quinine, and of foodstuffs containing caffeine **[5]**,
- Commission Directive 2004/77/EC of 29 April 2004 amending Directive 94/54/EC as regards the labeling of certain foods containing glycyrrhizinic acid and its ammonium salt **[6]** and
- Commission Regulation (EC) No 608/2004 of 31 March 2004 concerning labelling foods and food ingredients with added phytosterols, phytosterol esters, phytosterols, and/or phytostanol esters **[7]**.

The current legislation on additional mandatory particulars for specific types and categories of food is contained in Annex III of the FIC Regulation, where additional mandatory particulars for specific types and categories of food are listed in an exhaustive (closed) list. The European Commission is empowered by Article 10(1) of the FIC Regulation to establish the additional mandatory particulars for specific types and categories of food, by Article 10(1) of the FIC Regulation. The European Commission has adopted only one delegated Regulation under this empowerment about modifying the additional mandatory particulars for foods with added phytosterols and phytostanols and their esters **[8]**.

For the labelling of foods with additional mandatory particulars under Annex III of the FIC Regulation, the general requirements for the presentation of mandatory food information under the FIC Regulation (a form of presentation, availability, location, and presentation of mandatory particulars) apply. However, certain rules do not apply to the labeling of foods with additional mandatory particulars.

The first derogation concerns the form of the additional mandatory particulars. Mandatory food particulars under Article 9(1) of the FIC Regulation must be given in words and numbers. However, these particulars may additionally be expressed using pictograms and symbols. However, using pictograms and symbols instead of words or numbers is not allowed under Article 9(2) of the FIC Regulation. This requirement does not apply to the indication of additional mandatory particulars for specific food types and categories. The exact form of the additional mandatory particulars is set out in Annex III of the FIC Regulation. In addition, these particulars may not be expressed using pictograms and symbols. About the indication of additional mandatory particulars under Annex III of the FIC Regulation, neither can the European Commission adopt delegated and implementing acts allowing food business operators to express one or more additional mandatory particulars using pictograms or symbols instead of words or numbers.

The second derogation concerns the presentation of the additional mandatory particulars. Mandatory food particulars under Article 9(1) of the FIC Regulation shall be presented in such a way as to ensure their unambiguous legibility and in a font size such that the median font height (x-height) as defined in Annex IV of the FIC Regulation is greater than or equal to 1,2 mm. For foodstuffs with a wrapper or container whose largest surface area is less than 80 cm^2 (e.g. chewing gum, lollipops), the median font height shall be greater than or equal to 0,9 mm. These rules do not apply to the additional compulsory indications under Annex III of the FIC Regulation (e.g. the additional compulsory indication *'Packaged in a protective atmosphere.'* or the date of freezing or the date of first freezing). However, if an additional mandatory particular is made in the name of the food, for example, contains sweetener(s) - cola-flavored lemonade contains sweeteners, this additional mandatory claim must also comply with the minimum font size requirements of Article 13 of the FIC Regulation, as it is part of the mandatory claim under Article 9(1) of the FIC Regulation **[9]**.

Finally, about the indication of additional mandatory particulars, it should be noted that where specific (other) requirements for the form of indication, availability, location, or presentation of such particulars are set out in Annex III of the FIC Regulation, the specific provisions contained in Annex III of the FIC Regulation (lex specialis) apply instead of the general requirements for mandatory food information (lex generalis). This is because the specific legislation takes precedence in application over the general legislation **[10]**.

MATERIAL AND METHODOLOGY

The subject of this paper is the identification of the legislation at a national and supranational level that contains the legal regulation of additional mandatory particulars for specific types or categories of food under the FIC Regulation. The individual legal standards are obtained from www.eur-lex.eu, the official website of the European Union, for searching European Union legal acts, other acts, and official information published in the Official Journal of the European Union, and from www.slov-lex.sk, the official website of the Ministry of Justice of the Slovak Republic, which contains the Collection of Laws of the Slovak Republic. We draw in particular on the

FIC Regulation, the derogated Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labelling, presentation, and advertising of foodstuffs **[11]** and Council Directive 79/112/EEC of 18 December 1978 on the approximation of the laws of the Member States relating to the labelling, presentation and advertising of foodstuffs for sale to the ultimate consumer, as amended **[2]** and related legal acts. The source of information at the national level (Slovak Republic) is also Food Law 152/1995 Col. SR, as amended **[12]**, **[13]**. The subject matter of the uniform regulations is then analysed and interpreted using traditional methods of legal analysis and legal-hermeneutical methods, emphasising the linguistic and systematic interpretation of those provisions directly related to the indication of additional mandatory data. Domestic legislation is compared with supranational legislation to present it systematically within the logical structure of classical legal dogmatics as a systematic set of legal institutes, legal norms, and the consistent results of legal application and legal implementation practice. The findings of practice are subsumed under specific hypotheses of legal norms. At the same time, logical syllogism is used to deduce legally established dispositions and sanctions tied to the fulfilment of the hypotheses of legal norms. Given the interdisciplinary nature of the paper, the system of legal dogmatics of additional mandatory data is confronted and supplemented, where appropriate, with current legal and food doctrine.

RESULTS AND DISCUSSION

Additional mandatory particulars are provided for the following types or categories of food:

- foods packaged in certain gases,
- foods containing sweeteners,
- foods containing glycyrrhizinic acid or its ammonium salt,
- beverages with high caffeine content or foods with added caffeine,
- foods with added phytosterols, phytosterol esters, phytostanols, or phytostanol esters and
- frozen meat, frozen meat preparations and frozen unprocessed fishery products.

Foods packaged in certain gases

To extend the shelf life of food, the food industry uses modified atmosphere packaging. Packaging plays a crucial role by facilitating the containment, transportation, and logistics of fresh and processed commodities **[14]**. Modified atmosphere food packaging has a composition of gases surrounding the food that is different from that of air. Under reduced O_2 and high CO_2, the metabolic processes of the horticultural product and microbial activity are slowed down, contributing to extended shelf life **[14]**. Packaging of food in a modified atmosphere can take the form of vacuum packaging or packaging in a protective atmosphere **[15]**. Packaging gases are a functional class of food additives according to Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives **[16]**. Food additives are divided according to the function they perform into so-called functional classes. The functional classes of food additives are listed in Annex I of Regulation (EC) No 1333/2008 **[16]**. One of these functional classes is packaging gases. Packaging gases are used to package food in a protective atmosphere. Packaging gases are gases other than air, introduced into a container before, during, or after placing a foodstuff in that container. Packaging gases are listed in Table 1.

Table 1 Packaging gases.

E number	Name of the packaging gas
E 290	Carbon dioxide
E 938	Argon
E 939	Helium
E 941	Nitrogen
E 948	Oxygen
E 949	Hydrogen

Packaging gases used in the packaging of certain foods (e.g. packaged cut meat, meat products, or cheese) are not considered food ingredients within the meaning of the FIC Regulation and, therefore, do not appear in the list of food ingredients. However, consumers should be informed about the use of packaging gases in the packaging of foodstuffs, as foodstuffs packaged in this way have a longer shelf life than similar foodstuffs packaged in other ways. Legislation on the indication of additional mandatory particulars on foods whose shelf-life has been extended by the use of packaging gases has been adopted at the European Union level by Commission Directive 94/54/EC of 18 November 1994 concerning the compulsory indication on the labelling of certain foodstuffs of

particulars other than those provided for in Council Directive 79/112/EEC **[3]**. This legislation is now contained in Annex III of the FIC Regulation.

In the case of foodstuffs whose shelf life has been prolonged by the use of packaging gases, an additional mandatory particular 'Packaged in a protective atmosphere' must appear on the packaging or the label attached thereto. This additional mandatory particular is mandatory on the packaging or the label attached to the food and does not need to appear in the name of the food or in close proximity to the name of the food.

Foods containing sweeteners

Foods containing sweeteners must also contain additional mandatory particulars. This obligation was first established at the European Union level by Council Directive 96/21/EC of 29 March 1996, amending Commission Directive 94/54/EC concerning the compulsory indication on the labelling of certain foodstuffs of particulars other than those provided for in Directive 79/112/EEC **[4]**. Today, the legislation is contained in Annex III of the FIC Regulation. Sweeteners are a functional class of food additives under Annex I of Regulation (EC) No 1333/2008. Sweeteners are substances used to impart a sweet taste to foods or as table-top sweeteners. Artificial sweeteners are significantly sweeter than conventional sugar (sucrose) but have no calories. Thus, the popularity of artificial sweeteners in the global food market as substitutes for sugar has increased in recent years **[17]**. The use of artificial sweeteners is controversial due to concerns about carcinogenicity **[18]** and the potential for promoting obesity and type II diabetes **[19]**. Only substances listed in Annex II, Part B, point 2 may be used as sweeteners. The list of sweeteners is given in Table 2. The Scientific Committee on Food issued its first opinion on 14 September 1984 to review the safety of the use of certain sweeteners **[20]**.

Table 2 Sweeteners.

E number	Name of the sweetener
E 420	Sorbitol
E 421	Mannitol
E 950	Acesulfame K
E 951	Aspartame
E 952	Cyclamate
E 953	Isomalt
E 954	Saccharin
E 955	Sucralose
E 957	Thaumatin
E 959	Neohesperidin DC
E 960a	Steviol glycosides from Stevia
E 960c	Enzymatically produced steviol glycosides
E 961	Neotame
E 962	Aspartame-acesulfame salt
E 964	Polyglycitol syrup
E 965	Maltitol
E 966	Lactitol
E 967	Xylitol
E 968	Erythritol
E 969	Advantame

A food containing sweetener(s) shall bear the additional mandatory particular *'contains sweetener(s)'* in the name of the food. For example, cola-flavoured lemonade contains sweeteners. If a food contains added sugar(s) and sweetener(s), the name of the food must include the additional mandatory particular *'contains sugar(s) and sweetener(s)'*. For example, *'sterilized cucumbers in spicy sweet and sour brine, contains sugar and sweeteners'*.

Foods containing the sweeteners aspartame (E 951) or aspartame-acesulfame salts (E 962) shall bear the additional mandatory particular *'Contains aspartame (source of phenylalanine)'* on the packaging of the food or on the label attached to the food if aspartame or aspartame-acesulfame salts are listed in the list of ingredients of the food only as a reference to the E number. For example, if the ingredient *'E 951'* is listed in the composition of chewing gum (without the specific name of the sweetener), an additional mandatory particular *'Contains aspartame (source of phenylalanine)'* shall be indicated on the packaging of the chewing gum. Where aspartame

or aspartame-acesulfame salts are listed in the list of ingredients of the food under their specific name, the additional mandatory particular *'Contains phenylalanine source'* shall be indicated on the food packaging or the label attached thereto. For example, if the ingredient *'aspartame'* is listed in the composition of a lemonade, the packaging of that lemonade shall bear the additional mandatory particular *'Contains a source of phenylalanine'*. These additional mandatory particulars shall appear on the packaging or the label attached to the food and need not appear in or adjacent to the name of the food. A warning about the source of phenylalanine in foods is very important for phenylketonuria sufferers, as they cannot metabolise this amino acid **[21]**.

Food containing more than 10% added alcoholic sugars (polyols) must be labelled with the additional mandatory particular "Excessive consumption may have laxative effects" (e.g. some chewing gum). This additional mandatory particular shall appear on the packaging or the label attached to the food. Alcoholic sugars (polyols) are alcohols containing more than two hydroxyl groups. A list of polyols is given in Table 3 **[22]**.

Table 3 Polyols.

E number	Name of the polyol
E 420	Sorbitol
E 421	Mannitol
E 953	Isomalt
E 965	Maltitol
E 966	Lactitol
E 967	Xylitol
E 968	Erythritol

Foods containing glycyrrhizinic acid or its ammonium salt

Foods containing glycyrrhizinic acid or its ammonium salt shall also contain additional mandatory particulars. Glycyrrhizinic acid is found naturally in the plant *Glycyrrhiza glabra*, and its ammonium salt is produced from aqueous extracts from this plant. Glycyrrhizic acid has been known in China, Egypt, and Japan **[23]**. Licorice has a wide use in traditional Chinese and Japanese medicine. Over the past 50 years, the biological and therapeutic activity of glycyrrhizinic acid has been intensively studied in Asia and Europe **[24]**. There are numerous studies on the antiviral activity of glycyrrhizinic acid against various viruses **[25]**. Glycyrrhizic acid also has anti-inflammatory effects, as proven through many scientific studies **[26]**. Relevant scientific studies also point to the specificity of glycyrrhizinic acid for liver diseases. In particular, glycyrrhizinic acid has been found to exhibit a hepatoprotective effect, conditioned by the antioxidant activity of glycyrrhizinic acid **[27]**. Glycyrrhizinic acid also shows significant anti-cancer effects as it can induce apoptosis in tumour cells of different types of cancer **[28]**.

Exposure to glycyrrhizinic acid and its ammonium salt generally occurs through the consumption of liquorice confectionery, chewing gum, herbal teas, and other beverages. Mandatory labelling of certain foods (confectionery and beverages) with additional mandatory particulars was introduced at the European Union level by Commission Directive 2004/77/EC **[6]** based on the Opinion of the Scientific Committee on Food of 4 April 2003 on glycyrrhizinic acid and its ammonium salt of 4 April 2003 **[29]**. The legislation for this Directive is now contained in Annex III of the FIC Regulation.

Glycyrrhizic acid (FL 16.012) and its ammonium salt (FL 16.060) are food flavourings according to Regulation (EC) No 1334/2008 **[30]**. Glycyrrhizinic acid and its ammonium salt were added to the list of flavouring substances by Commission Implementing Regulation (EU) No 872/2012 **[31]** based on the Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials of the European Food Safety Authority of 22 May 2008 (EFSA-Q-2003-172B) **[32]**.

Confectionery or beverages containing glycyrrhizinic acid or its ammonium salt as a result of the addition of these substances or licorice, at a concentration of 100 mg/kg or 100 mg/l or more, shall contain, immediately after the list of ingredients, the additional mandatory particular *'Contains licorice'*. If the food does not bear a list of ingredients, the additional mandatory particular *'Contains licorice'* shall be added and shall appear next to the name of the food. The obligation to include this additional mandatory indication shall only apply if the term *'licorice'* is not already included in the list of ingredients. For example, the ingredient *'licorice extract'* is mentioned in the composition of the confectionery or the name of the food, e.g. licorice confectionery.

Confectionery containing glycyrrhizinic acid or its ammonium salt as a result of the addition of these substances or licorice, at a concentration of 4 g/kg or more, must include an additional mandatory particular immediately after the list of ingredients *'Contains licorice - people with high blood pressure should avoid*

excessive consumption'. For example, some pellets. In this case, it is irrelevant whether or not the term *'licorice'* appears in the list of ingredients or the name of the food. If the above conditions are fulfilled, the confectionery must contain this indication immediately after the list of ingredients. If the food does not bear a list of ingredients, an additional mandatory particular shall be given close to the name of the food.

Beverages containing glycyrrhizinic acid or its ammonium salt as a result of the addition of these substances or liquorice, at a concentration of 50 mg/l or more and, in the case of beverages containing more than 1,2 % alcohol by volume, at a concentration of 300 mg/l or more, shall contain the additional mandatory particular *'Contains licorice - people with high blood pressure should avoid excessive consumption'* immediately after the list of ingredients. This level applies to products prepared for direct consumption or after preparation according to the manufacturer's instructions. In this case, it is irrelevant whether or not the term *'licorice'* appears in the list of ingredients or the name of the food. If the above conditions are fulfilled, the confectionery must contain this indication immediately after the list of ingredients. If the food does not bear a list of ingredients, an additional mandatory particular shall be given close to the name of the food.

Beverages with high caffeine content or foods with added caffeine

Caffeine is a naturally occurring central nervous system (CNS) stimulant belonging to the methylxanthine class and is widely recognised as the most utilised psychoactive stimulant worldwide. Although this drug is most commonly sourced from coffee beans, it can also naturally occur in certain types of tea and cacao beans **[33]**. Additional mandatory particulars shall also be provided on caffeinated beverages and caffeinated foods. The original legislation was contained in Commission Directive 2002/67/EC **[5]**, the wording of which has been taken over in Annex III of the FIC Regulation. This legislation was adopted following the conclusions of the opinion of the Scientific Committee on Food on 21 January 1999 concerning caffeine, taurine, and D-glucuronolactone as ingredients in so-called energy drinks **[34]**. This legislation was based on the belief that consumers should be given information on the presence of caffeine, as well as a warning notice and information on the amount of caffeine in drinks, which are usually caffeine-free and where caffeine is more abundant.

Caffeine (FL 16.016) is a food flavouring according to Regulation (EC) No 1334/2008 **[30]**. It was added to the list of flavouring substances by Commission Implementing Regulation (EU) No 872/2012 **[31]**. Caffeine is a central nervous system and cardiovascular stimulant. It increases respiratory, heart, and blood pressure **[21]**. Caffeine is popular among athletes mainly due to its ergogenic effects on enhancing performance **[35]**. A scientific study examining caffeine's effects on muscles shows that caffeine affects muscles through an increase in intracellular calcium. Therefore, consuming caffeine improves muscle strength and endurance **[36]**. The Scientific Committee on Food issued the first opinion regarding caffeinated foods on 7 July 1983 **[37]**. Caffeine is a flavouring that is restricted to selected foods. Caffeine may be added to dairy products and ice cream in quantities not exceeding 70 mg/kg, to confectionery in quantities not exceeding 100 mg/kg, and to non-alcoholic beverages in quantities not exceeding 150 mg/kg, for example, energy drinks.

Beverages that are intended to be consumed unaltered and contain caffeine from any source above 150 mg/l (e.g. energy drinks) or are in concentrated or dried form and, when reconstituted, contain caffeine from any source over 150 mg/l shall contain the additional mandatory *claim 'Caffeinated. Not recommended for children or pregnant or lactating women'*, followed by the caffeine content expressed in mg/100 ml in brackets. The obligation to indicate the additional mandatory particulars on the food does not apply to coffee, tea, or coffee or tea extract-based beverages if the name of the food contains the term *'coffee'* or *'tea'*.

Foods other than beverages that have been added to for physiological purposes must bear the additional mandatory particular *'Contains caffeine. Not recommended for children or pregnant women'*. This additional mandatory particular shall be followed by a parenthetical indication of the caffeine content expressed in mg per 100 g/ml. For example, *'Contains caffeine. Not recommended for children or pregnant women (1596 mg/100 ml)'*. Regarding food supplements, the caffeine content shall be expressed per dose as recommended according to the daily consumption on the label.

The above additional mandatory particulars shall appear on the food in the same field of vision as the name of the food. The field of vision is all surfaces of the food packaging that can be read from a single point of view. At the same time, the legislator stresses that these additional mandatory particulars must be displayed in a prominent position so that they are easily visible and legible. They must not be hidden, obscured, distracted, or interfered with in any way by other text pictures, or other distracting material. However, these requirements apply to all mandatory food information under the FIC Regulation.

Finally, concerning caffeine as a food flavoring, it may be added that it must be mentioned by its name in the list of ingredients immediately after the term *'flavoring'* or *'flavorings'* when used for the manufacture or preparation of food. For example, in the list of ingredients of an energy drink, caffeine shall be listed alone as *'caffeine flavoring'* or together with other flavorings as *'flavorings (caffeine,...')'*.

Foods with added phytosterols, phytosterol esters, phytostanols or phytostanol esters

The obligation to provide additional particulars shall also apply to foods or food ingredients with added phytosterols and phytostanols and their esters. The term phytosterols (plant sterols) includes both phytosterols and phytostanols. These compounds are the most studied group of nutraceuticals whose health benefits have been known for more than seven decades **[38]**. Phytosterols and phytostanols, structurally and functionally related to cholesterol, are present in relatively high amounts in vegetable oils, nuts, and seeds, with total phytosterol content in some vegetable oils reaching values as high as 19 g/kg **[39]**. Phytosterols and phytostanols in plants have similar functions to cholesterol in humans. Phytosterols differ from cholesterol in that they have a different side-chain structure, whereas phytostanols are 5α-saturated derivatives of phytosterols **[40]**. SITO is the most common phytosterol found in foods, and as scientific conclusions suggest, it is SITO that is strongly associated with inhibition of several hallmarks of cancer, including suppression of cell death, maintenance of proliferative signalling, induction of angiogenesis and metastasis, **[41]**. Foods or food ingredients in which phytosterols or phytostanols are naturally present (e.g. vegetable oils, legumes) are not subject to additional mandatory disclosures. This obligation was established at the European Union level by Commission Regulation (EC) No 608/2004 of 31 March 2004 concerning the labeling of foods and food ingredients with added phytosterols, phytosterol esters, phytostanols and/or phytostanol esters **[7]**, which was adopted based on the conclusions of the opinion of the Scientific Committee on Food of 26 September 2002 entitled *'Overall view on the long-term effects of the intake of increased amounts of phytosterols from multiple food sources, with particular emphasis on the effects of ß-carotene'* **[42]**. This legislation has been incorporated into Annex III of the FIC Regulation.

Foods with added phytosterols and phytostanols and their esters must be labeled with the additional mandatory particular *'with added plant sterols'* or *'with added plant stanols'*. These additional mandatory particulars must appear on the food in the same field of vision as the name of the food. The field of vision is all the surfaces of the food packaging that can be read from a single point of view. However, there is no obligation to indicate them directly in the name of the food, although this practice may be encountered in food practice. For example, *'vegetable fat spread, with added plant sterols'*. In addition to the above additional mandatory particulars, the amount of added phytosterols, phytosterol esters, phytostanols, or phytostanol esters must be indicated in the list of ingredients of the food. The amount shall be expressed in % or grams of free plant sterols or stanols per 100 g or 100 ml of the food. For example, in the list of ingredients of a food supplement, the ingredient *'plant sterols (27,7 g per 100 g of product)'* shall be indicated.

Foods with added phytosterols and phytostanols and their esters shall also include the following additional mandatory particulars:

- a statement that the food is intended exclusively for people who want to lower their blood cholesterol level. For example, *'The product is not intended for people who do not need to check their blood cholesterol levels'*.
- A patient taking cholesterol-lowering medications should consume the product only under the supervision of a physician. For example, *'If you are taking a cholesterol-lowering medication, consult your physician about consuming the product'*.
- A claim that the food is nutritionally unsuitable for pregnant or lactating women and children under 5 years of age. For example, *'Products intended to lower cholesterol are not suitable for pregnant and lactating women and children under 5 years of age'*. This particular must be easily visible.
- Advice is that the food should be used as part of a balanced and varied diet, including regular consumption of fruit and vegetables, which helps to maintain carotenoid levels. For example, *'The product is suitable for use as part of a balanced and varied diet, including regular consumption of fruit and vegetables, which helps to maintain carotenoid levels'*.
- The claim that consumption of more than 3 g of added plant sterols/stanols per day should be avoided. For example, *'Consumption of more than 3 g of added plant sterols per day is not recommended'*. This claim must be made in the same field of vision as claim (a). The field of vision is all surfaces of the food packaging that can be read from a single point of view.

Frozen meat, frozen meat preparations, and frozen unprocessed fishery products

Meat and meat products play a crucial role in human diets due to their rich nutrients, such as protein, iron, zinc, selenium, vitamins, cholesterol, niacin, fats, etc. **[43]**. These nutrients supply large daily energy intakes for humans **[44]**. Meanwhile, these nutrients can also provide good growth conditions for various microorganisms, making meats prone to spoilage **[45]**. Preserving food to extend its shelf-life has been practised for several millennia. At its roots, food preservation involves altering the product's inherent properties, mainly pH and water activity (Aw), to inhibit the growth of pathogenic microorganisms, moulds, and spores **[46]**. According to Annexe III of the FIC Regulation, the date of freezing and the date of first freezing are additional compulsory particulars. The date of first freezing must be provided as an additional mandatory indication in cases where the food has been

frozen more than once. The date of freezing and the date of first freezing shall be provided as an additional mandatory particular according to Annex III of the FIC Regulation at:

- frozen meat,
- frozen meat preparations, and
- frozen unprocessed fishery products **[47]**.

The definitions of meat, meat preparations, and fishery products are based on Annex I to Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004, laying down specific hygiene rules for food of animal origin **[48]**. Meat is the edible parts of domestic ungulates, poultry, lagomorphs, wild game, farmed game, and small and large wild game, including blood (e.g. sliced boneless pork loin or minced beef). Meat preparations mean fresh meat, including meat that has been reduced to fragments, which has had foodstuffs, seasonings, or additives added to it, or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus eliminate the characteristics of fresh meat. (for example, slices of pork curry in a marinade for grilling or minced beef with salt and spices for making a hamburger). Fishery products mean all seawater or freshwater animals (except for live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods, and all mammals, reptiles, and frogs) whether wild or farmed and including all edible forms, parts, and products of such animals. The obligation to indicate the freezing date or first freezing shall apply only to unprocessed fishery products. The definitions of processing, unprocessed products, and processed products are based on Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs **[49]**.

Frozen food is not defined for food labelling by European Union law. The definition of frozen food is to be found in the legislation of the individual Member States of the European Union. The Slovak Republic has a definition of frozen food contained in the Decree of the Ministry of Agriculture and Rural Development of the Slovak Republic No 82/2018 Coll. on frozen food and frozen products **[50]**. According to the law of the Slovak Republic, frozen food is frozen by a process in which the phase of maximum formation of ice crystals is carried out as quickly as possible and is appropriate to the type of food being frozen. After reaching the final temperature, after its equilibration and stabilisation, a temperature of -12 °C or lower is permanently maintained at each point of the food. During transport, the temperature of the frozen food may be briefly raised by no more than 3 °C. The name of the frozen food must include or be accompanied by an indication of the physical state of the food - *'frozen'*. The obligation to include such an accompanying indication of the name of the frozen food shall apply only if its omission is likely to mislead the purchaser.

The date of freezing and the date of first freezing shall be indicated following Annex X of the FIC Regulation. The date of freezing and the date of first freezing shall consist of the day, month, and year. The sequence must be maintained, and the date must be given in uncoded form. This obligation shall not apply to deep-frozen meat preparations and unprocessed fishery products.

The date of freezing or the date of first freezing shall be indicated on the foodstuff by the word *'frozen'*. This word shall be followed by the date itself or a reference to where this date appears on the food. The obligation to indicate the date of freezing or first freezing shall apply only to pre-packaged frozen meat, meat preparations, and unprocessed fishery products. For non-packaged frozen meat, frozen meat preparations, and frozen unprocessed fishery products, there is no obligation to indicate their freezing date or first freezing. However, this obligation may be laid down for non-prepacked foodstuffs by individual Member States of the European Union using so-called national measures, according to the Communication from the Commission on questions relating to the application of Regulation (EU) No 1169/2011 on the provision of food information to consumers **[51]** individual member states of the European Union through so-called national measures.

CONCLUSION

Suppose the food is labelled with something other than additional mandatory particulars according to Annex III of the FIC Regulation. In that case, the food business operator commits an administrative offence under the provisions of Section 28(2)(f) of Act No. 152/1995 Coll. on Foodstuffs, as amended. For that administrative offense, the official food control authority shall impose a fine between EUR 100 and EUR 100 000.

The obligation to indicate additional mandatory particulars under Annex III of the FIC Regulation applies only to prepacked food. Non-prepacked foods do not have to bear the additional mandatory particulars under the FIC Regulation. The Slovak Republic has not adopted any national measures concerning the additional mandatory particulars contained in Annex III of the FIC Regulation, which would impose an obligation to indicate them for non-prepacked foodstuffs.

REFERENCES

1. Regulation (EU) 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004, OJ L 304, 22/11/2011, s. 18 – 63.
2. Council Directive 79/112/EEC of 18 December 1978 on the approximation of the laws of the Member States relating to the labeling, presentation, and advertising of foodstuffs for sale to the ultimate consumer, OJ L 33, 8.2.1979, p. 1–14.
3. Commission Directive 94/54/EC of 18 November 1994 concerning the compulsory indication on the labeling of certain foodstuffs of particulars other than those provided for in Council Directive 79/112/EEC, OJ L 300, 23/11/1994, p. 14–15.
4. Council Directive 96/21/EC of 29 March 1996 amending Commission Directive 94/54/EC concerning the compulsory indication on the labeling of certain foodstuffs of particulars other than those provided for in Directive 79/112/EEC, *OJ L 88, 05/04/1996, s. 5 – 6.*
5. Commission Directive 2002/67/EC of 18 July 2002 on the labeling of foodstuffs containing quinine, and of foodstuffs containing caffeine, *OJ L 191, 19.7.2002, p. 20–21.*
6. Commission Directive 2004/77/EC of 29 April 2004 amending Directive 94/54/EC as regards the labeling of certain foods containing glycyrrhizinic acid and its ammonium salt, OJ L 162, 30.4.2004, p. 76–77.
7. Commission Regulation (EC) No 608/2004 of 31 March 2004 concerning the labeling of foods and food ingredients with added phytosterols, phytosterol esters, phytostanols and/or phytostanol esters, OJ L 97, 1.4.2004, p. 44–45.
8. Commission Delegated Regulation (EU) No 78/2014 of 22 November 2013 amending Annexes II and III to Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers, as regards certain cereals causing allergies or intolerances and foods with added phytosterols, phytosterol esters, phytostanols or phytostanol esters, OJ L 27, 30.1.2014, p. 7–8.
9. Commission notice on questions and answers on the application of Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers, OJ C 196, 8.6.2018, s. 1 – 14.
10. Procházka, R., & Káčer, M. (2019). Teória práva [Theory of Law] (2nd ed.). C. H. Beck, 320 p., ISBN: 978-80-89603-73-2
11. Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to the labeling, presentation, and advertising of foodstuffs, OJ L 109, 6.5.2000, p. 29–42.
12. Food law 152/1995 Col. SR. Retrieved from https://www.slov-lex.sk/pravne-predpisy/SK/ZZ/1995/152/20220721.
13. Ševcová, K., Zajác, P., Čapla, J., & Čurlej, J. (2021). Development of the food act of the Slovak Republic from 1995 to 2021. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 982–994). HACCP Consulting. https://doi.org/10.5219/1689
14. Antmann, G., Ares, G., Lema, P., & Lareo, C. (2008). Influence of modified atmosphere packaging on sensory quality of shiitake mushrooms. In Postharvest Biology and Technology (Vol. 49, Issue 1, pp. 164–170). Elsevier BV. https://doi.org/10.1016/j.postharvbio.2008.01.020
15. Ducková, V., & Pavelková, A. (2018). Balenie a označovanie potravín [Food Packaging and Labeling] (2nd revised ed.). Slovenská poľnohospodárska univerzita v Nitre. 134 p.
16. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L 354, 31.12.2008, p. 16–33.
17. Sylvetsky, A. C., & Rother, K. I. (2016). Trends in the consumption of low-calorie sweeteners. In Physiology & Behavior (Vol. 164, pp. 446–450). Elsevier BV. https://doi.org/10.1016/j.physbeh.2016.03.030
18. Landrigan, P. J., & Straif, K. (2021). Aspartame and cancer – new evidence for causation. In Environmental Health (Vol. 20, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12940-021-00725-y
19. Rios-Leyvraz, M. & Montez, J. (2022). Health effects of the use of non-sugar sweeteners – a systematic review and meta-analysis. World Health Organization (WHO).

20. Food – science and techniques. (2024, September 02). In Reports of the Scientific Committee for Food. https://food.ec.europa.eu/document/download/8a8fb992-235b-4654-98d3-e4b4d66d3077_en?filename=sci-com_scf_reports_16.pdf.

21. Fikselová, M., Golian, J., & Zeleňáková, L. (2021). Zdravotná bezpečnosť potravín [Health Food Safety]. Slovenská poľnohospodárska univerzita v Nitre, p. 74.

22. Labeling of sweeteners sold to the final consumer - Czech Agriculture and Food Inspection Authority. (2024, September 02). Available at: https://www.szpi.gov.cz/clanek/postupy-a-stanoviska-szpi-oznacovani-oznacovani-sladidel-prodavanych-konecnemu-spotrebiteli.aspx.

23. Shibata, S. (2000). A Drug over the Millennia : Pharmacognosy, Chemistry, and Pharmacology of Licorice. In Yakugaku Zasshi (Vol. 120, Issue 10, pp. 849–862). Pharmaceutical Society of Japan. https://doi.org/10.1248/yakushi1947.120.10_849

24. Ming, L. J., & Yin, A. C. Y. (2013). Therapeutic Effects of Glycyrrhizic Acid. In Natural Product Communications (Vol. 8, Issue 3). SAGE Publications. https://doi.org/10.1177/1934578x1300800335

25. Selyutina, O. Yu., & Polyakov, N. E. (2019). Glycyrrhizic acid as a multifunctional drug carrier – From physicochemical properties to biomedical applications: A modern insight on the ancient drug. In International Journal of Pharmaceutics (Vol. 559, pp. 271–279). Elsevier BV. https://doi.org/10.1016/j.ijpharm.2019.01.047

26. Matsumoto, Y., Matsuura, T., Aoyagi, H., Matsuda, M., Hmwe, S. S., Date, T., Watanabe, N., Watashi, K., Suzuki, R., Ichinose, S., Wake, K., Suzuki, T., Miyamura, T., Wakita, T., & Aizaki, H. (2013). Antiviral Activity of Glycyrrhizin against Hepatitis C Virus In Vitro. In S. J. Polyak (Ed.), PLoS ONE (Vol. 8, Issue 7, p. e68992). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0068992

27. Orazizadeh, M., Fakhredini, F., Mansouri, E., & Khorsandi, L. (2014). Effect of glycyrrhizic acid on titanium dioxide nanoparticles-induced hepatotoxicity in rats. In Chemico-biological interactions (Vol. 220, pp. 214-221).

28. Su, X., Wu, L., Hu, M., Dong, W., Xu, M., & Zhang, P. (2017). Glycyrrhizic acid: A promising carrier material for anticancer therapy. In Biomedicine & Pharmacotherapy (Vol. 95, pp. 670–678). Elsevier BV. https://doi.org/10.1016/j.biopha.2017.08.123

29. Opinion of the Scientific Committee on Food on glycyrrhizinic acid and its ammonium salt (opinion expressed on 4 April 2003). (2024, September 02). Available at: https://food.ec.europa.eu/system/files/2020-12/sci com_scf_out186_en.pdf.

30. Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavorings and certain food ingredients with flavoring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC (Text with EEA relevance), OJ L 354, 31.12.2008, p. 34–50.

31. Commission Implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavoring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC Text with EEA relevance, OJ L 267, 2.10.2012, p. 1–161.

32. Flavouring Group Evaluation 35, (FGE.35) - Three quinine salts from the Priority list from chemical group 30 - Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food. (n.d.). [JD]. EFSA Journal, EFSA Journal. https://doi.org/10.2903/j.efsa.2009.739

33. Evans, J., Richards, J. R., & Battisti, A.S. (2024). Caffeine. In StatPearls. Available at: https://www.ncbi.nlm.nih.gov/books/NBK519490/

34. Opinion of the Scientific Committee on Food on Caffeine, Taurine and D-Glucurono - - Lactone as constituents of so-called "energy" drinks (expressed on 21 January 1999). (2024, September 02). https://food.ec.europa.eu/document/download/59ac8615-7965-4272-97d5-04f0e9802163_en?filename=sci-com_scf_out22_en.pdf.

35. Saunders, B., da Costa, L. R., de Souza, R. A. S., Barreto, G., & Marticorena, F. M. (2023). Caffeine and sport. In Advances in Food and Nutrition Research (pp. 95–127). Elsevier. https://doi.org/10.1016/bs.afnr.2023.03.002

36. Bilondi, H. T., Valipour, H., Khoshro, S., Jamilian, P., Ostadrahimi, A., & Zarezadeh, M. (2024). The effect of caffeine supplementation on muscular strength and endurance: A meta-analysis of meta-analyses. In Heliyon (Vol. 10, Issue 15, p. e35025). Elsevier BV. https://doi.org/10.1016/j.heliyon.2024.e35025

37. Reports of the Scientific Committee for Food (Fourteenth series) (2024, September 02) Available at: https://food.ec.europa.eu/document/download/ea4fa03b-8924-47b3-93e3-87a9370f4c94_en?filename=sci-com_scf_reports_14.pdf.

38. Kalliny, S., & Zawistowski, J. (2019). Phytosterols and Phytostanols. In Encyclopedia of Food Chemistry (pp. 289–299). Elsevier. https://doi.org/10.1016/b978-0-08-100596-5.21760-5

39. Yang, R., Xue, L., Zhang, L., Wang, X., Qi, X., Jiang, J., Yu, L., Wang, X., Zhang, W., Zhang, Q., & Li, P. (2019). Phytosterol Contents of Edible Oils and Their Contributions to Estimated Phytosterol Intake in the Chinese Diet. In Foods (Vol. 8, Issue 8, p. 334). MDPI AG. https://doi.org/10.3390/foods8080334

40. Gylling, H., & Simonen, P. (2015). Phytosterols, Phytostanols, and Lipoprotein Metabolism. In Nutrients (Vol. 7, Issue 9, pp. 7965–7977). MDPI AG. https://doi.org/10.3390/nu7095374

41. Cioccoloni, G., Soteriou, C., Websdale, A., Wallis, L., Zulyniak, M. A., & Thorne, J. L. (2020). Phytosterols and phytostanols and the hallmarks of cancer in model organisms: A systematic review and meta-analysis. In Critical Reviews in Food Science and Nutrition (Vol. 62, Issue 5, pp. 1145–1165). Informa UK Limited. https://doi.org/10.1080/10408398.2020.1835820

42. General view of the Scientific Committee on Food on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources, with particular attention to the effects on β-carotene (expressed on 26 September 2002). (2024, September 02). Available at: https://food.ec.europa.eu/document/download/7053cfc5-009a-4062-bdb4-af2c1e53c587_en?filename=sci-com_scf_out143_en.pdf.

43. Anzani, C., Boukid, F., Drummond, L., Mullen, A. M., & Álvarez, C. (2020). Optimising the use of proteins from rich meat co-products and non-meat alternatives: Nutritional, technological and allergenicity challenges. In Food Research International (Vol. 137, p. 109575). Elsevier BV. https://doi.org/10.1016/j.foodres.2020.109575

44. Laskowski, W., Górska-Warsewicz, H., & Kulykovets, O. (2018). Meat, Meat Products and Seafood as Sources of Energy and Nutrients in the Average Polish Diet. In Nutrients (Vol. 10, Issue 10, p. 1412). MDPI AG. https://doi.org/10.3390/nu10101412

45. Lu, N., Ma, J., & Sun, D.-W. (2022). Enhancing physical and chemical quality attributes of frozen meat and meat products: Mechanisms, techniques and applications. In Trends in Food Science & Technology (Vol. 124, pp. 63–85). Elsevier BV. https://doi.org/10.1016/j.tifs.2022.04.004

46. Dang, D. S., Bastarrachea, L. J., Martini, S., & Matarneh, S. K. (2021). Crystallization Behavior and Quality of Frozen Meat. In Foods (Vol. 10, Issue 11, p. 2707). MDPI AG. https://doi.org/10.3390/foods10112707 .

47. Venhartová, J., Rybnikár, S., Gábriš, T., et al. (2022). Potravinové právo [Food Law]. C. H. Beck, 344 p. ISBN: 978-80-8232-016-2.

48. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin, OJ L 139, 30.4.2004, p. 55–205.

49. Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs, OJ L 139, 30/04/2004, p. 1–54.

50. Decree of the Ministry of Agriculture and Rural Development of the Slovak Republic No. 82/2018 Coll. on frozen food and frozen products. Available at: https://www.slov-lex.sk/pravne-predpisy/SK/ZZ/2018/82/20180401.

51. Commission notice on questions and answers on the application of Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers, OJ C 196, 8.6.2018, s. 1 – 14.

Funds:

This research received no external funding.

Acknowledgments:

-

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

*Samuel Rybnikár, Trnava University in Trnava, Faculty of Law, Institute of Intellectual Property, Information Technology and Product Law, Hornopotočná, 23, 918 43, Trnava, Slovak Republic,
Tel.: +421335939635
E-mail: samuel.rybnikar@truni.sk
ORCID: https://orcid.org/0000-0002-8511-6509

Martin Pogádl, Trnava University in Trnava, Faculty of Law, Hornopotočná, 23, 918 43, Trnava, Slovak Republic,
Tel.: +421335939635
E-mail: marin.pogadl@tvu.sk
ORCID: https://orcid.org/0009-0003-3903-8057

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 21.8.2024
Revised: 20.10.2024
Accepted: 22.10.2024
Published: 22.10.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 899-918
https://doi.org/10.5219/2011
ISSN: 1337-0960 online
www.potravinarstvo.com

Analysis of the hard rennet cheese microbiota at different stages of the technological process

Olha Iakubchak, Olha Martynenko, Tetiana Taran, Oksana Pylypchuk, Tatyana Naumenko, Nina Tverezovska, Alina Menchynska, Inna Stetsyuk

ABSTRACT

The purpose of the research was microbiological screening using MALDI-TOF technology starting from bulk raw milk to the finished dairy product and analyzing microorganisms that were being detected during the technological process of production of Ukrainskyi hard rennet cheese and which were clinically significant for human and animal health. Methods. Microbial detection was performed by accumulation and inoculation using the sector inoculation method on differential media for aerobic and anaerobic microorganisms with further MALDI-TOF identification. Sampling was carried out at 7 stages of cheese production: starting from bulk raw milk to bactofugation, after bactofugation to a mixture normalized in fat content, a pasteurized mixture, a mixture prepared for coagulation, cheese after pressing, and cheese after maturation. Microflora studies were repeated three times, with 405 samples examined. Microbiological studies of Ukrainskyi hard rennet cheese using Maldi TOF technology starting from raw materials to finished dairy products showed the presence of microorganisms at all stages of production – from bulk milk to the finished product. During the entire period of experiments, 43 species of various microorganisms have been isolated and identified. However, the number and individual types of microorganisms differed at different stages of production. Some microorganisms that have been isolated in raw milk are also found in the final product, such as *Acinetobacter baumannii* and Escherichia coli. In total, 18 types of microorganisms have been isolated and identified in the final product – hard rennet cheese, including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*, which are of particular concern in the context of safe consumption of this cheese.

Keywords: MALDI-TOF, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, hard rennet cheese.

INTRODUCTION

Milk and dairy products are important components of human nutrition because they are an important source of protein, lactose, milk fat, and biologically active substances **[1]**. Due to its rich chemical composition and optimal physical properties favourable for most microorganisms, milk is an ideal environment for their growth and development.

The main part of the milk microbiota consists of mesophilic aerobic and facultatively anaerobic microorganisms that grow in the presence of oxygen. This group of bacteria is a microbiological indicator of food quality, showing the effectiveness of heat treatment and compliance with sanitary and hygienic requirements during production, primary raw milk processing, transportation, and storage. The presence of mesophilic aerobic microorganisms may also suggest sources of contamination during milk processing **[2]**.

In the dairy industry, the microbiological safety of raw milk is the basis for the technology of producing a proper-quality product. So, the permanent microflora of raw milk is represented by Corynebacterium spp. **[3]**,

certain species that can be pathogenic to animals and humans. These bacteria can also be found in finished dairy products, particularly cheeses, and this can also pose a danger to human health **[4]**.

Raw milk supplied to milk processing enterprises may contain various microorganisms **[5]**. Still, special attention should be paid to opportunistic and pathogenic ones since they can threaten the life and health of people and animals. One study shows the diversity of the microflora found in raw milk throughout the year and the dependence of milk quality indicators on the microflora composition. Bacterial contamination of milk was higher in May and June, with its lowest indicator in October and December. The following types of bacteria have been isolated: *Firmicutes*, and *Bacteroidote*. The most common genera were represented by *Pseudomonas*, *Acinetobacter*, *Streptococcus*, and *Lactobacillus* **[6]**.

Opportunistic pathogenic microorganisms, such as *Staphylococcus aureus*, can multiply in dairy products, affect their organoleptic parameters, and accumulate toxins **[7]**. Most often, the presence of this pathogen is registered in raw milk due to poor hygiene and sanitation on the farm, but hygiene during milking and hygiene of service personnel, as well as mastitis in cows, have an impact, too **[8]**.

Pathogenic microorganisms – pathogens of infectious diseases – in milk and dairy products can maintain their viability long and pose a danger to consumers. In particular, bacteria of the Enterococcus genus, isolated from raw milk and finished dairy products, can form a biofilm, which confirms the need for continuous monitoring of microbial adhesion in dairy production facilities **[9]**.

The bacteria that can be present in milk may cause a variety of bacterial infections. Pathogenic or opportunistic pathogenic microorganisms, such as *Acinetobacter* **[6]**, *Escherichia coli* **[7]**, *Klebsiella spp* **[8]**, and so on are especially dangerous for humans.

Klebsiella pneumoniae is one of the most common species of the *Klebsiella* genus. It is the causative agent of infectious diseases of animals, in particular, mastitis in cows **[9]**, **[10]**. Increasing antibiotic resistance of K. pneumoniae, especially strains producing extended-spectrum β-lactamases (ESBL) and/or carbapenems, is of worldwide concern today **[11]**, **[12]**.

In cattle, *Klebsiella spp* are transmitted through contact with udder teats with manure, bedding, and other agricultural accessories. The infection affects the epithelial cells of the teat and can persist in the udder for a long time. Besides, *Klebsiella spp* strains affect the safety and quality of milk, as well as the productivity of adult cows, and pose a threat to the survival of newborn calves **[13]**, **[14]**.

Over the past decade, the number of cases of *Klebsiella spp* detection in milk samples obtained from cows with mastitis has increased dramatically worldwide **[15]**, **[16]**.

Klebsiella pneumoniae is a zoonotic pathogen that often becomes a source of nosocomial infections **[17]**. *Klebsiella pneumoniae* mainly causes pneumonia, liver abscess, meningitis **[16]**,**[17]** urinary tract diseases **[18]**, toxemia, septicemia, and other symptoms of infection **[19]**, **[20]**. Researchers point out that in the United States of America, *Klebsiella pneumoniae* accounts for 3% to 8% of all nosocomial infections (nosocomium) - an infection that the patient did not have at the time of admission to a hospital or other healthcare facility **[19]**.

The World Health Organization (WHO) controls Klebsiella spp., carriers of many drug-resistance genes **[21]**, **[22]**.

Bacteria of the *Acinetobacter* genus are identified in milk as a result of contamination through milking equipment, which may contain water residues, or due to improper cleaning of milk pipelines or coolers, contaminated udders, and teats, non-compliance with hygienic requirements during the transportation and storage of milk, and improper cleaning of dairy equipment **[23]**, **[24]**. Most representatives of the Acinetobacter genus are opportunistic pathogenic clinically insignificant commensals with limited virulence. However, the severity of infections caused by Acinetobacter has recently increased due to the frequent use of mechanical breathing devices, venous catheters, and antibiotics, which pose a significant public health concern. *Acinetobacter baumannii (A. baumannii)* is an opportunistic pathogenic microorganism that causes various nosocomial infections **[25]**. Studies conducted in animal models and clinical data have shown that *A. baumannii* is a virulent species. It is a dangerous pathogen, especially due to the emergence of multi-resistant (MLR) strains and their association with many nosocomial and community-acquired infections **[26]**. Researchers claim that dairy products contaminated with *A. baumannii* can be community-acquired reservoirs as underestimated pathogens that pose health risks for immunocompromised adults and children **[27]**, **[28]**.

Escherichia coli, which causes subclinical or clinical mastitis in cattle, accounts for transmitting antimicrobial resistance through human consumption of raw milk or raw dairy products **[25]**.

The spread of E. coli's antimicrobial resistance has recently become increasingly recognised, with concern about human and animal health growing even more. In particular, the constant use of antimicrobial agents for the treatment and prevention of bovine mastitis has contributed to the emergence of antimicrobial resistance of E. coli due to genetic mutation or horizontal gene transfer that can potentially pose a health threat **[26]**.

One of the health protection problems worldwide is the presence of pathogenic bacteria *Escherichia coli* in milk and dairy products, which produces Shiga toxin (STEC) **[27]**, causing intestinal diseases. Therefore, monitoring milk and dairy products at all stages of production for contamination with *Escherichia coli* is important for ensuring the safety of dairy products **[28]**. Besides, *Escherichia coli* can form biofilms, which pose a significant risk during dairy production. Also, researchers leave open the possibility that biofilms can withstand milk pasteurisation regimes **[29]**.

Raw milk may also contain other microorganisms that risk human and animal health **[30]**. To neutralise unwanted microorganisms during raw milk processing, it is exposed to a number of technological factors, such as bactofugation, pasteurisation, and the like **[31]**.

Scientific Hypothesis

Dairy products and their production chains can become a depot for the transmission of bacteria that contaminate raw milk, circulate in processing plants, live in finished dairy products, and pose a threat to consumer health. That is why it was our objective to conduct microbiological screening using Maldi TOF technology, starting from raw milk to the finished dairy product, and to conduct an analysis of microorganisms that are being detected during the technological process of Ukrains hard rennet cheese's production and are clinically significant for human and animal health.

MATERIAL AND METHODOLOGY

Samples

Samples of bulk raw milk were taken at Haisyn Dairy Plant LLC, located in Haisyn Town, Vinnytsia Oblast. Raw milk was supplied from five dairy farms. The plant's technological capacity allows it to produce 300 tons of hard and 180 tons of soft cheeses, 540 tons of butter and spreads, and 360 tons of dry dairy products monthly. The company has implemented a food safety management system to the international standard ISO 22000 requirements.

Chemicals

Nutrient media, reagents, and materials were used in the work.

Blood Agar (BA). Produced by BioMérieux, France.
Buffered Peptone Water (BPW). Produced by HiMedia, India.
Baird Parker Agar (BPA). Produced by HiMedia, India.
Endo Agar (Endo). Produced by Farmactiv, Ukraine.
Pseudomonas Agar (Pseudo). Produced by HiMedia, India.
Enterococcus Agar. Produced by Farmactiv, Ukraine.
Bacillus Cereus Agar. Produced by HiMedia, India.
Bismuth Sulfite Agar (BCA). Produced by HiMedia, India.
Xylose-lysine deoxycholate Agar (XLD Agar). Produced by HiMedia, India.
Packages for creating anaerobic conditions. Produced by BioMérieux, France.
HCCA mass spectrometer matrix (art. 255344). Produced by Bruker, Germany.
Bacterial calibrator (art. 255343). Produced by Bruker, Germany.
Peptone salt solution (PSS). Produced in Ukraine.

Materials

Loops made of platinum/iridium or nickel/chromium, with a diameter of 3 mm. Produced in Ukraine.
Graduated glasses. Produced by Simax, Czech Republic.
Volumetric flasks. Produced by Duran, Germany.
Test tubes P2 16x150 mm. Produced by Sklopryład, Ukraine.
Petri dishes, with a diameter of 90 mm. Produced by Vorwarts Diagnostic, Ukraine.
Plastic sterile Pasteur pipettes. Produced by Labexpert, China.
Sterile glass vials, 500 ml. Produced by Simax, Czech Republic.
Sterile tips with filter for Eppendorf dispensers for 0,1-10 µL. Produced by Eppendorf, Germany.
Metal chips for MALDI-TOF. Produced by Bruker Daltonics, Germany.

Instruments

During the study, we used the equipment as follows:

MALDI-TOF MS mass spectrometer. Produced by Bruker Daltonics, Germany.

Wet sterilisation device (autoclave), capable of maintaining temperatures from 120□C to 134□C (vertical autoclave 5050 ELV D-line). Produced by Tuttnauer, Israel.

The drying chamber maintains a temperature of 160ºC (dry heat steriliser ED115). Produced by Binder, Germany.

Incubator (thermostat) capable of maintaining temperatures of 30 and 37ºC - 1ºC (incubator with natural convection, Binder BD 115). Produced by Binder, Germany.

pH meter with calibration accuracy of 0.1 units of hydrogen index for 25 ºC (laboratory pH meter/ionomer, Mettler Toledo). Produced by Mettler Toledo, Switzerland.

Variable volume piston dispenser for 0.1-2.5 µL. Produced by Eppendorf, Germany.

Loop steriliser. Produced by SteriMaks, Germany.

Vortex (vibration mixer), Biosan. Produced by Biosan, Latvia.

Leica DM500 LED binocular microscope. Produced by Leica, Germany.

Refrigerator and freezer compartments. Produced by Liebherr, Switzerland.

Laboratory Methods

Microbiological milk screening was performed using the MALDI-TOF PV.BLS 7.2-08.15 method **[32]**. The method's principle lies in detecting existing microorganisms in any group of foods, feed, and water by accumulation and inoculation on differential media for aerobic and anaerobic microorganisms, with identification conducted on the MALDI-TOF device. The sector inoculation method was applied following PV to count microorganisms.BLS 7.2-09/08 Investigation of biological fluids by microbiological method (semi-quantitative method) **[33]**.

Identification of microorganisms was carried out according to RI.BLS 7.2-09.13 Standard Operating Procedure "Working with MALDI-TOF Bruker Biotyper" **[34]**. Pathogenic/opportunistic pathogenic bacteria were isolated with their identification on MALDI-TOF **[35]**, which corresponds to the DSTU ISO 16140:2006 Standard **[36]**.

The preparation of media was carried out according to the manufacturer's instructions: preparation of samples for microorganism identification and preparation of standard solutions of reagents.

To prepare 1 cm^3 of basic solvent (OS), we added to the Eppendorf microtube 1.5 cm^3 475 µL of ultrapure deionised water; 500 µL of acetonitrile (ACN); 25 µL of 100% trifluoroacetic acid (TFA). The solvent was thoroughly mixed;

Preparation of the matrix (Bruker IVD HCCA Matrix): we added 250 (±5) µL of OS to a test tube containing 2.5 mg of IVD HCCA matrix (or its analogue) (in a 1:100 ratio, with a final concentration of 10 mg of matrix/cm3), and closed the test tube tightly. The solvent was thoroughly mixed on the vortex (vibration mixer) until the crystals were completely dissolved. The finished solution was stored at room temperature (20-25°C) in a place protected from light for up to one week (test tubes with the precipitate in the form of a crystal are no longer suitable for use);

Preparation of a working solution of the Bruker Bacterial Test Standard (BTS, cat. No. 255343): we added 50 µL of basic solvent (OS) to a BTS test tube. We dissolved it by pipetting about 20 times at room temperature. We piped slowly (about once every 2 seconds) with the tip in the solution during the procedure. We kept it for 5 minutes at room temperature and repeated the procedure as indicated above. We centrifuged it at 13,000 rpm for 2 minutes at room temperature if necessary. Then, we counted the number of colonies growing in different sectors (Table 1).

Table 1 Determining the degree of bacterial contamination by the number of isolated colonies.

A	Number of CFU per sector			Number of CFU in 1 cm^3 of the product
	I	II	III	
1 - 6	-	-	-	up to 1,000
8 - 20	-	-	-	3,000
20 - 30	-	-	-	5,000
30 - 60	-	-	-	10,000
70 - 80	-	-	-	50,000
100 - 150	5 - 10	-	-	100,000
-	20 - 30	-	-	500,000
-	40 - 60	-	-	1 million (10^9)
-	100 - 140	10-20	-	5 million ($5*10^9$)
-	-	30-40	-	10 million (10^{10})
-	-	60-80	single colonies	100 million (10^{11})

Each time, before starting work with a new chip, the device was calibrated with a 100% concentration bacterial test standard (BTS) per the operating instructions to control the compliance and intensity of peaks.

Sector inoculation method: The sample was thoroughly mixed before inoculation. The cup containing agar was conventionally divided into 4 sectors. We used a platinum loop with a diameter of 2 mm and a capacity of

0.005 ml to inoculate the sample (30-40 streaks) on Sector A of a petri dish containing blood agar. After that, the loop was sterilised 4 streak inoculations were made from Sector A to Sector I and similarly – from Sector I to Sector II, and from Sector II to Sector III. The cups were incubated at 37°C for 18-24 hours.

Description of the Experiment

Samples were taken at various technological stages of production: raw milk before bactofugation, raw milk after bactofugation, a normalised mixture from the tank, a pasteurised mixture, a mixture from the cheese maker prepared for coagulation, Ukrainskyi cheese after pressing, Ukrainskyi hard cheese after ripening. At each stage of production, 15 samples were taken for microbiological studies. The studies were repeated three times. A total of 405 samples were taken at seven stages of hard rennet cheese production to study the microflora. Water (10 samples) and brine were examined before and after pasteurisation (10 samples each).

Sample preparation: For sampling milk at Haisyn Dairy Plant LLC, we used sterile disposable plastic dishes with a volume of 100 cm^3. The sampling site was flamed, and the first portion of milk was drained into a separate container; then, we took the samples for examination in a disposable sterile container tightly closed. Immediately after sampling, all samples were placed into a container with a temperature of 2°C and delivered within 2.5 hours to the Biolights Expert Centre for Diagnostics and Laboratory Support LLC, Ternopil City (accreditation according to ISO/IEC 17025).

Appropriate reagents and solutions were prepared before starting work.

The samples to be examined were in liquid form (milk before and after bactofugation, normalised and pasteurised mixture) and semi-solid form (cheese mixture from the cheese maker and cheese after pressing).

Dilutions in a solvent (10 g of product per 90 cm^3) were prepared in peptone salt solution (PSS). This dilution was the source for direct inoculation of samples on cups with blood agar performed by sector inoculation method and further incubated under aerobic and anaerobic conditions at a temperature of 37±1 °C within 24±1 hours.

The liquid product was directly inoculated on the surface of blood agar (by the sector inoculation method) and incubated under aerobic and anaerobic conditions at 37±1°C within 24±1 h. To accumulate a small amount of microflora in the 10 g product sample (liquid or solid), we added up to 90 cm^3 of buffered peptone water (BPW). We incubated it at a temperature of 37±1°C within 24±1 h.

After accumulation, the sample was once again inoculated into differential diagnostic media by sector inoculation method (Baird-Parker Agar, XLD Endo Agar, Pseudomonas Agar, Enterococcus Agar, Bacillus Cereus Agar) and grown within 24±1 or 48±1 h at a temperature of 37±1 or 30±1°C (depending on the requirements of incubation of the nutrient medium).

After cultivation, we examined the inoculations, with the resulting colonies identified on MALDI TOF according to RI.BLS 7.2-09.13 "Working with MALDI-TOF Bruker Biotyper" **[37]**.

An isolated colony (1-2 µL in volume) was taken from a petri dish using a loop or toothpick. In a circular motion, the bacterial mass was evenly applied in a thin layer directly to the surface of the chip hole. After drying up, an automatic dispenser applied a matrix solution in a volume of 1 µL to the sample.

The chip was transferred to MALDI-TOF, and isolated cultures were identified using MBT Compass MALDI Biotyper 3.1 and Compass 1.4 for FLEX—Volume 1 and 2 Software and Manuals (Bruker Daltonik, Bremen, Germany). The studies' outcomes included bacteria identified with a scope value of 2.00.

Number of samples analyzed: We analysed 12 samples.

Number of repeated analyses: All measurements of instrument readings were performed two times.

Number of experiment replications: The number of repetitions of each experiment to determine one value was two times.

Design of the experiment: At the initial stage, samples of milk and dairy products were taken during the technological process for microbiological screening to study microorganisms identified in raw milk and detected in subsequent technological processes. After the first experiment, it became clear that individual microorganisms found in raw milk were also found in the final product. It should be noted that spore-forming microorganisms appear after milk pasteurisation. Therefore, at the next stages, we investigated the probable sources of contamination of the intermediate and final product at the processing stages. For this purpose, we conducted microbiological studies of water and brine. These studies aimed to exclude or confirm the bacteria circulation at the milk processing stages in the milk processing plant. The research findings were subjected to statistical processing and analysis.

Statistical Analysis

Statistical processing of the obtained results was performed using the ANOVA program, with the data in the tables presented as x ± SD (mean ± standard deviation). The difference between the groups was probable at $P < 0.05$ (considering the Bonferroni correction).

RESULTS AND DISCUSSION

At the first stage of the technological process of making Ukrainskyi hard rennet cheese, 18 types of microorganisms were isolated during the study of samples of bulk raw milk before bactofugation (Table 2).

Table 2 Types of bacteria isolated from raw milk at the first stage of the technological process – before bactofugation.

Types of microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/cm^3
Escherichia coli	100	$2.5\pm0.2\times10^4$
Kurthia gibsonii	33	$1\pm0.1\times10^1$
Acinetobacter baumannii	17	$1\pm0.01\times10^1$
Lactococcus lactis	17	$1\pm0.04\times10^1$
Enterobacter bugandensis	33	$1\pm0.02\times10^1$
Hafnia alvei	17	$1\pm0.001\times10^1$
Acinetobacter nosocomialis	17	$1\pm0.03\times10^1$
Lactococcus garvieae	66	1±0.04×101
Citrobacter freundii	17	$1\pm0.02\times10^2$
Staphylococcus aureus	66	$1\pm0.01\times10^1$
Streptococcus uberis	66	$1\pm0.02\times10^1$
Streptococcus uberis	17	$1\pm0.01\times10^1$
Enterococcus faecalis	50	$1\pm0.001\times10^1$
Citrobacter break	17	$1\pm0.05\times10^1$
Macrococcus caseolyticus	17	$1\pm0.001\times10^1$
Enterobacter cloacae	33	$1\pm0.03\times10^1$
Enterococcus faecium	17	$1\pm0.002\times10^1$
Enterobacter ludwigii	33	$1\pm0.004\times10^1$

Note: M±m, n=45.

A gram-negative bacteria, *Escherichia coli*, was isolated in all raw milk samples, which indicates its significantly dominant amount over the rest of the microflora. However, apart from *Escherichia coli*, *Streptococcus uberis,* and *Staphylococcus aureus* were isolated in raw milk in 66% of cases, and mastitis pathogens in cows. *Streptococcus uberis* is considered non-pathogenic to humans, possibly due to difficulties in identifying this type of infection. Besides, *Streptococcus uberis* is identified by classical microbiology as *Staphylococcus aureus* **[38]**.

A significant amount of the microflora identified in milk was of fecal origin. *Enterococcus faecalis* was identified in 50% of the samples, with *Enterobacter cloacae* and *Enterobacter bugandensis* identified in 33%. This speaks for non-compliance with hygienic requirements when obtaining raw milk on farms supplying it to the milk processing plant **[39]**, **[40]**. Other bacteria have also been identified: *Kurthia gibsonii*, *Lactococcus lactis*, *Hafnia alvei*, *Acinetobacter nosocomialis*, *Lactococcus garvieae*, *Citrobacter freundii*, *Enterobacter kobei*, *Citrobacter braaki*, *Macrococcus caseolyticus*, *Enterococcus faecium*, *Enterobacter ludwigii*. Among them, clinically significant human diseases *Citrobacter braaki*i, since it is classified as wound microflora (especially the content of wounds) **[41]**, and *Acinetobacter baumannii*, which is the causative agent of infections of the respiratory tract, blood, abdominal cavity, urinary tract, traumatic infections, central nervous system infections, skin infections, accompanied by the risk of severe complications. This bacterium is resistant to antibiotics and disinfectants, soitoftenbecomes a nosocomial flora **[42]**, **[43]**.

In the second stage of the investigation, raw milk samples were taken after their bactofugation. At this technological stage, 13 microorganisms were identified (Table 3).

Table 3 Bacteria isolated in raw milk at the second stage of the technological process – after bactofugation.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	100	$1.6 \pm 0.1 \times 10^3$
Kurthia gibsonii	33	$1 \pm 0.01 \times 10^1$
Lactococcus lactis	33	$1 \pm 0.01 \times 10^1$
Enterobacter bugandensis	17	$1 \pm 0.03 \times 10^1$
Citrobacter freundii	17	$1 \pm 0.001 \times 10^1$
Staphylococcus aureus	33	$1 \pm 0.01 \times 10^1$
Enterococcus faecalis	66	$1 \pm 0.01 \times 10^1$
Enterobacter cloacae	50	$1 \pm 0.02 \times 10^1$
Enterococcus faecium	50	$1 \pm 0{,}01 \times 10^2$
Enterobacter ludwigii	50	$1 \pm 0.02 \times 10^1$
Staphylococcus chromogens	17	$1 \pm 0.01 \times 10^1$
Streptococcus gallolyticus	17	$1 \pm 0.01 \times 10^1$
Chryseobacterium bovis	17	$1 \pm 0.001 \times 10^1$

Note: M±m, n=45.

Several bacteria were detected at the first stage of research, in particular: *Acinetobacter baumannii*, *Hafnia alvei*, *Acinetobacter nosocomialis*, *Lactococcus garvieae*, *Streptococcus uberis*, *Enterobacter kobei*, *Citrobacter braaki*, Macrococcus caseolyticus. However, bacteria that had not been detected at the first research stage and isolated, such as *Staphylococcus chromogenes*, and *Streptococcus gallolyticus*.

In 100% of the samples taken after bactofugation, the gram-negative bacterium *Escherichia coli* was isolated, but in concentrations slightly lower than in raw milk, by 1.6 times. Also, the causative agent of cow mastitis, *Staphylococcus aureus,* and faecal contamination flora, *Enterobacter cloacae,* was isolated in 33% of milk samples, with *Enterococcus faecalis* isolated in 66%. We should note that in the second stage, *Staphylococcus aureus* was isolated 2 times from several samples. *Enterobacter cloacae* was isolated 1.5 times, with *Enterococcus faecalis* isolated 1.3 times from larger samples. The increase in the number of samples from which *Enterobacter cloacae* and *Enterococcus faecalis* were isolated may be due to the formation of biofilms of these microorganisms on the equipment or due to its unsatisfactory hygiene **[44]**.

We identified *Streptococcus gallolyticus* (old name Streptococcus bovis) in 15% of the samples. This opportunistic pathogenic microorganism can occasionally enter the human bloodstream and cause various diseases. As a rule, this microorganism only colonises pregnant women in the intestines and genitourinary tract. However, there is evidence that this bacterium is associated with infectious endocarditis and human colon cancer. It has the property of adhering to the extracellular matrix, such as collagen, fibronectin, and fibrin, which is a pathogenesis mechanism **[45]**.

Streptococcus gallolyticus is part of the bovine rumen biota and causes diseases in ruminants, particularly mastitis in cows. That is why there is a reason to believe this bacterium got into raw milk **[46]**.

Other bacteria were detected in a smaller percentage of samples. Still, in general, the isolation of 13 types of microorganisms after bactofugation without a significant decrease in their number may indicate unsatisfactory hygienic treatment of technological equipment or the presence of biofilms **[47]**.

Observe in Table 4 the absence of bacteria that were detected at the first stage, before bactofugation, and were not identified after bactofugation: *Acinetobacter baumannii*, *Hafnia alvei*, *Acinetobacter nosocomialis*, *Lactococcus garvieae*, *Streptococcus uberis*, *Enterobacter kobei*, *Citrobacter braaki*, *Macrococcus caseolyticus*. However, we observed the appearance of bacteria that were not detected at the first stage but were fixed after bactofugation: *Staphylococcus chromogenes*, *Streptococcus gallolyticus*.

Table 4 Comparative table: Bacteria isolated before and after bactofugation.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
	before bactofugation		after bactofugation	
Escherichia coli	100	$2.5\pm0.2\times10^4$	100	$1.6\pm0.1\times10^3$
Kurthia gibsonii	33	$1\pm0.1\times10^1$	33	$1\pm0.01\times10^1$
Lactococcus lactis	17	$1\pm0.04\times10^1$	33	$1\pm0.01\times10^1$
Enterobacter bugandensis	33	$1\pm0.02\times10^1$	17	$1\pm0.03\times10^1$
Citrobacter freundii	17	$1\pm0.001\times10^1$	17	$1\pm0.001\times10^1$
Staphylococcus aureus	33	$1\pm0.01\times10^1$	33	$1\pm0.01\times10^1$
Enterococcus faecalis	50	$1\pm0.001\times10^1$	66	$1\pm0.01\times10^1$
Enterobacter cloacae	33	$1\pm0.03\times10^1$	50	$1\pm0.02\times10^1$
Enterococcus faecium	17	$1\pm0.002\times10^1$	50	$1\pm0,01\times10^2$
Enterobacter ludwigii	33	$1\pm0.004\times10^1$	50	$1\pm0.02\times10^1$
Staphylococcus chromogens	-	-	17	$1\pm0.01\times10^1$
Streptococcus gallolyticus	-	-	17	$1\pm0.01\times10^1$
Chryseobacterium bovis	-	-	17	$1\pm0.001\times10^1$
Acinetobacter nosocomialis	17	$1\pm0.03\times10^1$	-	-
Acinetobacter baumannii	17	$1\pm0.01\times10^1$	-	-
Hafnia alvei	17	$1\pm0.001\times10^1$	-	-
Lactococcus garvieae	66	1±0.04×101	-	-
Streptococcus uberis	66	$1\pm0.02\times10^1$	-	-
Citrobacter braaki	17	$1\pm0.05\times10^1$	-	-
Macrococcus caseolyticus	17	$1\pm0.001\times10^1$	-	-

Note: M±m, n=45.

The next step was to study samples of milk mixture normalised in fat content. The study results showed that 18 types of microorganisms had been re-isolated at this stage of production (Table 5).

At this research stage, a gram-negative bacteria *Escherichia coli* was isolated from 66% of samples in a milk mixture normalised in fat content, which, in contrast to the previous stage, is 34% lower. *Streptococcus uberis*, isolated in raw milk after bactofugation and in the normalized mixture, was not detected. *Staphylococcus aureus*, found in raw milk and after bactofugation, was also not detected in the the normalized mixture. In 33% of the normal samples, we found *Enterococcus faecalis*, which was 50% lower than the indicator of the previous stage. The number of *Enterobacter cloacae* and *Enterobacter ludwigii* had also reduced, which might be explained by the suppression of some bacterial species by others.

We also noted the appearance of microorganisms that had yet to be isolated at the previous two stages, namely: *Lelliottia amnigena*, *Streptococcus parauberis*, and *Citrobacter gillenii*. This might be due to both the presence of biofilms and the increase in the quantity of some bacterial species and the decrease in the number of others, and the suppression of some bacteria, depending on their number or the entry of these bacteria from washing water or other potential sources in the milk processing plant.

Special attention should be paid to *Lelliottia amnigena* – a gram-negative facultative anaerobic bacterium. It is usually detected in water sources and then in food (onions, cream, unpasteurised milk, and Spanish pork sausages), which, under favourable conditions, may cause infectious diseases in humans, especially in immunocompromised patients. Several cases of human infection have been described in published research papers, such as endophthalmitis, urinary tract infections, pyonephrosis, and sepsis **[48]**.

In the next, third stage, we studied pasteurised mixtures. Types of microorganisms were detected in pasteurised milk (Table 6).

Table 5 Bacteria isolated from samples of milk mixture normalized in fat content.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	66	$1\pm0.1\times10^4$
Kurthia gibsonii	50	$1\pm0.1\times10^1$
Enterobacter bugandensis	33	$1\pm0.01\times10^1$
Hafnia alvei	33	$1\pm0.04\times10^1$
Lactococcus garvieae	33	$1\pm0.02\times10^1$
Citrobacter freundii	50	$1,4\pm0.7\times10^4$
Enterococcus faecalis	66	$3.4\pm0.4\times10^2$
Citrobacter braaki	66	$1\pm0.3\times10^3$
Macrococcus caseolyticus	50	$1\pm0.01\times10^1$
Enterobacter cloacae	33	$1\pm0.01\times10^1$
Enterobacter ludwigii	17	$1\pm0.02\times10^1$
Moraxella osloensis	17	$1\pm0.01\times10^1$
Buttiauxella gaviniae	17	$1\pm0.001\times10^1$
Aeromonas media	17	$1\pm0.03\times10^1$
Citrobacter koseri	17	$1\pm0.01\times10^1$
Lelliottia amnigena	33	$1\pm0.02\times10^1$
Streptococcus parauberis	17	$1\pm0.002\times10^1$
Citrobacter gillenii	17	$1\pm0.02\times10^4$

Note: M±m, n=45.

Table 6 Bacteria isolated from pasteurized milk samples.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	17	$1\pm0.01\times10^3$
Kurthia gibsonii	17	$1\pm0.02\times10^1$
Hafnia alvei	17	$1\pm0.01\times10^1$
Enterococcus faecalis	17	$1\pm0.01\times10^1$
Streptococcus gallolyticus	17	$1\pm0.02\times10^1$
Acinetobacter Pitti	17	$1\pm0.01\times10^1$
Bacillus cereus	33	$1\pm0.02\times10^1$
Bacillus subtilis	33	$1\pm0.001\times10^1$
Bacillus licheniformis	66	$1\pm0.01\times10^1$
Bacillus megaterium	17	$1\pm0.002\times10^1$

Note: M±m, n=45.

We found *Escherichia coli* again in 17% of the studied samples but in a much smaller amount ($1\pm0.01\times10^3$).

Streptococcus gallolyticus was isolated and identified in 83% of the samples, which is a gram-positive, opportunistic pathogen that can cause bacteremia and endocarditis in humans.

Also, after the pasteurisation process, the appearance of various types of bacteria of the Bacillus family was detected (*Bacillus cereus, Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus megaterium*), which had not been identified in samples taken during previous technological processes. *Bacillus cereus* is a spore-forming microorganism, a common contaminator of dairy products (Table 7). Because the microorganism is widespread in the environment, it can contaminate milk during the milking process and enter dairy products at every stage of processing, storage, and hard rennet cheese ripening. Pasteurisationofmilkisineffectiveifitiscontaminatedwith *Bacillus cereus*. Moreover, it may act as a spore germination activator **[49]**.

In one example, *Acinetobacter pitty*, which belongs to the *Moraxellaceae* family, was isolated. *Acinetobacter pitty*, in association with other species of *Acinetobacter*, may cause various infectious diseases in humans, such as pneumonia, bacteremia, wound infections, meningitis, and urinary tract infections. *Acinetobacter* types of bacteria have a natural resistance to antibiotics and easily acquire this resistance, with their clinical isolates being able to spread rapidly among patients and survive in a hospital environment **[50]**.

Table 7 Comparative table: Bacteria isolated before and after pasteurization.

Microorganisms that have been identified	**% of samples with isolated bacteria**	**Number of microorganisms, CFU/ cm^3**	**% of samples with isolated bacteria**	**Number of microorganisms, CFU/ cm^3**
	before pasteurization		after pasteurization	
Escherichia coli	66	$1\pm0.1\times10^4$	17	$1\pm0.01\times10^3$
Kurthia gibsonii	50	$1\pm0.1\times10^1$	17	$1\pm0.02\times10^1$
Enterobacter bugandensis	33	$1\pm0.01\times10^1$	-	-
Hafnia alvei	33	$1\pm0.04\times10^1$	17	$1\pm0.01\times10^1$
Lactococcus garvieae	33	$1\pm0.02\times10^1$	-	-
Citrobacter freundii	50	$1,4\pm0.7\times10^4$	-	-
Enterococcus faecalis	66	$3.4\pm0.4\times10^2$	17	$1\pm0.01\times10^1$
Citrobacter braaki	66	$1\pm0.3\times10^3$	-	-
Macrococcus caseolyticus	50	$1\pm0.01\times10^1$	-	-
Enterobacter cloacae	33	$1\pm0.01\times10^1$	-	-
Enterobacter ludwigii	17	$1\pm0.02\times10^1$	-	-
Moraxella osloensis	17	$1\pm0.01\times10^1$	-	-
Buttiauxella gaviniae	17	$1\pm0.001\times10^1$	-	-
Aeromonas media	17	$1\pm0.03\times10^1$	-	-
Citrobacter koseri	17	$1\pm0.01\times10^1$	-	-
Lelliottia amnigena	33	$1\pm0.02\times10^1$	-	-
Streptococcus parauberis	17	$1\pm0.002\times10^1$	-	-
Citrobacter gillenii	17	$1\pm0.02\times10^4$	-	-
Streptococcus gallolyticus	-	-	17	$1\pm0.02\times10^1$
Acinetobacter Pitti	-	-	17	$1\pm0.01\times10^1$
Bacillus cereus	-	-	33	$1\pm0.02\times10^1$
Bacillus subtilis	-	-	33	$1\pm0.001\times10^1$
Bacillus licheniformis	-	-	66	$1\pm0.01\times10^1$
Bacillus megaterium	-	-	17	$1\pm0.002\times10^1$

Note: M±m, n=45.

Data from the literature also indicate the fixation of bacteria of the Bacillus family in pasteurized milk **[51]**. Pasteurisation of milk is ineffective in reducing contamination and may instead act as an activator of spore germination. **[52]**, **[53]**. In the next, fourth stage, we studied the mixture from the cheese maker prepared for curdling. At this stage, we isolated three types of microorganisms. The main bacterial component in all samples was the bacterium *Lactococcus lactis*, which was isolated at $1.7\pm0.02\times10^4$. *Escherichia coli* was isolated in 17% of samples, and Enterobacter cloacae was isolated in 33% of samples, $1\pm0.01\times10^1$.

The next, fifth stage, was dedicated to studying cheese after pressing. At this stage, 15 types of bacteria were isolated (Table 8).

Table 8 Bacteria isolated from samples of pressed cheese mixture.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	83	$7.4 \pm 0.01 \times 10^3$
Kurthia gibsonii	17	$1 \pm 0.1 \times 10^1$
Acinetobacter baumannii	50	$1 \pm 0.01 \times 10^1$
Lactococcus lactis	66	$6 \pm 0.02 \times 10^1$
Enterococcus faecalis	50	$1 \pm 0.01 \times 10^1$
Citrobacter break	17	$1 \pm 0.01 \times 10^1$
Macrococcus caseolyticus	33	$1 \pm 0.01 \times 10^1$
Enterobacter cloacae	33	$5.05 \pm 0.01 \times 10^2$
Enterococcus faecium	50	$1.3 \pm 0.01 \times 10^1$
Bacillus cereus	33	$1 \pm 0.01 \times 10^1$
Klebsiella pneumoniae	100	$1 \pm 0.01 \times 10^3$
Enterobacter xiangfangensis	17	$1 \pm 0.01 \times 10^1$
Pseudomonas putida	17	$1 \pm 0.02 \times 10^1$
Enterobacter hormaechei	17	$1 \pm 0.1 \times 10^1$
Klebsiella variicol	17	$1 \pm 0.01 \times 10^1$

Note: M±m, n=45.

After pressing, *Klebsiella pneumoniae was isolated in* 100% of the mixture samples. *Escherichia coli* and various forms of enterococci (*Enterococcus faecalis*, *Enterococcus faecium*) were isolated in most samples (83%). Half of the studied samples had *Acinetobacter baumannii*, and various forms of Enterobacteria (*Enterobacter cloacae*, *Enterobacter hormaechei*, *Enterobacter xiangfangensis*).

Unlike previous samples, *Klebsiella pneumoniae*, *Klebsiella varietal*, *Enterobacter xiangfangensis*, *Pseudomonas putida*, and *Enterobacter hormaechei* were found in the mixture for making cheese after pressing, which might indicate internal contamination during cheese production.

Special attention should be paid to *Klebsiella pneumonia*, as it is a zoonotic pathogen that generally causes various infectious conditions in both humans and animals **[54]**. We are concerned that this pathogen is detected in all samples at the penultimate stage of cheese production, which suggests that *Klebsiella pneumoniae* has entered the final product.

Considering that microorganisms can also enter milk at all technological stages with water, we examined 15 samples of water used in production. No microorganisms were detected in all the studied samples.

Studies were also conducted on the presence of microorganisms in the brine for salting cheese before and after pasteurisation. We identified 7 types of microorganisms (Table 9).

Table 9 Bacteria isolated from cheese brine samples.

Microorganisms that have been identified	Number of microorganisms in brine before pasteurization, CFU/cm^3	Number of microorganisms in brine after pasteurization, CFU/cm^3
Escherichia coli	$2 \pm 0.01 \times 10^5$	$2 \pm 0.02 \times 10^5$
Lactococcus garvieae	$1 \pm 0.01 \times 10^1$	
Enterococcus faecalis	$1 \pm 0.01 \times 10^1$	$1 \pm 0.001 \times 10^1$
Enterobacter cloacae	$1 \pm 0.02 \times 10^3$	$2 \pm 0.03 \times 10^3$
Enterococcus faecium	$1 \pm 0.001 \times 10^1$	
Klebsiella pneumoniae	$1 \pm 0.02 \times 10^3$	$1 \pm 0.01 \times 10^3$
Enterobacter xiangfangensis		$1 \pm 0.001 \times 10^1$

Note: M±m, n=15.

Study Shav found is contaminated with various bacteria, particularly *Klebsiella pneumoniae*, which we have previously found in cheese samples after pressing. Also, brine, both before and after pasteurization, contains *Escherichia coli* in a fairly large number of 2×10^5 CFU/cm^3.

The final stage was studying the cheese after it had matured for 30 days. Eighteen types of microorganisms were identified in the finished hard rennet cheese (Table 10).

Table 10 Bacteria isolated from samples of Ukrainskyi hard rennet cheese.

Microorganisms that have been identified	% of samples with isolated bacteria	Number of microorganisms, CFU/ cm^3
Escherichia coli	83	$5.6\pm0.01\times10^2$
Kurthia gibsonii	33	$1\pm0.02\times10^1$
Acinetobacter baumannii	50	$1\pm0.002\times10^1$
Lactococcus lactis	66	$1.28\pm0.02\times10^2$
Enterococcus faecalis	66	$1\pm0.002\times10^1$
Macrococcus caseolyticus	33	$1\pm0.002\times10^1$
Enterobacter cloacae	33	$5\pm0.06\times10^2$
Enterococcus faecium	50	$1.33\pm0.001\times10^1$
Bacillus cereus	50	$1\pm0.002\times10^1$
Klebsiella pneumoniae	100	$1\pm0.05\times10^3$
Enterobacter xiangfangensis	17	$1\pm0.02\times10^1$
Pseudomonas putida	17	$1\pm0.01\times10^1$
Micrococcus luteus	17	$1\pm0.03\times10^1$
Corynebacterium flavescens	17	$1\pm0.02\times10^1$
Staphylococcus hominins	17	$1\pm0.01\times10^1$
Lysinibacillus sphaericus	17	$1\pm0.01\times10^1$
Enterobacter asburiae	17	$1\pm0.01\times10^1$

Note: M±m, n=15.

Considering the above, the finished product also contains many microorganisms. According to the search findings, a high content of *Escherichia coli* was detected—10^2. *Klebsiella pneumoniae* was identified in cheese after pressing and in finished cheese in 100% of samples, although this bacterium had not been detected at previous stages. Most likely, the brine was contaminated with bacteria at the final stages of production.

In total, 18 types of microorganisms were isolated and identified in the final product – Ukrainskyi hard rennet cheese, including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, which are of particular concern in terms of the safety of cheese consumption.

It should be noted that at the final stage, bacteria that had not been identified at the previous stages of milk processing wereisolated, such as *Micrococcus luteus*, *Corynebacterium flavescens*, *Staphylococcus hominis*, *Lysinibacillus sphaericus*, *Enterobacter asburiae*, *Corynebacterium flavescens*. Most likely, they were part of the starter cultures of microorganisms added during cheese production. However, *Lysinibacillus sphaericus* may be found in the human gastrointestinal tract, but most commonly it is found in soil. Also, this bacterium is classified as a causative agent of insect diseases, which is present everywhere in the environment. They contaminate bacteria, which relatively rarely cause infectious diseases in humans **[55]**.

So, bulk raw milk that had been delivered for processing contained 18 types of microorganisms, including fecal contamination bacteria – *Enterobacter cloacae*, *Enterobacter ludwigii*, *Citrobacter braaki*, *Enterobacter kobei* and pathogens of cow mastitis: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus uberis*. This speaks for the inadequate sanitary quality of raw milk. Of particular concern are bacteria that are of clinical significance to humans, which may cause various infectious diseases, such as *Lactococcus garvieae* **[54]**, *Acinetobacter baumannii* **[56]**, *Enterobacter bugandensis* **[57]**, *Enterobacter ludwigii* **[58]**, *Acinetobacter baumannii* and *Enterococcus faecalis* **[59]**. All of them relate to bacteria that can become resistant to antibiotics pose a serious threat to people in various infectious conditions and circulate as nosocomial infections. It is dangerous that Acinetobacter identified the very first stage – in raw milk before bactofugation, and then it appears at the final stages of the technological process more often than at the beginning. This fact indicates the possible circulation

of these bacteria in the equipment or auxiliary accessories of the milk processing plant. The bacterium's ability to resist disinfectants allows it to survive even when milk pipelines and other equipment are thoroughly washed and disinfected. Controlling only coliform bacteria (CB) as sanitary indicative microorganisms may not show the real situation with bacterial contamination of equipment, pipelines, water, etc. We should also note that the bacterium retains its viability in cheese after 30 days of storage, even with salt. *Acinetobacter* spp. Bacteria can transmit genes resistant to other bacteria, thus posing a significant risk to humans. However, due to the difficulty of isolation and the absence of official standard methods, there is a lack of work on epidemiological data on foodborne diseases caused by this microorganism.

The Infectious Diseases Society of America (IDSA) classified *A. baumannii* as ESKAPE type (acronym of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter)* which cause most nosocomial infections in the United States and around the world, being usually antibiotic-resistant **[60]**.

Enterococcus faecalis was isolated at all stages of the technological process except for the mixture prepared for curdling. *Enterococcus faecium* was isolated at the beginning of the technological process (before bactofugation and after bactofugation) and at the end of the technological process (cheese after pressing, cheese after maturation). Apart from the fact that enterococci are one of the external pathogens of mastitis, the opportune pathogenic bacteria are part of humans' and animals' normal physiological intestinal flora. However, in recent years, they have become one of the main pathogens that cause numerous infections in humans, mainly nosocomial ones, such as bacteremia and infections of the urinary tract, skin, soft tissues, abdominal cavity, pelvis, and central nervous system. These infections are mostly caused by *E. faecalis* (about 80.0%) and *E. faecium* (10.0%–15.0%) **[61]**. The high resistance of enterococci to adverse conditions allows them to survive in the environment, particularly, in slaughterhouses. Potential mammary gland infections caused by the bacteria are normally mild. It is typical for Enterococci to have high resistance to many anti-bacterial substances, both by internal and acquired mechanisms. Due to their ability to acquire and transmit genes that determine resistance to other bacteria, they are perceived as a good indicator of antimicrobial resistance in the environment. The possibility of enterococci transmission through milk to humans raises concern **[62]**. *Streptococcus gallolyticus* was isolated after bactofugation.

This opportunistic pathogenic microorganism can occasionally enter the human bloodstream and cause various diseases. As a rule, this microorganism only colonises pregnant women's intestines and genitourinary tract. However, there is evidence that this bacterium is associated with infectious endocarditis and human colon cancer. It can adhere to the extracellular matrix, such as collagen, fibronectin, and fibrin, a pathogenesis mechanism. *Streptococcus gallolyticus* is part of the rumen biota, but it also causes various diseases of ruminants. In particular, it can cause mastitis in cows. That is why there is a reason to suggest that this bacterium was present in raw milk **[63]**.

Special attention should be paid to Lelliottiaamnigena, isolated in a mixture of normal fat content. It is a gram-negative facultative anaerobic bacterium, usually found in water sources and then in food (onions, cream, unpasteurisedmilkandSpanish pork sausages), which, under favourable conditions, can cause infectious diseases in humans, especially in immunocompromised patients. Several cases of human infection with endophthalmitis, urinary tract infections, pionephrosis, and sepsis have been highlighted in the published research papers **[61]**. Since this microorganism was not detected in previous studies, we may assume it got into samples from potential sources at the milk processing plant.

After pasteurisation, we detected the appearance of various types of bacteria belonging to the Bacillus family (*Bacillus cereus, Bacillus licheniformis*, *Bacillus subtilis*, and *Bacillus megaterium*). These bacteria were not isolated in samples taken during previous processes. *Bacillus cereus* is a spore-germinating microorganism that is ten isolated in dairy products. Since the microorganism is widespread in the environment, it can contaminate milk during the milking process. It can enter the dairy product at every cheese processing, storage, and maturation stage. Pasteurisation of milk is not effective for *Bacillus cereus.* Instead, pasteurisation may act as a spore germination activator **[62]**.

Acinetobacter pitty, belonging to the *Moraxellaceae f*amily, has also been identified. In association with other Acinetobacter species, Acinetobacter pitty causes various infectious conditions in humans, such as pneumonia, bacteremia, wound infections, meningitis, and urinary tract infections. Acinetobacter types of bacteria have a natural resistance to antibiotics and easily acquire this resistance, with their clinical isolates being able to spread rapidly among patients and survive in a hospital environment **[42]**.

Also of great concern is the appearance of *Klebsiella pneumonia*, which is detected at the last two stages of the technological process and found in the final product after maturation.

Table 11. Bacteria isolated from raw milk to final product (Ukrainskyi hard rennet cheese).

Microorganisms that have been identified	Raw milk	After bactofugation	Milk mixture normalized	After pasteurization	Pressed cheese mixture	Final product
	% of samples with isolated bacteria					
Escherichia coli	100	100	66	17	17	83
Kurthia gibsonii	33	33	50	17	17	17
Acinetobacter baumannii	17				50	33
Lactococcus lactis	17	50			66	66
Enterobacter bugandensis	33	17	33			
Hafnia alvei	17	33	17			
Acinetobacter nosocomialis	17					
Lactococcus garvieae	66		33			
Citrobacter freundii	17	17	50			
Staphylococcus aureus	66	50				
Streptococcus uberis	33					
Enterobacter kobei	17					
Enterococcus faecalis	50	66	66	17	50	50
Citrobacter braaki	17		66			
Macrococcus caseolyticus	17		50		33	33
Enterobacter cloacae	33	50	33		33	33
Enterococcus faecium	17	50			50	17
Enterobacter ludwigii	33	50	17			
Staphylococcus chromogens		17				
Streptococcus gallolyticus		17		100		
Chryseobacterium bovis		17				
Moraxella osmosis			17			
Buttiauxella gaviniae			17			
Aeromonas media			17			
Citrobacter koseri			17			
Lelliottia amnigena			33			
Streptococcus parauberis			17			
Citrobacter genii			17			
Acinetobacter Pitti				17		
Bacillus cereus				33	33	50
Bacillus subtilis				33		
Bacillus licheniformis				66		
Bacillus megaterium				17		
Klebsiella pneumoniae					100	100
Enterobacter xiangfangensis					17	17
Pseudomonas putida					17	17
Enterobacter hormaechei					17	
Klebsiella varietal					17	
Micrococcus luteus						17
Corynebacterium flavescens						17
Staphylococcus hominins						17
Lysinibacillus sphaericus						17
Enterobacter asburiae						17

Note: n=45.

A total of 43 species of various microorganisms have been isolated and identified during the entire experimental period (Table 11). We have found the following bacteria: *Escherichia coli*, *Kurthia gibsonii*, *Acinetobacter baumannii*, *Lactococcus lactis*, *Enterobacter bugandensis*, *Hafnia alvei*, *Acinetobacter nosocomialis*, *Lactococcus garvieae*, *Citrobacter freundii*, *Staphylococcus aureus*, *Streptococcus uberis*, *Enterobacter kobei*, *Enterococcus faecalis*, *Citrobacter braaki*, *Macrococcus caseolyticus*, *Enterobacter cloacae*, *Enterococcus faecium*, *Enterobacter ludwigii*, Staphylococcus chromogenes, *Streptococcus gallolyticus*, *Chryseobacterium bovis*, *Moraxella osloensis*, *Buttiauxella gaviniae*, *Aeromonas media*, *Citrobacter koseri*,

Lelliottia amnigena, *Streptococcus parauberis*, *Citrobacter gillenii*, *Acinetobacter pittii*, *Bacillus cereus*, *Bacillus subtilis, Bacillus licheniformis*, *Bacillus megaterium*, *Klebsiella pneumoniae*, *Enterobacter xiangfangensis*, *Pseudomonas putida*, *Enterobacter hormaechei*, *Klebsiella variicol*, *Micrococcus luteus*, *Corynebacterium flavescens*, *Staphylococcus hominis*, *Lysinibacillus sphaericus*, *Enterobacter asburiae*.

For a more in-depth understanding of the sources of microbial entry into samples at the stages of the technological process of cheese production, the study of the microbiota of equipment, starter cultures, and brine for salting cheese, as well as the sensitivity of the most significant microorganisms to antibacterial agents is looking rather promising.

CONCLUSION

Microbiological screening of Ukrainskyi hard rennet cheese using MALDI-TOF technology, starting from bulk raw milk to the finished dairy product, showed the presence of microorganisms at all stages of production.

During the experiments, 43 species of various microorganisms were isolated and identified. However, the number and individual types of microorganisms differed at different stages of production. Of the 43 types of microorganisms identified during the entire experiment, only 18 were fixed at the first production stage, that is, in raw milk. The other 25 types of bacteria were detected at the next stages of production. Some microorganisms isolated in raw milk are also identified in the final product: *Acinetobacter baumannii* and *Escherichia coli*. In this regard, the production conditions must monitor these microorganisms to control them and prevent their appearance in the finished product, hard cheese.

A clinically significant microorganism such as *Klebsiella pneumonia* most likely got into the final product from brine. This is caused by improper control during production and insufficient sanitary actions to avoid contamination with foreign microflora.

This experiment requires further research, particularly the study of the sensitivity of isolated bacteria to antibiotics and how technological processes affect their significance. The fact that bacteria that can cause various diseases get into the final product will pose an even greater threat if these bacteria remain resistant to antibiotics.

In total, 18 types of microorganisms were isolated and identified in the final product—Ukrainskyi hard rennet cheese—including *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli*, which are of particular concern regarding the safe consumption of cheese.

The presence of opportunistic microorganisms in finished dairy products can lead to: the risk of potentially dangerous bacteria entering the human body, spoilage of products before the expiration date, deterioration of the taste qualities of products, and, accordingly, economic losses for the manufacturer. Based on the experimental data, it is possible to recommend food industry manufacturers conduct additional monitoring (microbiological screening) during the technological process to identify potential sources of contamination by various types of microorganisms. According to the obtained data, it can be concluded that more is needed to control microbiological indicators regulated by legislation. In the production process, a wide list of microorganisms can circulate, which are not included in the basic indicators of the control of dairy products.

REFERENCES

1. Mikulec, N., Špoljarić, J., Plavljanić, D., Lovrić, N., Oštarić, F., GajdošKljusurić, J., Sarim, K. Mohd., Zdolec, N., &Kazazić, S. (2024). MALDI-TOF Mass Spectrometry-Based Identification of Aerobic Mesophilic Bacteria in Raw Unpreserved and Preserved Milk. In Processes (Vol. 12, Issue 4, p. 731). MDPI AG. https://doi.org/10.3390/pr12040731
2. Hahne, J., Kloster, T., Rathmann, S., Weber, M., & Lipski, A. (2018). Isolation and characterization of Corynebacterium spp. from bulk tank raw cow's milk of different dairy farms in Germany. In L. A. Maldonado Manjarrez (Ed.), PLOS ONE (Vol. 13, Issue 4, p. e0194365). Public Library of Science (PLoS). https://doi.org/10.1371/journal.pone.0194365
3. Yuan, H., Han, S., Zhang, S., Xue, Y., Zhang, Y., Lu, H., & Wang, S. (2022). Microbial Properties of Raw Milk throughout the Year and Their Relationships to Quality Parameters. In Foods (Vol. 11, Issue 19, p. 3077). MDPI AG. https://doi.org/10.3390/foods11193077
4. Borena, B. M., Gurmessa, F. T., Gebremedhin, E. Z., Sarba, E. J., & Marami, L. M. (2023). *Staphylococcus aureus* in cow milk and milk products in Ambo and Bako towns, Oromia, Ethiopia: prevalence, associated risk factors, hygienic quality, and antibiogram. In International Microbiology (Vol. 26, Issue 3, pp. 513–527). Springer Science and Business Media LLC. https://doi.org/10.1007/s10123-022-00317-x
5. Gajewska, J., Chajęcka-Wierzchowska, W., Byczkowska-Rostkowska, Z., & Saki, M. (2023). Biofilm Formation Capacity and Presence of Virulence Determinants among Enterococcus Species from Milk and Raw Milk Cheeses. In Life (Vol. 13, Issue 2, p. 495). MDPI AG. https://doi.org/10.3390/life13020495

6. Tamma, P. D., Aitken, S. L., Bonomo, R. A., Mathers, A. J., van Duin, D., & Clancy, C. J. (2021). Infectious Diseases Society of America Guidance on the Treatment of AmpC β-Lactamase–Producing Enterobacterales, Carbapenem-Resistant Acinetobacter baumannii, and Stenotrophomonas maltophilia Infections. In Clinical Infectious Diseases (Vol. 74, Issue 12, pp. 2089–2114). Oxford University Press (OUP). https://doi.org/10.1093/cid/ciab1013
7. Enciso-Martínez, Y., González-Aguilar, G. A., Martínez-Téllez, M. A., González-Pérez, C. J., Valencia-Rivera, D. E., Barrios-Villa, E., & Ayala-Zavala, J. F. (2022). Relevance of tracking the diversity of Escherichia coli pathotypes to reinforce food safety. In International Journal of Food Microbiology (Vol. 374, p. 109736). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2022.109736
8. Crippa, C., Pasquali, F., Rodrigues, C., De Cesare, A., Lucchi, A., Gambi, L., Manfreda, G., Brisse, S., & Palma, F. (2023). Genomic features of Klebsiella isolated from artisanal ready-to-eat food production facilities. In Scientific Reports (Vol. 13, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-023-37821-7
9. Zheng, Z., Gorden, P. J., Xia, X., Zheng, Y., & Li, G. (2021). Whole-genome analysis of <scp>Klebsiella pneumoniae</scp> from bovine mastitis milk in the U.S. In Environmental Microbiology (Vol. 24, Issue 3, pp. 1183–1199). Wiley. https://doi.org/10.1111/1462-2920.15721
10. Ágredo-Campos, Á. S., Fernández-Silva, J. A., & Ramírez-Vásquez, N. F. (2023). *Staphylococcus aureus*, Escherichia coli, and *Klebsiella spp*. Prevalence in bulk tank milk of Colombian herds and associated milking practices. In Veterinary World (pp. 869–881). Veterinary World. https://doi.org/10.14202/vetworld.2023.869-881
11. Bonardi, S., Cabassi, C. S., Fiaccadori, E., Cavirani, S., Parisi, A., Bacci, C., Lamperti, L., Rega, M., Conter, M., Marra, F., Crippa, C., Gambi, L., Spadini, C., Iannarelli, M., Paladini, C., Filippin, N., & Pasquali, F. (2023). Detection of carbapenemase- and ESBL-producing Klebsiella pneumoniae from bovine bulk milk and comparison with clinical human isolates in Italy. In International Journal of Food Microbiology (Vol. 387, p. 110049). Elsevier BV. https://doi.org/10.1016/j.ijfoodmicro.2022.110049
12. Song, J., Xiang, W., Wang, Q., Yin, J., Tian, T., Yang, Q., Zhang, M., Ge, G., Li, J., Diao, N., Liu, F., Shi, K., Cai, R., Du, R., & Gong, Q. (2023). Prevalence and risk factors of *Klebsiella spp*. in milk samples from dairy cows with mastitis—A global systematic review. In Frontiers in Veterinary Science (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fvets.2023.1143257
13. Song, J., Xiang, W., Wang, Q., Yin, J., Tian, T., Yang, Q., Zhang, M., Ge, G., Li, J., Diao, N., Liu, F., Shi, K., Cai, R., Du, R., & Gong, Q. (2023). Prevalence and risk factors of *Klebsiella spp*. in milk samples from dairy cows with mastitis—A global systematic review. In Frontiers in Veterinary Science (Vol. 10). Frontiers Media SA. https://doi.org/10.3389/fvets.2023.1143257
14. Schukken, Y., Chuff, M., Moroni, P., Gurjar, A., Santisteban, C., Welcome, F., &Zadoks, R. (2012). The "Other" Gram-Negative Bacteria in Mastitis. In Veterinary Clinics of North America: Food Animal Practice (Vol. 28, Issue 2, pp. 239–256). Elsevier BV. https://doi.org/10.1016/j.cvfa.2012.04.001
15. Gao, J., Li, S., Zhang, J., Zhou, Y., Xu, S., Barkema, H. W., Nobrega, D. B., Zhu, C., & Han, B. (2019). Prevalence of Potential Virulence Genes in *Klebsiella spp*. Isolated from Cows with Clinical Mastitis on Large Chinese Dairy Farms. In Foodborne Pathogens and Disease (Vol. 16, Issue 12, pp. 856–863). Mary Ann Liebert Inc. https://doi.org/10.1089/fpd.2019.2657
16. Zhu, J., Wang, T., Chen, L., & Du, H. (2021). Virulence Factors in Hypervirulent Klebsiella pneumoniae. In Frontiers in Microbiology (Vol. 12). Frontiers Media SA. https://doi.org/10.3389/fmicb.2021.642484
17. Liu, J., Liu, Y., Li, C., Peng, W., Jiang, C., Peng, S., & Fu, L. (2024). Characteristics of Klebsiella pneumoniae pyogenic liver abscess from 2010–2021 in a tertiary teaching hospital in South China. In Journal of Global Antimicrobial Resistance (Vol. 36, pp. 210–216). Elsevier BV. https://doi.org/10.1016/j.jgar.2023.12.024
18. Euler, C. W., Raz, A., Hernandez, A., Serrano, A., Xu, S., Andersson, M., Zou, G., Zhang, Y., Fischetti, V. A., & Li, J. (2023). PlyKp104, a Novel Phage Lysin for the Treatment of Klebsiella pneumoniae, Pseudomonas aeruginosa, and Other Gram-Negative ESKAPE Pathogens. In Antimicrobial Agents and Chemotherapy (Vol. 67, Issue 5). American Society for Microbiology. https://doi.org/10.1128/aac.01519-22
19. Ashurst, J. V., & Dawson, A. (2023). Klebsiella Pneumonia. In-Stat Pearls. Stat Pearls Publishing.
20. Prestinaci, F., Pezzotti, P., &Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. In Pathogens and Global Health (Vol. 109, Issue 7, pp. 309–318). Informa UK Limited. https://doi.org/10.1179/2047773215y.0000000030
21. Cao, H., Yan, Y., Wang, L., Dong, L., Pang, X., Tang, S., Li, A., Xiang, A., Zhang, L., & Zheng, B. (2021). High-Throughput Sequencing Reveals Bacterial Diversity in Raw Milk Production Environment and

Production Chain in Tangshan City of China. In Food Science of Animal Resources (Vol. 41, Issue 3, pp. 452–467). Korean Society for Food Science of Animal Resources. https://doi.org/10.5851/kosfa.2021.e10

22. Rangel, K., & De-Simone, S. G. (2024). Treatment and Management of Acinetobacter Pneumonia: Lessons Learned from Recent World Event. In Infection and Drug Resistance: Vol. Volume 17 (pp. 507–529). Informa UK Limited. https://doi.org/10.2147/idr.s431525
23. Mohamed, H. M. A., Abd-Elhafeez, H. H., Al-Jabr, O. A., & El-Zamkan, M. A. (2022). Characterization of Acinetobacter baumannii Isolated from Raw Milk. In Biology (Vol. 11, Issue 12, p. 1845). MDPI AG. https://doi.org/10.3390/biology11121845
24. Amorim, A. M. B. de, & Nascimento, J. dos S. (2017). Acinetobacter: an underrated foodborne pathogen? In The Journal of Infection in Developing Countries (Vol. 11, Issue 02, pp. 111–114). Journal of Infection in Developing Countries. https://doi.org/10.3855/jidc.8418
25. Yoon, S., & Lee, Y. J. (2022). Molecular characteristics of Escherichia coli from bulk tank milk in Korea. In Journal of Veterinary Science (Vol. 23, Issue 1). The Korean Society of Veterinary Science. https://doi.org/10.4142/jvs.21084
26. Yihunie, F. B., Belete, M. A., Fentahun, G., & Dubie, T. (2024). Molecular detection and antibiogram of Shiga toxin-producing Escherichia coli (STEC) from raw milk in and around Bahir Dar town dairy farms, Ethiopia. In Heliyon (Vol. 10, Issue 7, p. e28839). Elsevier BV. https://doi.org/10.1016/j.heliyon.2024.e28839
27. Shanina, O., Minchenko, S., Gavrysh, T., Sukhenko, Y., Sukhenko, V., Vasyliv, V., Miedviedieva, N., Mushtruk, M., Stechyshyn, M., & Rozbytska, T. (2020). Substantiation of basic stages of gluten-free steamed bread production and its influence on quality of finished product. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 189–201). HACCP Consulting. https://doi.org/10.5219/1200
28. Rosario, A. I. L. S., Castro, V. S., Santos, L. F., Lisboa, R. C., Vallim, D. C., Silva, M. C. A., Figueiredo, E. E. S., Conte-Junior, C. A., & Costa, M. P. (2021). Shiga toxin-producing Escherichia coli isolated from pasteurized dairy products from Bahia, Brazil. In Journal of Dairy Science (Vol. 104, Issue 6, pp. 6535–6547). American Dairy Science Association. https://doi.org/10.3168/jds.2020-19511
29. Machado, M. A. M., Castro, V. S., da Cunha-Neto, A., Vallim, D. C., Pereira, R. de C. L., dos Reis, J. O., de Almeida, P. V., Galvan, D., Conte-Junior, C. A., & Figueiredo, E. E. de S. (2023). Heat-resistant and biofilm-forming Escherichia coli in pasteurized milk from Brazil. In Brazilian Journal of Microbiology (Vol. 54, Issue 2, pp. 1035–1046). Springer Science and Business Media LLC. https://doi.org/10.1007/s42770-023-00920-8
30. Birlutiu, V., Birlutiu, R.-M., &Dobritoiu, E. S. (2023). Lelliottiaamnigena and *Pseudomonas putida* Coinfection Associated with a Critical SARS-CoV-2 Infection: A Case Report. In Microorganisms (Vol. 11, Issue 9, p. 2143). MDPI AG. https://doi.org/10.3390/microorganisms11092143
31. Sharan, M., Vijay, D., Dhaka, P., Bedi, J. S., & Gill, J. P. S. (2022). Biofilms as a microbial hazard in the food industry: A scoping review. In Journal of Applied Microbiology (Vol. 133, Issue 4, pp. 2210–2234). Oxford University Press (OUP). https://doi.org/10.1111/jam.15766
32. Tirloni, E., Stella, S., Celandroni, F., Mazzantini, D., Bernardi, C., &Ghelardi, E. (2022). *Bacillus cereus* in Dairy Products and Production Plants. In Foods (Vol. 11, Issue 17, p. 2572). MDPI AG. https://doi.org/10.3390/foods11172572
33. PV.BLS 7.2–08.15. Microbiological screening of food products, feed, and water PV.BLS 7.2-09/08 Study of biological fluids by microbiological method (semi-quantitative method).
34. RI.BLS 7.2-09.13 Work with MALDI-TOF Bruker Biotyper
35. PV.BLS 7.2-09/10 Isolation of pathogenic/conditionally pathogenic bacteria with identification by MALDI-TOF
36. DSTU ISO 16140:2006 Microbiology of food products and animal feed. Alternative methods validation protocol
37. Valentiny, C., Dirschmid, H., &Lhotta, K. (2015). *Streptococcus uberis* and *Staphylococcus aureus* forefoot and bloodstream co-infection in a hemodialysis patient: a case report. In BMC Nephrology (Vol. 16, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12882-015-0069-6
38. Zheplinska, M., Mushtruk, M., Shablii, L., Shynkaruk, V., Slobodyanyuk, N., Rudyk, Y., Chumachenko, I., Marchyshyna, Y., Omelian, A., & Kharsika, I. (2022). Development and shelf-life assessment of soft-drink with honey. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 114–126). HACCP Consulting. https://doi.org/10.5219/1738
39. Soman, R., & Eashwernath, R. (2020). Bacteremia due to *Streptococcus gallolyticus*: A Name with an Ominous Significance? In Indian Journal of Critical Care Medicine (Vol. 24, Issue 10, pp. 901–902). Jaypee Brothers Medical Publishing. https://doi.org/10.5005/jp-journals-10071-23623

40. Birlutiu, V., Birlutiu, R.-M., &Dobritoiu, E. S. (2023). Lelliottiaamnigena and *Pseudomonas putida* Coinfection Associated with a Critical SARS-CoV-2 Infection: A Case Report. In Microorganisms (Vol. 11, Issue 9, p. 2143). MDPI AG. https://doi.org/10.3390/microorganisms11092143

41. Tirloni, E., Stella, S., Celandroni, F., Mazzantini, D., Bernardi, C., &Ghelardi, E. (2022). *Bacillus cereus* in Dairy Products and Production Plants. In Foods (Vol. 11, Issue 17, p. 2572). MDPI AG. https://doi.org/10.3390/foods11172572

42. Lázaro-Díez, M., Navascués-Lejarza, T., Remuzgo-Martínez, S., Navas, J., Icardo, J. M., Acosta, F., Martínez-Martínez, L., & Ramos-Vivas, J. (2016). Acinetobacter baumannii and A. pittii clinical isolates lack adherence and cytotoxicity to lung epithelial cells in vitro. In Microbes and Infection (Vol. 18, Issue 9, pp. 559–564). Elsevier BV. https://doi.org/10.1016/j.micinf.2016.05.002

43. Chooduang, S., Surya, W., Torres, J., Boonserm, P. (2018). An aromatic cluster in LysinibacillussphaericusBinB is involved in toxicity and proper in-membrane folding. In Archives of Biochemistry and Biophysics (Vol. 660, pp. 29–35). Elsevier BV. https://doi.org/10.1016/j.abb.2018.10.006

44. Dawadi, P., Odari, R., Poudel, R. C., Pokhrel, L. R., & Bhatt, L. R. (2023). Isolation of *Lactococcus garvieae* NEP21 from raw cow (Bos indicus) milk in Nepal. In Science of The Total Environment (Vol. 861, p. 160641). Elsevier BV. https://doi.org/10.1016/j.scitotenv.2022.160641

45. Adamchuk, L., Sukhenko, V., Akulonok, O., Bilotserkivets, T., Vyshniak, V., Lisohurska, D., Lisohurska, O., Slobodyanyuk, N., Shanina, O., & Galyasnyj, I. (2020). Methods for determining the botanical origin of honey. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 483–493). HACCP Consulting. https://doi.org/10.5219/1386

46. Mohamed, H. M. A., Abd-Elhafeez, H. H., Al-Jabr, O. A., & El-Zamkan, M. A. (2022). Characterization of Acinetobacter baumannii Isolated from Raw Milk. In Biology (Vol. 11, Issue 12, p. 1845). MDPI AG. https://doi.org/10.3390/biology11121845

47. Rogoskii, I., Mushtruk, M., Titova, L., Snezhko, O., Rogach, S., Blesnyuk, O., Rosamaha, Y., Zubok, T., Yeremenko, O., & Nadtochiy, O. (2020). Engineering management of starter cultures in study of temperature of fermentation of sour-milk drink with apiproducts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 1047–1054). HACCP Consulting. https://doi.org/10.5219/1437

48. Pati, N. B., Doijad, S. P., Schultze, T., Mannala, G. K., Yao, Y., Jaiswal, S., Ryan, D., Suar, M., Gwozdzinski, K., Bunk, B., Mraheil, M. A., Marahiel, M. A., Hegemann, J. D., Spröer, C., Goesmann, A., Falgenhauer, L., Hain, T., Imirzalioglu, C., Mshana, S. E., … Chakraborty, T. (2018). *Enterobacter bugandensis*: a novel enterobacterial species associated with severe clinical infection. In Scientific Reports (Vol. 8, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-018-23069-z

49. Paikra, S. K., Panda, J., Sahoo, G., & Mishra, M. (2022). Characterization of exopolysaccharide derived from *Enterobacter ludwigii* and its possible role as an emulsifier. In 3 Biotech (Vol. 12, Issue 9). Springer Science and Business Media LLC. https://doi.org/10.1007/s13205-022-03279-z

50. Różańska, H., Lewtak-Piłat, A., Kubajka, M., & Weiner, M. (2019). Occurrence of enterococci in mastitic cow's milk and their antimicrobial resistance. In Journal of Veterinary Research (Vol. 63, Issue 1, pp. 93–97). Walter de Gruyter GmbH. https://doi.org/10.2478/jvetres-2019-0014

51. Malta, R. C. R., Ramos, G. L. de P. A., & Nascimento, J. dos S. (2020). From food to hospital: we need to talk about Acinetobacter spp. In Germs (Vol. 10, Issue 3, pp. 210–217). AsociatiaPentruCrestereaVizibilitatiiCercetariiStiintifice (ACVCS). https://doi.org/10.18683/germs.2020.1

52. Soman, R., &Eashwernath, R. (2020). Bacteremia due to *Streptococcus gallolyticus*: A Name with an Ominous Significance? In Indian Journal of Critical Care Medicine (Vol. 24, Issue 10, pp. 901–902). Jaypee Brothers Medical Publishing. https://doi.org/10.5005/jp-journals-10071-23623

53. Birlutiu, V., Birlutiu, R.-M., &Dobritoiu, E. S. (2023). Lelliottiaamnigena and *Pseudomonas putida* Coinfection Associated with a Critical SARS-CoV-2 Infection: A Case Report. In Microorganisms (Vol. 11, Issue 9, p. 2143). MDPI AG. https://doi.org/10.3390/microorganisms11092143

54. Bal'-Prylypko, L. V., Derevyanko, L. P., Slobodyanyuk, N. M., Starkova, E. R., & Androshchiuk, O. S. (2018). Using of the Ampullaria glauca snails' caviar for correction of the effects of the ionizing radiation exposure in small dose. In Nuclear Physics and Atomic Energy (Vol. 19, Issue 2, pp. 159–165). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka) (Publications). https://doi.org/10.15407/jnpae2018.02.159

55. Tirloni, E., Stella, S., Celandroni, F., Mazzantini, D., Bernardi, C., &Ghelardi, E. (2022). *Bacillus cereus* in Dairy Products and Production Plants. In Foods (Vol. 11, Issue 17, p. 2572). MDPI AG. https://doi.org/10.3390/foods11172572

56. Patel, N., Prasanna, V., Rana, R., &Daunaria, D. (2022). Bacteremia due to carbapenem-resistant *Citrobacter braaki*i. In Journal of Family Medicine and Primary Care (Vol. 11, Issue 6, p. 3395). Medknow. https://doi.org/10.4103/jfmpc.jfmpc_1685_21

57. Lee, C.-R., Lee, J. H., Park, M., Park, K. S., Bae, I. K., Kim, Y. B., Cha, C.-J., Jeong, B. C., & Lee, S. H. (2017). Biology of Acinetobacter baumannii: Pathogenesis, Antibiotic Resistance Mechanisms, and Prospective Treatment Options. In Frontiers in Cellular and Infection Microbiology (Vol. 7). Frontiers Media SA. https://doi.org/10.3389/fcimb.2017.00055

58. Iakubchak, O., Adamenko, L., Taran, T., Sydorenko, O., Rozbytska, T., Tverezovska, N., Israelian, V., Holembovska, N., Menchynska, A., &Ivaniuta, A. (2023). The study of the cytotoxic effect of disinfectants. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 82–95). HACCP Consulting. https://doi.org/10.5219/1822

59. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/1711

60. Zheplinska, M., Mushtruk, M., & Salavor, O. (2021). Cavitational Impact on Electrical Conductivity in the Beet Processing Industry. In Lecture Notes in Mechanical Engineering (pp. 755–762). Springer International Publishing. https://doi.org/10.1007/978-3-030-68014-5_73

61. Kukhtyn, M. D., Horiuk, Y. V., Salata, V. Z., Klymyk, V. T., Vorozhbit, N. M., & Rushchinskaya, T. M. (2021). Staphylococcus aureus of raw cow's milk. In Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies (Vol. 23, Issue 102, pp. 53–59). Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. https://doi.org/10.32718/nvlvet10208

62. Calahorrano-Moreno, M. B., Ordoñez-Bailon, J. J., Baquerizo-Crespo, R. J., Dueñas-Rivadeneira, A. A., B. S. M. Montenegro, M. C., & Rodríguez-Díaz, J. M. (2022). Contaminants in the cow's milk we consume? Pasteurization and other technologies in the elimination of contaminants. In F1000Research (Vol. 11, p. 91). F1000 Research Ltd. https://doi.org/10.12688/f1000research.108779.1

63. Sarba, E. J., Wirtu, W., Gebremedhin, E. Z., Borena, B. M., & Marami, L. M. (2023). Occurrence and antimicrobial susceptibility patterns of Escherichia coli and Escherichia coli O157 isolated from cow milk and milk products, Ethiopia. In Scientific Reports (Vol. 13, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-023-43043-8

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Dr. Larysa Bal-Prylypko

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Olha Iakubchak, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Veterinary Hygiene, Vystavkova, Str., 16, 03041, Kyiv, Ukraine
E-mail: olga.yakubchak@gmail.com
ORCID: https://orcid.org/0000-0002-9390-6578

Olha Martynenko,"ECOLINE CORP" LIMITED LIABILITY COMPANY, Bandery Stepana avenue, building 21-A, 04073, Kyiv, Ukraine
E-mail: olmar@ukr.net
ORCID: https://orcid.org/0009-0001-8239-2583

Tetiana Taran, National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Veterinary Hygiene, Vystavkova, Str., 16, 03041 Kyiv, Ukraine
E-mail: ttaran@ukr.net
ORCID: https://orcid.org/0000-0002-9370-8539

***Oksana Pylypchuk**, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Management of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Vystavkova Str. 16, 03127, Kyiv, Ukraine, Tel.: +38(096) 961-20-83
E-mail: pilipchuk_os@ukr.net
ORCID: https://orcid.org/0000-0002-2757-6232

Tatyana Naumenko, National University of Life and Environmental Sciences of Ukraine, Faculty of foodtechnologies and quality management of products of agricultural products, Department of Standardization and certifying of agricultural products, Vystavkova, Str., 16, 03041, Kyiv, Ukraine, Tel.: +38(093) 921-96-80
E-mail: roirf1991@gmail.com
ORCID: https://orcid.org/0000-0003-0098-927X

Nina Tverezovska, National University of Life and Environmental Sciences of Ukraine, Faculty of Humanities and Pedagogy, Department of Social Work and Rehabilitation, Heroes of Defense, Str.15, 03041 Kyiv, Ukraine, Tel.: +38(044) 527-83-57
E-mail: tverezovskaya@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-0672-9308

Alina Menchynska, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technology of meat, fish and marine products, Vystavkova, Str., 16, 03040, Kyiv, Ukraine, Tel.: +38(097)658-38-88
E-mail: menchynska@ukr.net
ORCID: https://orcid.org/0000-0001-8593-3325

Inna Stetsyuk, National University of Life and Environmental Sciences of Ukraine, FacultyofFoodTechnologyandQualityControlofAgriculturalProducts, Department of Technology of meat, fish and marine products, Vystavkova, Str., 16, 03040, Kyiv, Ukraine, Tel.: +38(096)145-48-00
E-mail: stetsyukinna8513@ukr.net
ORCID: https://orcid.org/0000-0001-8392-6527

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 5.9.2024
Revised: 17.10.2024
Accepted: 23.10.2024
Published: 24.10.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 919-934
https://doi.org/10.5219/2018
ISSN: 1337-0960 online
www.potravinarstvo.com

Global trends in halal food detection

Anni Faridah, Adrian Arif, Durain Parmanoan, Ruhul Fitri Rosel, Abror Abror, Asmar Yulastri, Nurul Huda

ABSTRACT

The growth of the halal food industry has had a positive impact on the global economy. Unfortunately, halal food adulteration is a prevalent issue that has prompted researchers to develop new tools for halal food detection. This study aimed to examine the trends in halal food detection tools. To achieve this, we conducted a global trend analysis of halal food detection tools by reviewing their development, assessing research reference relationships, and identifying future research directions. This study utilised 170 articles published between 2006 and 2024 that focused on halal food detection tools. This study employed both qualitative and quantitative methods using bibliometric analysis. *Biblioshiny* and *Vos Viewer* software were used for data analysis. Polymerase Chain Reaction (PCR) has emerged as the most widely used halal food detection tool. Among these institutions, *Universiti Putra Malaysia* produced the largest number of publications. Malaysia and Indonesia conducted the most research and were frequently cited as references. Rohman was the author with the highest number of citations (277). The goal of this bibliometric analysis was to provide valuable scientific insights that will support future research in the field of halal food detection. Additional studies are strongly recommended to develop more effective tools for halal food detection.

Keywords: Global Trends, Halal Food Detection,

INTRODUCTION

The global halal food industry is experiencing significant growth and increased awareness and demand for products that meet halal standards. The halal market extends beyond the food sector to include cosmetics, pharmaceuticals, medical equipment, and service sectors, such as tourism, logistics, marketing, print and electronic media, packaging, and branding **[1]**. The global community's growing interest in halal products is not solely driven by religious beliefs but also by the assurance of superior quality in terms of ethics, health, safety, and environmental sustainability **[2]**. The increasing availability of safe halal food products for the Muslim population, the largest global population, is of paramount importance. The emerging halal food sector presents new opportunities to propel global economic growth and development. With its substantial population and growing demand for halal products, Indonesia has the potential to become a frontrunner in the halal industry. This is not limited to countries with a Muslim majority but also extends to nations with minority Muslim communities that contribute to the growth of the halal industry. According to the State of the Global Islamic Economy (SGIE) report, Indonesia's halal industry is projected to rank third by 2023, following Malaysia and Saudi Arabia.

Enhancements in halal criteria, monitoring procedures, and fundamental principles have led to a growing demand for halal commodities, particularly in the food sector **[3]**. Over the years, the scope of halal food has expanded beyond animal slaughter, alcohol use, and pork use. It now encompasses the entire process from

creation to consumption. Therefore, the assurance of halal food is imperative. This can be realized by understanding the certification process comprehensively and confirming the authenticity of halal products. Halal certification not only pertains to halal claims, but also ensures that production materials, equipment, processes, storage, distribution, and the Halal Guarantee System adhere to Islamic law, involving several procedures **[4]**. With advances in food processing and technological progress, it has become increasingly difficult to directly determine the halalness of a product because of the variety and number of food additives (BTP) used in the production process, whose halal qualities are not yet clear. Moreover, haram materials, such as pork, are diverse and easier to process than similar materials, such as cows, buffalo, sheep, and goats. All pig parts can be processed into various products, and pig breeding is faster and less expensive than other animals. Human limitations also play a significant role, as detecting halal foods using the senses takes considerable time. Therefore, a halal-food detection tool is required.

Halal food detection tools are important considering the high number of cases of food adulteration that have occurred for a long time. Halal food counterfeiting is common, and there are various ways to counterfeit halal food. The halal food detection tools used varied according to the food's characteristics. However, no research on the types of halal detection devices has been thoroughly studied. *How many lots and what types of halal detection devices have been studied?* The more types of halal food detection devices are studied, the better the diverse and complex characteristics of processed food. Ideally, a halal food detection tool should be easy and fast to use to increase the effectiveness and efficiency of the testing process and ensure compliance with established halal standards.

Research question:

R1: Which halal detection tools are most widely used?

R2: What types of halal food detection tools have been used thus far?

Therefore, halal detection tools are important to ensure that the products to be consumed are by Islamic Sharia law. This study aimed to evaluate the extent of research developments related to halal food detection tools that have been applied to food products. This study uses bibliometric analysis to answer these questions. It is hoped that this study will guide research areas that have not yet been explored to encourage the expansion and progress of the halal food industry.

This study investigated the progress and application of halal food detection instruments using bibliometric analysis. Using Scopus, it provides a thorough overview of the scientific developments, trends, and key contributors in halal food detection in recent years. The novelty of this study lies in its systematic and quantitative methods to comprehensively understand the landscape of halal food detection instruments, offering valuable insights into their evolution, significant research areas, and future directions for food safety and religious compliance. By mapping intellectual structure and research dynamics, this bibliometric study addresses a gap in the literature regarding the lack of comprehensive reviews on the advancements and effectiveness of various halal food detection methods. This study also highlights the need for future research to develop more user-friendly, cost-effective, and accurate detection tools to identify non-halal components in complex food matrices. Such research can significantly inform policymaking and enhance the overall reliability and accessibility of halal food-detection technologies.

METHODS

This study applied a bibliometric analysis. Bibliometric analysis is a quantitative method used to describe the characteristics of a group of published literature, such as journal articles or conference proceedings reviews, book chapters, etc. This method involves collecting bibliographic data from publications, including information about the author, journal, and year of publication, and applying statistical techniques to analyse and interpret the data **[5]** and **[6]**. This study aimed to identify reputable international articles related to the study of biomaterials for *Halal Food Detection*. Data were collected from the Scopus database between 2006 and 2024. Research articles were searched using the applications *publish or perish*. Data were collected in

June 2024. The keywords used were TITLE-ABS-KEY ("Halal Food Detection") AND PUB YEAR >2006 and PUB YEAR <2024. The software packages *Biblioshiny* **[7]** and *Vosviewer* 2024 were used in social network analysis for network visualisation. After obtaining raw data (CSV format) from Scopus, the data were converted to. xls format before analysis.

This study revealed several findings based on methodological steps. In identifying the relevant studies step, 181 articles were found using halal food detection keywords. The first criterion was the Scopus category, which applied the inclusion and exclusion criteria. Searches can only include the article abstract, title, and keywords. This study refined the initial results from the subject area and reduced the findings from 181 articles to 170. This can be seen in Figure 1

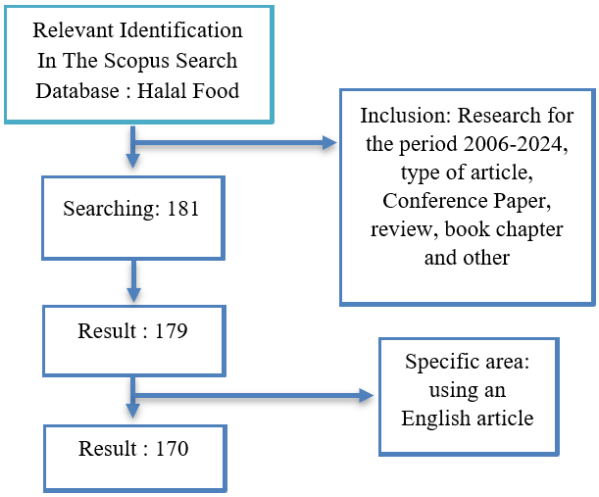


Figure 1 Prisma Flow Diagram.

RESULTS AND DISCUSSION

Halal food detection

With the increase in food a3) dulteration, halal detection tools are needed to guarantee that our food is safe. Some of the tools used in this study include the HPLC method carried out by **[8]** to classify and differentiate pork fat from other animal fats, such as chicken, goat, and beef, based on the composition of triacylglycerol (TAG), which is known to be very important in the differentiation of meat species. Some authors **[9]** and **[10]** used high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) to detect lupine (*Lupinus angustifolius*), peas (*Pisum sativum*), and soybeans (*Glycine maxima*) in meat products with high sensitivity. The PCA analysis of the spectral data showed that spectrometers FT-NIR in *mode-attenuated total internal reflection* can differentiate chicken, pork, and turkey meat based on their infrared spectrum **[11]**. However, only FTIR combined with attenuated total reflectance (ATR) regression and partial least squares (PLS) regression has been used to detect lard in chocolate formulations **[12]** and **[13]**.

"Halal food detection" or "halal food verification technology" refers to various methods and technologies used to authenticate the halal status of food products, ensuring that they comply with Islamic law **[14]**. Halal food detection involves various analytical techniques to ensure food products are free of non-halal components, particularly pork derivatives **[15]**.

Next, in this discussion, food detection tools with more than 3 articles are explained, as shown in the following table

Table 1 Halal food detection use by researchers.

No	Halal Food Detection	Publications (2006-2024)
1	Polymerase Chain Reaction (PCR)	70
2	Biosensors	26
3	Spectroscopy	18
4	Clustered Regularly Interspaced Short Palindromic Repeats	15
5	Near-infrared (NIR) Spectroscopy	9
6	Electronic Nose	8
7	Gas Chromatography (GC)	5
8	High-Pressure Liquid Chromatography (HPLC)	4

From the table above, it can be explained that most researchers who publish their work in Scopus use the halal food detection tool Polymerase Chain Reaction (PCR), with 70 publications from 2006-2024, followed by Biosensors with 26 publications, Spectroscopy with 18, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) with 15, Near-Infrared (NIR) Spectroscopy with 9 publications, Electronic Nose with 8 publications, Gas Chromatography (GC) with 5 publications, and High-Pressure Liquid Chromatography (HPLC) with 4 publications. Meanwhile, less than four publications are not discussed in this research. Next, a discussion of each food detection tool used by researchers is presented as follows:

1) Polymerase Chain Reaction (PCR), a molecular biology technique, is based on specific oligonucleotide hybridisation with the target DNA and its synthesis **[16]**. PCR can detect even 1% pork in a mixture of other meats and has proven effective in identifying pork adulteration in food **[17]** and **[18]**. PCR's sensitivity of PCR in detecting specific DNA segments, identifying animal species in food products, and its accuracy in detecting animal-derived ingredients in food are its key advantages. However, PCR requires specialised training, limits user accessibility without specific expertise, and incurs high operational costs.

2) Biosensors are analytical devices used to detect biological molecules, such as DNA samples, through various processes such as denaturation, hybridisation, and voltammetric analysis **[19]**. Biosensors are highly sensitive and specific for detecting target molecules, provide rapid results, and are cost-effective compared to traditional analytical methods **[19]** and **[20]**. However, biosensors are less accurate in complex sample matrices, require skilled personnel, and require proper storage conditions to maintain performance over time **[18]**, **[19]** and **[21]**.

3) Spectroscopy is a technique used to study the interaction between matter and electromagnetic radiation, providing valuable information about the composition, structure, and properties of substances **[22]** and **[23]**. Spectroscopy allows for the efficient confirmation of DNA amplification and provides reliable results for detecting forbidden animal DNA in food samples **[24]**. This tool offers rapid analysis, non-destructive testing, and cost-effectiveness **[23]**. However, it is less accurate and has the risk of missing contaminant detection **[25]**; **[26]**.

4) CRISPR is a tool for detecting infectious diseases quickly, sensitively, and specifically with its nucleic acid target. CRISPR has a wide range of applications in genetic engineering, including gene therapy, agriculture, and biotechnology **[27]**. CRISPR technology is also capable of cutting DNA. However, CRISPR has the potential for off-target effects that can cause unwanted genetic modifications and safety concerns.

5) Near-infrared (NIR) spectroscopy is a technique based on measuring the absorption of electromagnetic radiation in the wavelength range of 750–2500 nm. Several studies have demonstrated the capability of NIR spectroscopy to evaluate meat quality, including predicting chemical components, technological parameters, sensory properties, carcass fat quality, and meat product quality, as well as classifying and identifying various types of meat **[20]**. NIR Spectroscopy offers rapid analysis and cost-effectiveness as it does not require

chemicals. However, data interpretation is complex, and sample preparation variability affects the results' accuracy and reproducibility, necessitating standardisation for consistent analysis **[28]**.

6) Electronic nose (e-nose) uses chemical gas sensors to mimic human olfaction, recognise complex aromas, and evaluate volatile compounds using statistical methods **[29]**. E-nose requires minimal sample preparation, offers rapid analysis, has high specificity for oxidised compounds, and is inexpensive. However, the e-nose is less selective for certain compounds, operates at high temperatures, has limited sensitivity to low-molecular-weight gases, and has high power consumption **[30]**, **[22]** and **[31]**.

7) Gas Chromatography (GC) separates and analyses volatile compounds without decomposition. GC is often used in meat adulteration to determine product purity and identify fatty acid composition, a specific indicator of adulteration **[32]**. GC offers high separation efficiency and sensitive detection of compounds, even at low concentrations. GC is suitable for volatile compounds (low molecular weight). 8) High-Performance Liquid Chromatography (HPLC): The advantages of HPLC in pork fat analysis include high sensitivity, selective detection with UV detection, and the ability to analyse triglycerides (TGA) and fatty acids quantitatively. Integration with chemometrics, such as PCA, enhances discriminant analysis between beef and pork meatballs **[33]**. However, HPLC has drawbacks, such as being slow, expensive, invasive, and lacking a universal detector. Its separation efficiency is lower than that of Gas Chromatography (GC), and column costs are high **[28]** and **[33]**. With the various halal detection tools that already exist, it is hoped that simpler detection tools that are still capable of detecting the presence of non-halal components in low concentrations within complex food matrices can be developed in the future.

According to the Scopus Database, using the keywords "halal food detection," we found 170 documents.

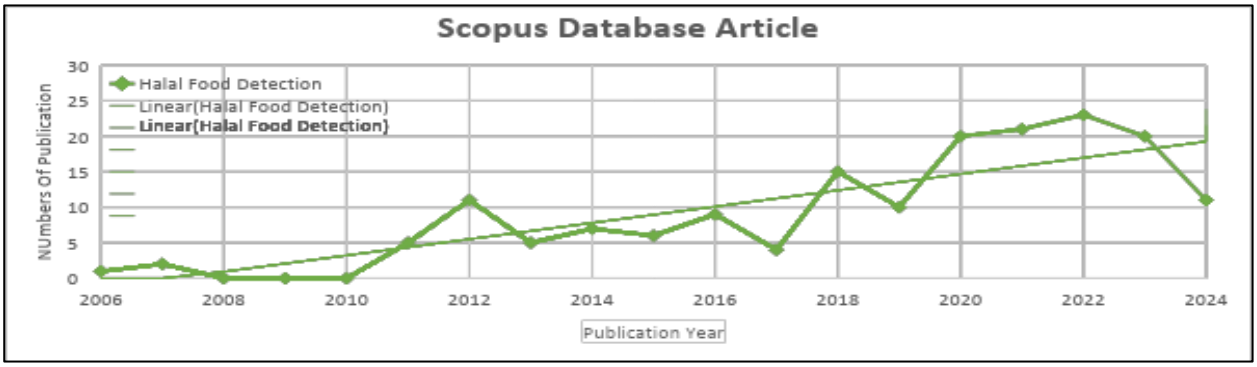


Figure 2 "Halal Food Detection" Research Graph from 2006-2024.

The characteristics of research results on halal food detection are related to the number of articles published annually from 2006 to 2024, as shown in Figure 2. Figure 2 shows that in 2006 there was research on Halal Food Detection, which increased in 2007. However, from 2008 to 2010, no one had researched it; in 2012, there was a sharp increase. In 2013-2017, there was a decline and a significant increase in 2020. This follows the opinion of **[34]** and **[35]**, halal food is becoming an increasingly interesting topic, especially due to the discovery that Many foods that are supposed to be halal contain pork. This has increased concern and attention to the authenticity of food products and the importance of ensuring that consumed food complies with halal principles. Figure 1 shows a linear increase, indicating that the growth rate of the publications was sustainable. The relationship between x and y was $y = 1.2211x - 2477.2$ ($R^2 = 0.7$) for Halal Food Detection. The coefficient of determination of each search shows that the linear regression line is consistent with the actual results, indicating that the number of publications continues to increase. In 2024, there will only be around 10 articles because this year hasn't ended yet.

Publication based on keywords

Keywords in an article serve as a reflection of its core content. As observed in Figure 3, Figure 4, and Figure 5, the keywords can be classified into dominant colour-coded groups: red, green, blue, and yellow. Each group represents a specific domain related to halal food detection, adulteration, and biomaterial analysis.

The Red Group focuses on adulteration and includes keywords like "Halal Authentication," "Analytical Method," and "Mass Spectrometry." These terms relate to verifying food compliance with halal standards, utilising analytical techniques such as mass spectrometry to examine food components. The red group also contains "Principal Component Analysis (PCA)," a statistical technique for data dimensionality reduction, and "Chemometrics," which applies mathematical and statistical methods for experimental design and chemical data analysis. This group emphasises analytical methods like High-Performance Liquid Chromatography (HPLC) and Fourier Transform Infrared Spectroscopy (FTIR), essential for detecting food adulteration and ensuring food quality.

The Blue Group represents halal food detection tools and is dominated by molecular detection techniques. Keywords include "PCR (Polymerase Chain Reaction)," "mitochondrial DNA," and "processed foods," which highlight the molecular tools used to identify halal food authenticity. These techniques are crucial for examining genes and DNA, especially in processed foods, to verify compliance with halal standards.

The Green Group focuses on biomaterials and incorporates terms like "biomaterials," "mammals," "non-human," "cattle," and "pig," reflecting the study of animal-based products in the context of food production. This group is particularly important in halal food analysis, as it detects animal ingredients that might conflict with halal or kosher requirements.

Lastly, the Yellow Group serves as a linking group between the other three categories, illustrating the connections between molecular detection techniques (blue group), analytical methods (red group), and biomaterials (green group). Keywords such as "DNA," "genes," and "food adulteration" bridge these concepts, highlighting the interdisciplinary approach required for halal food detection, adulteration analysis, and biomaterial identification.

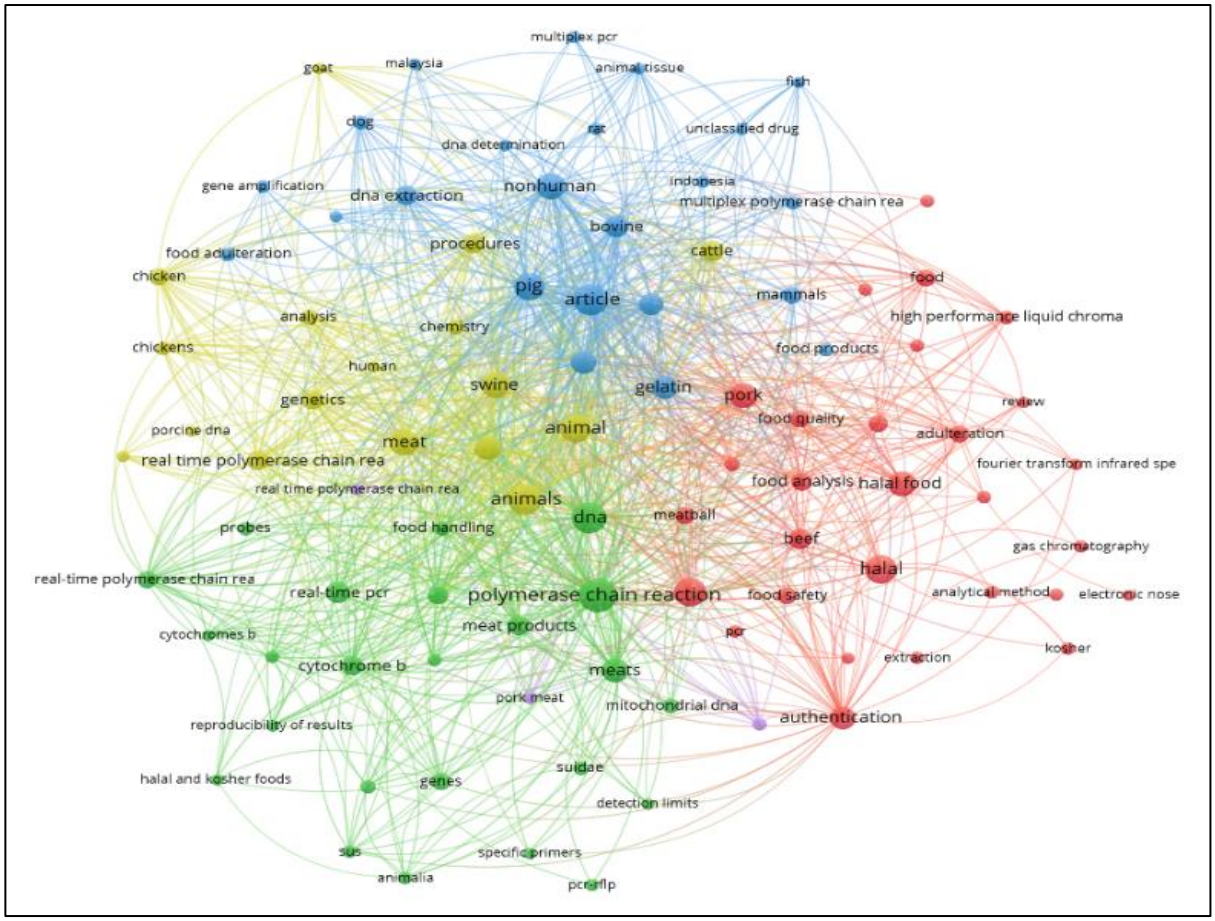


Figure 3 *VosViewer* Keyword Halal Food Detection.

Figure 4 shows a network visualisation of the relationship between various keywords in research *on Halal Food Detection*.

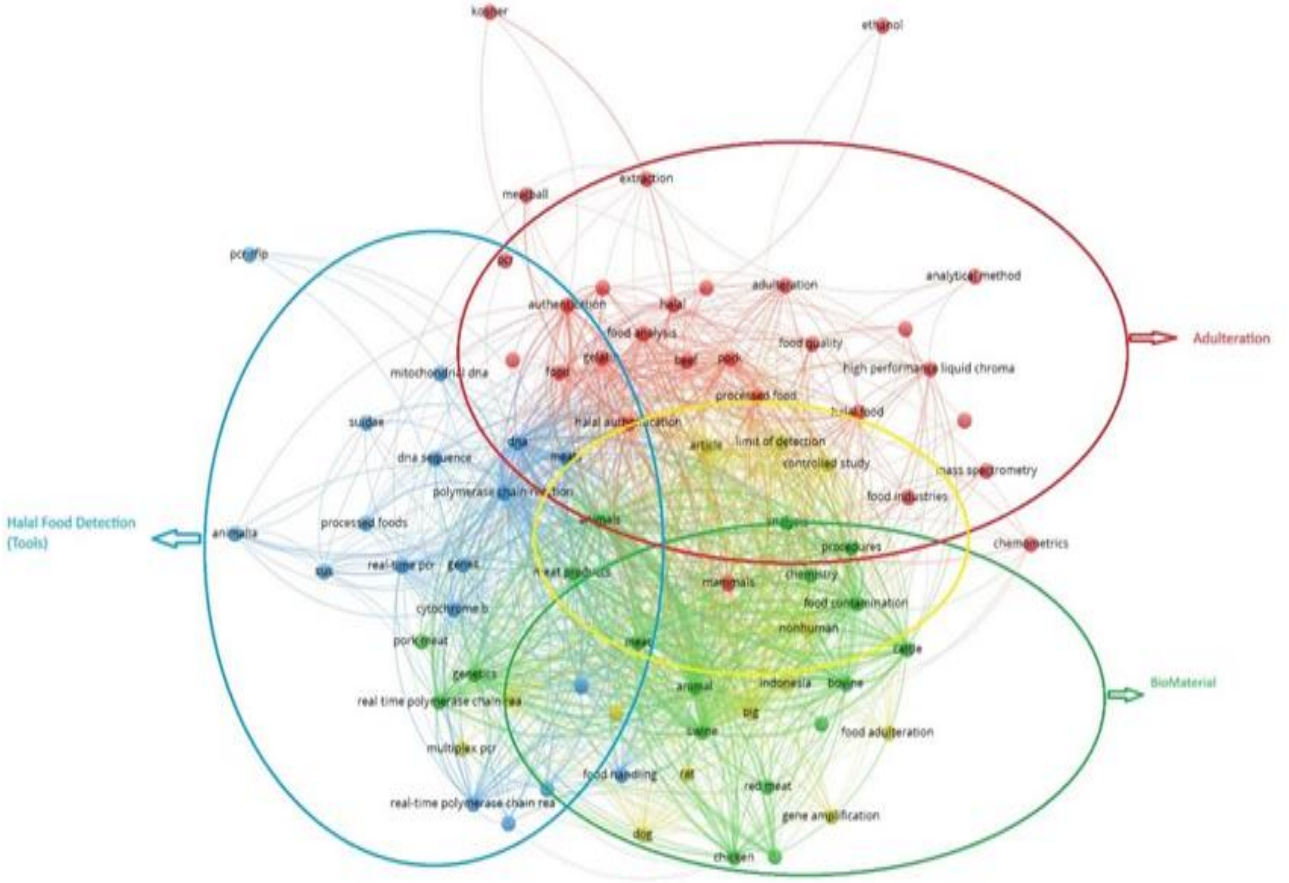


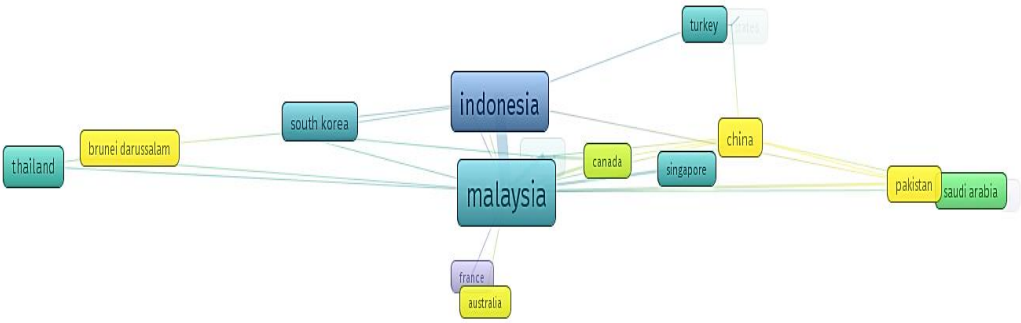
Figure 4 Keywords based on Color Clusters.

The green group focuses on terms like "*Food Contamination*," which relates to the contamination of food by harmful or undesirable substances, and terms such as "*Swine," "Cattle," "Bovine,"* and "*Pig*," which are often the subject of food analysis. Types of meat such as "*Red Meat," "Pork,*" and "*Meat*" Meat are often analysed for their safety or authenticity. "Food Adulteration" refers to adding impure or fake ingredients to food; at the same time, a "Controlled Study" is a scientific study that uses controls to ensure the validity of the results. The yellow group includes terms such as "Non-human," "Rat," and "Dog," which are often used in food analyses. "Gene Amplification" is the process of increasing the number of copies of a particular gene for further analysis, and "DNA Extraction" is the process of taking DNA from a sample for analysis. Specificity" measures the performance of a diagnostic test, with sensitivity referring to the test's ability to detect positives and specificity to detect negatives correctly. "Limit of Detection" is the lowest concentration of a substance that can be detected but is not necessarily quantified as an exact value.

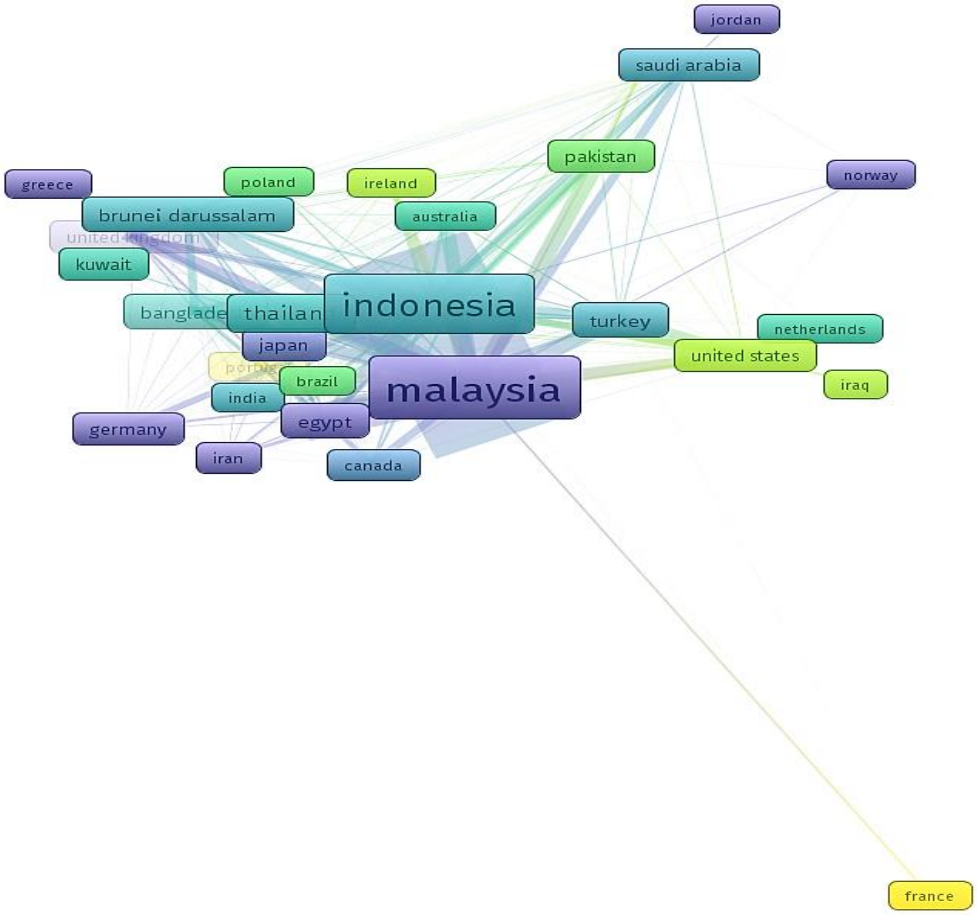
The blue group focuses on molecular biology techniques, such as “Real-Time Polymerase Chain Reaction (PCR),” which amplifies and simultaneously measures the amount of target DNA in a sample. DNA Sequence refers to the sequence of bases in DNA that determines genetic information.

“Genes” and “Genetics” refer to the study of genes and heredity. "Mitochondrial DNA" is the DNA in mitochondria that is often used to analyse heredity and evolution. “Reproducibility” indicates the ability of an experiment or study to be reproduced with consistent results, and “Cytochrome b” is a protein in the electron transport chain often used in phylogenetic analysis. Thus, it can be concluded that this image shows the complex relationship between various aspects of food analysis and authentication, demonstrating how different scientific methods are used to ensure the safety and authenticity of food products, particularly meat and animal products.

Bibliometric analysis on Scopus database



A



B

Figure 5 Number of publications by country (A), bibliographic coupling, and cooperation between countries in this research (B).

Based on the visualisation of images A and B (Figure 5), it can be explained that Indonesia and Malaysia are the two countries with the strongest links in Halal Food Detection research **[36]** and **[23]**, which can be seen from the large size of the nodes and the many lines connecting these two countries with other countries. These two countries have many research references compared to other Asian countries. Indonesia and Malaysia also have significant links with Southeast Asian countries, such as Thailand, Brunei Darussalam, and Bangladesh, indicating strong regional references in Halal Food Detection research. Additionally, there are clear links between Southeast Asian countries and countries in the Middle East, such as Saudi Arabia, Pakistan, and Iran, indicating great cooperation and attention toward Halal Food Detection in countries with significant Muslim populations **[37]**. Several Western countries, such as the United States, Germany, and the Netherlands, are also involved in this research network, showing that Halal Food Detection research is not only limited to

Muslim-majority countries but is also attracting global attention. According to **[1]**, halal food is a global market opportunity where various countries aim to position themselves strategically in the global economy and take advantage of the growing Muslim consumer base. Although France has weaker links than Western countries, it remains connected to the research network. Other countries such as Japan, India, Australia, and Brazil were also involved in this research **[38]**, showing wide geographic diversity in Halal Food Detection research references. Overall, this linkage map illustrates that Halal Food Detection research is a global issue with broad references between countries on various continents, reflecting the importance of this issue to Muslim communities worldwide and concerns about the safety and authenticity of food products.

Word TreeMap and Word Cloud Halal Food Detection

TreeMap was used to identify the available keywords. Figure 6 shows that the most dominant keywords in halal food detection were "polymerase chain reaction" and "DNA," each with a percentage of 6%. Correspondingly, word clouds (Figure 7) were used to identify the focus of this study. Larger words in a word cloud indicate a higher frequency of occurrence in the scientific literature.

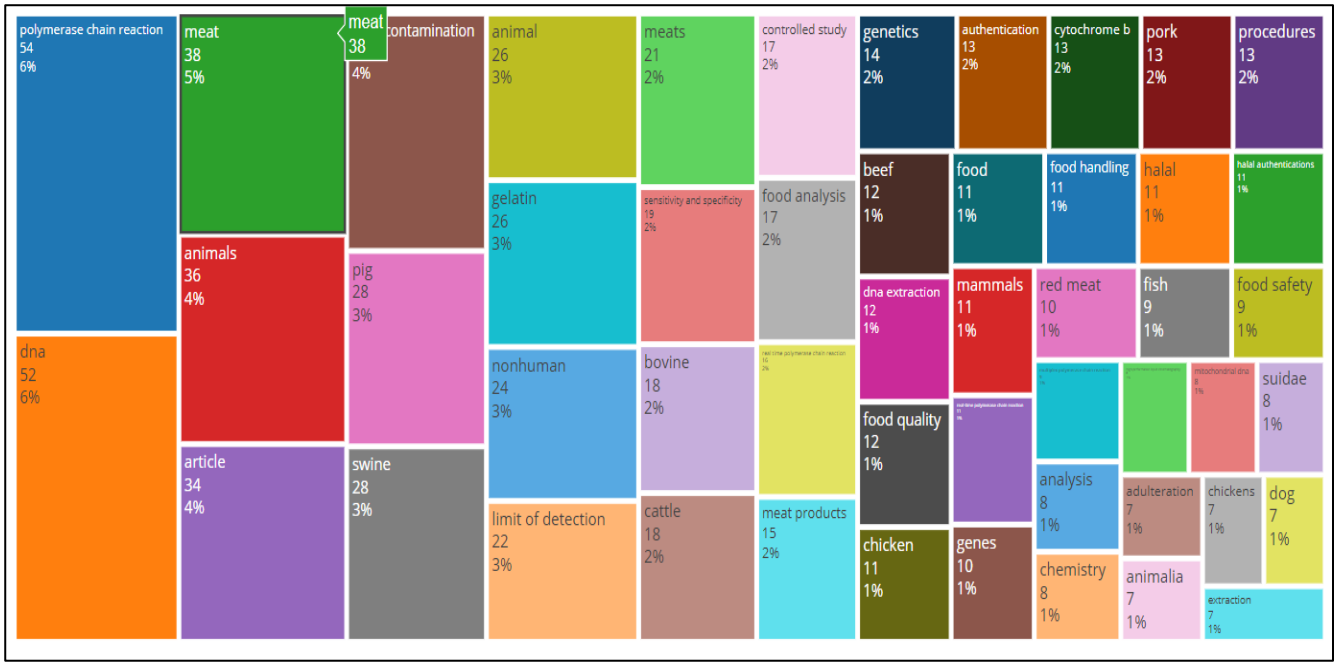


Figure 6 Word TreeMap Halal Food Detection.

Figure 7 Word Cloud Halal Food Detection.

Development and future research directions

With technological advances, halal food detection tools are expected to become more sophisticated, accurate, practical, and rapid. Technological innovations such as spectroscopy, DNA analysis, and portable devices will simplify the halal verification process in real time and at various stages of production. Developing software that can quickly process data and provide reliable results is also helpful. With halal food detection tools, it can become more economical and can be accessed by producers and consumers. Reducing halal detection tools' production and distribution costs will enable small- and medium-sized businesses to utilize them, thereby increasing quality standards and consumer confidence in halal products. This has the potential to open new markets and increase exports of halal products to countries with large Muslim populations, thus positively impacting the global economy.

The need for halal products continues to increase with the growth of the Muslim population worldwide and greater awareness of the importance of consuming products that comply with halal principles. This will help ensure that the halal products circulating in the market meet specified requirements, providing consumers with security and trust.

Another hope is that halal food detection tools will be easier to use, even for those without a technical background. Easy-to-operate tools will enable more people to detect halal products independently at home and in business.

Legislation

Halal certification has become a key component in food regulation across many countries. The legislation regarding halal certification varies depending on the country and local policies. At the regional level, institutions such as the Department of Islamic Development Malaysia (JAKIM), the Indonesian Council of Ulama (MUI) through the Halal Product Assurance Agency (BPJPH), and the Islamic Religious Council of Singapore (MUIS) play an important role in ensuring that products circulating both in domestic and international markets comply with Islamic law. JAKIM, for instance, sets strict standards concerning animal slaughter processes, cleanliness in production, and food processing. These standards apply for local consumption and export, especially to Middle Eastern countries that require halal certification for imported products.

At the global level, international trade standards, as regulated by the World Trade Organization (WTO), affirm that importing countries must respect local regulations regarding halal certification. Many non-Muslim countries also implement halal certification standards due to the increasing demand for halal products worldwide, driven by both the growth of the Muslim population and increasing consumer awareness of halal products, which are considered cleaner and safer.

In Indonesia, halal certification is regulated by Law No. 33 of 2014 on Halal Product Assurance (JPH), strengthened by Government Regulation No. 31 of 2019. This law mandates that all food, beverage, cosmetic, and pharmaceutical products circulating in Indonesia must have a halal certificate issued by BPJPH with a halal fatwa from MUI. Besides the domestic market, this certification is also crucial for enhancing the competitiveness of Indonesian products in international markets. Implementing halal certification requires halal detection tools, such as Polymerase Chain Reaction (PCR) and mass spectrometry, which detect non-halal contaminants, such as pork. This technology is becoming increasingly relevant to ensure compliance with legal requirements in local and export markets. According to research published in the “Food Control Journal“ and “Comprehensive Reviews in Food Science and Food Safety“, these technologies have improved accuracy in detecting non-halal contamination and support the enforcement of halal standards.**[39] [22].** Certification bodies such as JAKIM in Malaysia, MUI in Indonesia, and MUIS in Singapore have become the main references for many countries in setting their halal standards.

Certification

The halal certification process not only focuses on the final product but also includes the certification of management systems to ensure that every stage of production and distribution complies with Islamic principles. This certification is known as the Halal Assurance System (HAS), which is implemented to ensure

that the entire halal supply chain, from raw materials to final distribution, is properly monitored. In Malaysia, the Department of Islamic Development Malaysia (JAKIM) oversees the implementation of HAS as part of the halal quality management system **[40]**. This halal management system certification includes worker training, separation of production facilities to avoid cross-contamination with non-halal materials, and comprehensive documentation of every process. This system is applied across various sectors, such as the food, cosmetics, pharmaceutical, and logistics industries, and is a mandatory requirement for companies seeking JAKIM halal certification. In Indonesia, halal certification also includes management system oversight through the Indonesian Council of Ulama (MUI) and the Halal Product Assurance Agency (BPJPH) under Law No. 33 of 2014.

Halal food certification is a key focus of certification bodies. This certification involves rigorous audits of raw materials, production processes, and the distribution chain for food and beverage products. Ingredients used in halal products must not contain forbidden substances (such as pork and its derivatives, carrion, blood, alcohol, or ingredients from animals not slaughtered according to Islamic law, as well as toxic plants). The Islamic method of animal slaughter involves invoking the name of Allah, cutting the throat and major arteries, and requiring that the person performing the slaughter is a Muslim. In Indonesia, products that have been halal-certified by BPJPH receive a special logo or mark indicating that the product has passed the halal audit. This mark assures consumers that the product is safe and halal for consumption. In Malaysia, products certified by JAKIM are also labelled with a globally recognised halal logo, which is round and contains the word "HALAL" in Arabic and "Malaysia Halal" at the bottom.

This certification is important in international trade, as many countries, especially in the Middle East, require imported products to have globally recognised halal labels. The certification process involves several stages: application, audit and inspection, laboratory testing (if needed), halal fatwa issuance, and certification. As food materials, processes, and technology become more complex, accurate, easy, and affordable detection tools are increasingly necessary. Halal detection tools in certification have also advanced, with technologies such as Polymerase Chain Reaction (PCR) and mass spectrometry frequently recommended for detecting traces of non-halal substances, such as pork or alcohol, during halal certification audits, although they are not always mandatory. However, certification bodies like JAKIM, BPJPH, and the Halal Monitoring Committee (HMC) actively encourage using these tools to improve accuracy and transparency in the certification process. According to a study in the *Food Control Journal*, these technologies enhance the effectiveness of halal certification by supporting audits and inspections of products for both domestic consumption and export markets.
[39].

The global halal certification management system involves complex steps involving multiple parties (certification bodies to industry), from standards and testing to supervision. With the increasing demand for halal products, especially in the global market, this halal certification system is becoming increasingly important. It continues to evolve with the support of technology and the harmonisation of standards worldwide. Cooperation between countries and halal certification bodies ensures that halal products are recognised and traded globally without regulatory barriers. Standards such as those developed by OIC/SMIIC and JAKIM play an important role in maintaining the quality and consistency of halal products in the global market.

Laboratory testing

Laboratory testing ensures that a product's raw materials, additives, production processes, and facilities do not contain forbidden elements. The involvement of laboratories in halal testing must comply with ISO/IEC 17025 standards, which play an important role in ensuring consistency, accuracy, and international recognition of test results. Adherence to this standard ensures that laboratories possess the technical competence and effective quality management systems, making test results, such as the detection of non-halal substances, reliable on a global scale. **[41]**. This is crucial in international trade, where various countries recognise halal certificates. In Malaysia, the Department of Islamic Development Malaysia (JAKIM) requires laboratories involved in halal testing to have ISO 17025 accreditation, while in Indonesia, the Indonesian Council of Ulama (MUI) mandates the same through cooperation with the Institute for Food, Drug, and Cosmetic Studies

(LPPOM) **[39]**. This standard ensures that laboratory methods, such as Polymerase Chain Reaction (PCR) for detecting pork DNA, provide consistent and valid results.

For example, accredited laboratories like the Chemistry Department in Malaysia, recognised by JAKIM, and BPJPH laboratories accredited by the National Accreditation Committee (KAN) in Indonesia, recognized by MUI, use advanced methods in halal analysis **[22]**. In the Middle East, countries such as the United Arab Emirates also accredit halal testing laboratories based on ISO 17025 through local certification bodies. This laboratory accreditation covers a range of testing, from raw material identification to final product testing, to ensure the absence of non-halal contamination. Laboratory methods such as PCR and mass spectrometry are the primary techniques for detecting forbidden substances, such as pork contamination, with minimal measurement uncertainty. The measurement uncertainty governed by ISO 17025 is key to ensuring that test results are reproducible and validated with high confidence, which is an essential requirement for halal certification **[41]**. According to a study published in the "Journal of Halal Research", laboratories that meet ISO 17025 standards ensure the accuracy of results and guarantee international recognition for the halal products tested, enhancing consumer confidence and facilitating access to global markets **[41]**.

CONCLUSION

Based on search results, research trends in halal food detection have identified 17 different detection tools, with many researchers currently focusing on Polymerase Chain Reaction (PCR), Spectroscopy, and Liquid Chromatography–Mass Spectrometry (LCMS). This bibliometric analysis, the first to explore global trends in halal food detection using Scopus-based data over the past 18 years, offers valuable insights for future research. PCR stands out due to its high sensitivity and specificity in detecting unwanted DNA components, such as pork, even in minute quantities. However, its limitations include high costs, the need for advanced laboratory equipment, the risk of cross-contamination, and its inability to detect non-DNA components like oils. Therefore, future research should focus on enhancing the precision of bibliometric analyses and providing more comprehensive information to improve the accuracy and reliability of halal food detection methods.

REFERENCES

1. Azam, M. S. E., & abdullah, M. A. (2020). Global halal industry: realities and opportunities. In International Journal of Islamic Business Ethics (Vol. 5, Issue 1, p. 47). Universitas Islam Sultan Agung. https://doi.org/10.30659/ijibe.5.1.47-59
2. Yuli, S. B. C., & Wojtyla, E. (2020). Challenges and strategies in developing human resources for the halal industry: Evidence from Indonesia. In Journal of Innovation in Business and Economics (Vol. 4, Issue 02, pp. 77–86). Universitas Muhammadiyah Malang. https://doi.org/10.22219/jibe.v4i02.8270
3. Ruhaeni, N., & Aqimuddin, E. an. (2023). Halal food certification as an exception clause under the rule of the WTO-GATT: An Indonesia experience. In Cogent Social Sciences (Vol. 9, Issue 2). Informa UK Limited. https://doi.org/10.1080/23311886.2023.2260160
4. Warto, W., & Samsuri, S. (2020). Sertifikasi Halal dan Implikasinya Bagi Bisnis Produk Halal di Indonesia. In Al Maal: Journal of Islamic Economics and Banking (Vol. 2, Issue 1, p. 98). Universitas Muhammadiyah Tangerang. https://doi.org/10.31000/almaal.v2i1.2803
5. Nyagadza, B. (2022). Search engine marketing and social media marketing predictive trends. In Journal of Digital Media & Policy (Vol. 13, Issue 3, pp. 407–425). Intellect. https://doi.org/10.1386/jdmp_00036_1
6. Büyükkidik, S. (2022). A Bibliometric Analysis: A Tutorial for the Bibliometrix Package in R Using IRT Literature. In Eğitimde ve Psikolojide Ölçme ve Değerlendirme Dergisi (Vol. 13, Issue 3, pp. 164–193). Egitimde ve Psikolojide Olcme ve Degerlendirme Dergisi. https://doi.org/10.21031/epod.1069307
7. L. Marlina, A. S. Rusydiana, P. Hidayat, and N. Firdaus, "Twenty years of Islamic banking in Indonesia: A bibliophily application," Libr. Philos. Pract., vol. 4999, 2021. Available at: https://digitalcommons.unl.edu/libphilprac/4999?utm_source=digitalcommons.unl.edu%2Flibphilprac%2F4999&utm_medium=PDF&utm_campaign=PDFCoverPages
8. Rohman, K. Triyana, S. Sismindari, and Y. Erwanto, "Differentiation of lard and other animal fats based on triacylglycerols composition and principal component analysis.," 2012. Available at: http://www.ifrj.upm.edu.my/19%20(02)%202012/(14)IFRJ-2012%20Rohman.pdf
9. Wood, J. D., Enser, M., Fisher, A. V., Nute, G. R., Sheard, P. R., Richardson, R. I., Hughes, S. I., & Whittington, F. M. (2008). Fat deposition, fatty acid composition, and meat quality: A review. In Meat Science (Vol. 78, Issue 4, pp. 343–358). Elsevier BV. https://doi.org/10.1016/j.meatsci.2007.07.019

10. Hoffmann, B., Münch, S., Schwägele, F., Neusüß, C., & Jira, W. (2017). A sensitive HPLC-MS/MS screening method for the simultaneous detection of lupine, pea, and soy proteins in meat products. In Food Control (Vol. 71, pp. 200–209). Elsevier BV. https://doi.org/10.1016/j.foodcont.2016.06.021
11. N. H. Harun, M. A. N. H. Abdullah, M. S. Mohd Ashmir Wong, N. Mamat, and S. Moidin, "Halal training: issues and challenges from trainers' perspectives in Halal Products Research Institute (HPRI)," 2021. Available at: https://ir.uitm.edu.my/id/eprint/54252
12. S. S. Bahri and Y. B. Che Man, "Rapid detection of lard in chocolate and chocolate-based food products using Fourier transform infrared spectroscopy.," 2016. https://doi.org/10.1016/j.foodchem.2004.05.029
13. Aida, A. A., Che Man, Y. B., Wong, C. M. V. L., Raha, A. R., & Son, R. (2005). Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. In Meat Science (Vol. 69, Issue 1, pp. 47–52). Elsevier BV. https://doi.org/10.1016/j.meatsci.2004.06.020
14. N. Salamah, A. Guntarti, and L. H. Nurani, "Analysis of soft gelatin capsule with real-time polymerase chain reaction for halal authentication". http://dx.doi.org/10.12928/pharmaciana.v13i1.25694
15. Aini, S. R., Rohman, A., Mulyanto, Erwanto, Y., Ansar, Handayani, B. R., & Irnawati. (2023). The metabolomics approach used for halal authentication analysis of food and pharmaceutical products: a review. In Food Research (Vol. 7, Issue 3, pp. 180–187). Rynnye Lyan Resources. https://doi.org/10.26656/fr.2017.7(3).986
16. Fajardo, V., González, I., Rojas, M., García, T., & Martín, R. (2010). A review of current PCR-based methodologies for the authentication of meats from game animal species. In Trends in Food Science & Technology (Vol. 21, Issue 8, pp. 408–421). Elsevier BV. https://doi.org/10.1016/j.tifs.2010.06.002
17. Chisholm, J., Sánchez, A., Brown, J., & Hird, H. (2008). The Development of Species-specific Real-time PCR Assays for the Detection of Pheasant and Quail in Food. In Food Analytical Methods (Vol. 1, Issue 3, pp. 190–194). Springer Science and Business Media LLC. https://doi.org/10.1007/s12161-008-9026-3
18. Ali, M. E., Hashim, U., Mustafa, S., Che Man, Y. B., Dhahi, Th. S., Kashif, M., Uddin, Md. K., & Abd Hamid, S. B. (2012). Analysis of pork adulteration in commercial meatballs targeting porcine-specific mitochondrial cytochrome b gene by TaqMan probe real-time polymerase chain reaction. In Meat Science (Vol. 91, Issue 4, pp. 454–459). Elsevier BV. https://doi.org/10.1016/j.meatsci.2012.02.031
19. Hartati, Y. W., Suryani, A. A., Agustina, M., Gaffar, S., & Anggraeni, A. (2019). A Gold Nanoparticle–DNA Bioconjugate–Based Electrochemical Biosensor for Detection of Sus scrofa mtDNA in Raw and Processed Meat. In Food Analytical Methods (Vol. 12, Issue 11, pp. 2591–2600). Springer Science and Business Media LLC. https://doi.org/10.1007/s12161-019-01593-6
20. Schmutzler, M., Beganovic, A., Böhler, G., & Huck, C. W. (2015). Methods for detection of pork adulteration in veal product based on FT-NIR spectroscopy for laboratory, industrial and on-site analysis. In Food Control (Vol. 57, pp. 258–267). Elsevier BV. https://doi.org/10.1016/j.foodcont.2015.04.019
21. Flauzino, J. M. R., Alves, L. M., Rodovalho, V. R., Madurro, J. M., & Brito Madurro, A. G. (2022). Application of biosensors for detection of meat species: A short review. In Food Control (Vol. 142, p. 109214). Elsevier BV. https://doi.org/10.1016/j.foodcont.2022.109214
22. Mortas, M., Awad, N., & Ayvaz, H. (2022). Adulteration detection technologies used for halal/kosher food products: an overview. In Discover Food (Vol. 2, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1007/s44187-022-00015-7
23. Lestari, D., Rohman, A., Syofyan, S., Yuliana, N. D., Abu Bakar, N. K. Bt., & Hamidi, D. (2022). Analysis of beef meatballs with rat meat adulteration using Fourier Transform Infrared (FTIR) spectroscopy in combination with chemometrics. In International Journal of Food Properties (Vol. 25, Issue 1, pp. 1446–1457). Informa UK Limited. https://doi.org/10.1080/10942912.2022.2083637
24. Akhtar, M. T., Samar, M., Shami, A. A., Mumtaz, M. W., Mukhtar, H., Tahir, A., Shahzad-ul-Hussan, S., Chaudhary, S. U., & Kaka, U. (2021). 1H-NMR-Based Metabolomics: An Integrated Approach for the Detection of the Adulteration in Chicken, Chevon, Beef and Donkey Meat. In Molecules (Vol. 26, Issue 15, p. 4643). MDPI AG. https://doi.org/10.3390/molecules26154643
25. Siddiqui, M. A., Khir, M. H. M., Witjaksono, G., Ghumman, A. S. M., Junaid, M., Magsi, S. A., & Saboor, A. (2021). Multivariate Analysis Coupled with M-SVM Classification for Lard Adulteration Detection in Meat Mixtures of Beef, Lamb, and Chicken Using FTIR Spectroscopy. In Foods (Vol. 10, Issue 10, p. 2405). MDPI AG. https://doi.org/10.3390/foods10102405
26. C. D. P. Hardiyanto, "Pemahaman dan Preferensi Masyarakat dalam Memilih Makanan Halal (Studi pada Masyarakat Perum Griyashanta RW 12 Kota Malang)," *J. Ilm. Mhs. FEB*, vol. 5, no. 2, 2017. Available at: https://jimfeb.ub.ac.id/index.php/jimfeb/article/view/3758
27. Denyingyhot, A., Phraephaisarn, C., Vesaratchavest, M., Dahlan, W., & Keeratipibul, S. (2021). A new tool for quality control to monitor contamination of six non-halal meats in food industry by multiplex high-resolution melting analysis (HRMA). In NFS Journal (Vol. 25, pp. 31–40). Elsevier BV. https://doi.org/10.1016/j.nfs.2021.09.002

28. Alsaqri, S. N., Mabood, F., Boqué, R., Jabeen, F., Ahmad, A., Hussain, J., Sohail, M., Syed, M. G., Melhi, S., Shahzad, A., Khan, M. N., Al-Amri, I., Shah, R., & Din, I. U. (2023). Rapid detection of pork gelatin in ice cream samples by using non-destructive FT-NIR spectroscopy and Partial least squares-discriminant analysis. In Food Chemistry Advances (Vol. 2, p. 100215). Elsevier BV. https://doi.org/10.1016/j.focha.2023.100215
29. Sarno, R., Triyana, K., Sabilla, S. I., Wijaya, D. R., Sunaryono, D., & Fatichah, C. (2020). Detecting Pork Adulteration in Beef for Halal Authentication Using an Optimized Electronic Nose System. In IEEE Access (Vol. 8, pp. 221700–221711). Institute of Electrical and Electronics Engineers (IEEE). https://doi.org/10.1109/access.2020.3043394
30. Che Man, Y. B., Aida, A. A., Raha, A. R., & Son, R. (2007). Identification of pork derivatives in food products by species-specific polymerase chain reaction (PCR) for halal verification. In Food Control (Vol. 18, Issue 7, pp. 885–889). Elsevier BV. https://doi.org/10.1016/j.foodcont.2006.05.004
31. Ab Mutalib, N. A., Jaswir, I., & Akmeliawati, R. (2013). IIUM-fabricated portable electronic nose for halal authentication in beverages. In 2013 5th International Conference on Information and Communication Technology for the Muslim World (ICT4M). 2013 5th International Conference on Information and Communication Technology for the Muslim World 2013 (ICT4M). IEEE. https://doi.org/10.1109/ict4m.2013.6518899
32. Mansur, A. R., Oh, J., Lee, H. S., & Oh, S. Y. (2022). Determination of ethanol in foods and beverages by magnetic stirring-assisted aqueous extraction coupled with GC-FID: A validated method for halal verification. In Food Chemistry (Vol. 366, p. 130526). Elsevier BV. https://doi.org/10.1016/j.foodchem.2021.130526
33. Ahda, M. (2016). Application of HPLC (High-Pressure Liquid Chromatography) for Analysis of Lard in the Meatball Product Combined with PCA (Principal Component Analysis). In Asian Journal of Pharmaceutical and Clinical Research (Vol. 9, Issue 6, p. 120). Innovare Academic Sciences Pvt Ltd. https://doi.org/10.22159/ajpcr.2016.v9i6.13831
34. Windarsih, A., Riswanto, F. D. O., Bakar, N. K. A., Yuliana, N. D., Dachriyanus, & Rohman, A. (2022). Detection of Pork in Beef Meatballs Using LC-HRMS Based Untargeted Metabolomics and Chemometrics for Halal Authentication. In Molecules (Vol. 27, Issue 23, p. 8325). MDPI AG. https://doi.org/10.3390/molecules27238325
35. Vichasilp, C., & Poungchompu, O. (2014). Feasibility of Detecting Pork Adulteration in Halal Meatballs Using Near Infrared Spectroscopy (NIR). In Chiang Mai University Journal of Natural Sciences (Vol. 13, Issue 1). Chiang Mai University. https://doi.org/10.12982/cmujns.2014.0052
36. Rohman, A., Sismindari, Erwanto, Y., & Che Man, Y. B. (2011). Analysis of pork adulteration in beef meatball using Fourier transform infrared (FTIR) spectroscopy. In Meat Science (Vol. 88, Issue 1, pp. 91–95). Elsevier BV. https://doi.org/10.1016/j.meatsci.2010.12.007
37. Ramachandran, K., Daoudi, K., Kacem, H. H., Columbus, S., Benaoum, H., & Gaidi, M. (2022). Rapid and ultra-sensitive detection of pork DNA with surface-enhanced Raman spectroscopy for onsite food quality inspection. In Sensing and Bio-Sensing Research (Vol. 38, p. 100524). Elsevier BV. https://doi.org/10.1016/j.sbsr.2022.100524
38. Cheubong, C., Takano, E., Kitayama, Y., Sunayama, H., Minamoto, K., Takeuchi, R., Furutani, S., & Takeuchi, T. (2021). Molecularly imprinted polymer nanogel-based fluorescence sensing of pork contamination in halal meat extracts. In Biosensors and Bioelectronics (Vol. 172, p. 112775). Elsevier BV. https://doi.org/10.1016/j.bios.2020.112775
39. Ahmad, A. N., Ungku Zainal Abidin, U. F., Othman, M., & Abdul Rahman, R. (2018). Overview of the halal food control system in Malaysia. In Food Control (Vol. 90, pp. 352–363). Elsevier BV. https://doi.org/10.1016/j.foodcont.2018.02.035
40. Z. Zakaria and S. Z. Ismail, "The trade description act 2011: regulating'halal'in Malaysia," 2014. Available at: http://eprints.um.edu.my/id/eprint/13062
41. A. N. B. Ahmad and N. BINTI, "Implementation of halal food management system in small and medium enterprises food manufacturers," 2018. Available at: http://psasir.upm.edu.my/id/eprint/79184/1/IPPH%202018%2010%20ir.pdf
42. Republik Indonesia. (2014). Undang-Undang No. 33 Tahun 2014 tentang Jaminan Produk Halal. Lembaran Negara Republik Indonesia Tahun 2014 Nomor 295. Available at: https://peraturan.bpk.go.id/Details/38709/uu-no-33-tahun-2014
43. Republik Indonesia. (2019). Peraturan Pemerintah No. 31 Tahun 2019 tentang Pelaksanaan Undang-Undang No. 33 Tahun 2014 tentang Jaminan Produk Halal. Lembaran Negara Republik Indonesia Tahun 2019 Nomor 88. Available at: https://peraturan.bpk.go.id/Details/161941/pp-no-31-tahun-2019

Funds:
This research received no external funding.
Acknowledgments: -
Conflict of Interest:
The authors declared that they have no conflict of interest.
Ethical Statement:
This article does not contain any studies that would require an ethical statement.

Contact Address:
***Anni Faridah**, Universitas Negeri Padang, Faculty of Tourism and Hospitality, Department of Tourism and Hospitality, Jl. Prof. Dr. Hamka, Air Tawar Barat, Kec. North Padang, Padang City, West Sumatra 25171, Indonesia,
Tel.: +62 811-6650-258
E-mail: faridah.anni@fpp.unp.ac.id
ORCID: https://orcid.org/0000-0003-4561-6645

Adrian Arif, Universitas Negeri Padang, Faculty of Tourism and Hospitality, Department of Tourism and Hospitality, Jl. Prof. Dr. Hamka, Air Tawar Barat, Kec. North Padang, Padang City, West Sumatra 25171, Indonesia,
Tel.: +62 812-6696-2425
E-mail : arif.adrian@fpp.unp.ac.id
ORCID: https://orcid.org/0000-0002-1235-3677

Durain Parmanoan, Universitas Negeri Padang, Faculty of Engineering, Mechanical Engineering Department, Jl. Prof. Dr. Hamka, Air Tawar Barat, Kec. North Padang, Padang City, West Sumatra 25171, Indonesia,
Tel.: +62 813-6384-6058
E-mail : durain_siregar@unp.ac.id
ORCID: https://orcid.org/0009-0000-2438-5150

Ruhul Fitri Rosel, Universitas Negeri Padang, Faculty of Tourism and Hospitality, Department of Tourism and Hospitality, Jl. Prof. Dr. Hamka, Air Tawar Barat, Kec. North Padang, Padang City, West Sumatra 25171, Indonesia,
Tel.: +6281277611382
E-mail: ulierosel09@gmail.com
ORCID: https://orcid.org/0009-0005-1145-810X

Abror Abor, Universitas Negeri Padang, Faculty Of Economics And Business, Management Study Program, Jl. Prof. Dr. Hamka, Air Tawar Barat, Kec. North Padang, Padang City, West Sumatra 25171, Indonesia,
Tel.: +62 812-7737-1977
E-mail: abror094@fe.unp.ac.id
ORCID: https://orcid.org/0000-0002-6061-1597

Asmar Yulastri, Universitas Negeri Padang, Faculty of Tourism and Hospitality, Department of Tourism and Hospitality, Jl. Prof. Dr. Hamka, Air Tawar Barat, Kec. North Padang, Padang City, West Sumatra 25171, Indonesia,
Tel.: +62 812-6617-208
E-mail : yulastri.asmar@fpp.unp.ac.id
ORCID: https://orcid.org/0000-0001-5737-0094

Nurul Huda, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Kota Kinabalu, Malaysia,
Tel.: +60 12-484 3144
E-mail : drnurulhuda@ums.edu.my
ORCID: https://orcid.org/0000-0001-9867-6401

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 22.8.2024
Revised: 25.9.2024
Accepted: 7.10.2024
Published: 30.10.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 935-950
https://doi.org/10.5219/2012
ISSN: 1337-0960 online
www.potravinarstvo.com

Influence of starter cultures of lactic acid bacteria on microbiological parameters and shelf life of sausages

Snizhana Lokes, Larysa Shevchenko, Kyrylo Doronin, Vita Mykhalska, Valentyna Israelian, Nataliia Holembovska, Nina Tverezovska, Oleksandr Savchenko

ABSTRACT

The main spoilage microorganisms of the vacuum-packaged sausages on the first day of chilled storage are the bacteria of the following families: Enterobacteriaceae (*Raoultella planticola, Raoultella ornithinolytica*, and *Citrobacter freundii*), Morganellaceae (*Morganella morganii*) and Staphylococcaceae (*Macrococcus caseolyticus*), and at the end of the shelf life (on the twenty-first day) - Enterobacteriaceae (*Proteus mirabilis, Moellerella wisconsensis* and *Serratia liquefaciens*). An appearance of cloudy juice, surface slime and delamination of the vacuum packaging characterises the sausage spoilage. QMAFAnM in the sausages was increased by 1.09 lg CFU/g and 1.53 lg CFU/g on the first day of storage, by 1.18 lg CFU/g and 1.54 lg CFU/g on the twelfth day, by 0.92 lg CFU/g and 1.96 lg CFU/g on the eighteenth day, respectively, compared to the control sample, because "Vienna sausages with chicken fillet" were treated with starter culture SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging. Because the sausages were treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis, the lactic-acid microorganisms were increased by 0.63 lg CFU/g and 0.53 lg CFU/g on the twenty-fifth and thirtieth days, respectively, compared to the sausages that were treated with SafePro BLC-48. During the entire shelf life, no pathogenic and opportunistic pathogenic bacteria, in particular *S. aureus, L. monocytogenes, Salmonella spp., E. coli*, coliform bacteria, as well as yeast and mold, were detected in the sausages under all treatment options. The use of starter culture SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) increases the shelf life of the vacuum-packaged sausages if they are kept in a refrigerator for up to 30 days, which is 12 days longer than their shelf life without treatment. The sausage treatment with the mixed starter cultures of the lactic-acid microorganisms may be promising if the development of the aerobic spoilage bacteria is confirmed.

Keywords: safety, spoilage, vacuum packaging, minced meat, cooked meat products

INTRODUCTION

Animal meat is a valuable source of protein, lipids and biologically active substances **[1]**. It is processed into cold smoked meats, meatballs, sausages, nuggets and meat loaves, but the sausages remain one of the most popular products for consumers. Cooked meat products like sausages have a short shelf life and can spoil quickly. This not only leads to an increased risk to the consumer's health but also causes economic losses for the meat industry. The shelf life of the sausages depends on the quality and microbial contamination of raw materials, the casing type, and the packaging and storage conditions **[2]**.

The spoilage microflora of the meat products, in particular the sausages, consists of *Staphylococcus aureus, Streptococcus sp., Escherichia coli, Campylobacter jejuni*, *Shigella spp., Salmonella spp*., *Listeria monocytogenes, Clostridium botulinum/perfringens, Bacillus cereus*, which can be dangerous to the consumer health **[3]**, **[4]**. The microflora sources of the sausage products are the raw materials and technological operations of preparation and processing: carcass cutting, deboning, trimming, salt pickling, making of minced meat, filling of sausage casing with minced meat, as well as the air environment, staff hands, and technological equipment.

Reduced microbial contamination of sausages is the main condition for maintaining their quality, consumer demand, and safety **[5]**, **[6]**, **[7]**.

The most common preservation method of cooked meat products is to add chemicals to the raw materials. Synthetic chemical preservatives are widely used in the food industry to prevent spoilage caused by increased microorganisms, enzyme activity, and oxidative processes in cooked sausages. However, their consumer appeal is declining due to potential health risks and changes in organoleptic properties caused by synthetic preservatives in food products, particularly sausages **[8]**.

One of the most promising methods of reducing microbial contamination of meat [9] and meat products is using microbial cultures of lactic acid microorganisms that can produce bacteriocins that inhibit the growth and reproduction of spoilage bacteria **[10]**. The bacteriocins can interact with the microbial cell surface, increasing the permeability of its membrane, inhibiting the formation of the cell wall components, and synthesising the nucleic acid and protein **[11]**, **[12]**.

The most common bacteria used in the meat industry are the genus *Lactobacillus* **[13]**. They include *Lactobacillus curvatus*, a facultative anaerobe capable of synthesizing the lactic acid from sugars **[14]**, **[15]**. In addition, *L. curvatus* has a variety of genes that determine the bacteriocin synthesis **[16]** and is often used as a biological protective agent in fermented meat products that can inhibit the growth of spoilage bacteria. In addition, an equally important factor is the possibility of using the bacteriocin produced by this microorganism by spraying it onto a plastic film for active food packaging **[17]**. In addition to the bacteriocins, organic acids and hydrogen peroxide, produced by *L. curvatus* metabolism, can reduce the pH-value of the medium when the meat products are fermented and thus reduce their nitrite content **[18]**, **[19]**.

Due to the synthesis of the bacteriocins or antibacterial proteins the lactic-acid bacteria are highly effective against food-borne pathogens such as *Staphylococcus aureus, Pseudomonas fluorescens, P. aeruginosa, Salmonella typhi, Shigella flexneri, Listeria monocytogenes, Escherichia coli* O157:H7 and *Clostridium botulinum* **[20]**. Furthermore, the results show that the bacteriocins can effectively inhibit the formation of the biofilms of the pathogenic microorganisms **[21]**. It has been established that *L. curvatus* has a specific antimicrobial activity against *Campylobacter jejuni* ATCC 33560, *Camp. jejuni* NCTC 11168, *Listeria monocytogenes* ATCC 7644 and *Bacillus subtilis* ATCC 8633 **[22]**.

It is known that one of the prerequisites for the shelf life of cooked meat products, in particular the sausages, to be extended is the creation of anaerobic conditions, which are provided by the vacuum packaging, a modified gas environment **[23]**, as well as various oxygen absorbers based on iron nanocompounds, which are aimed at reducing the intensity of oxidative processes in the food products **[24]**, **[25]**. However, in that case this raises the question of controlling the residual content of any chemical components in the food product, particularly the sausages **[26]**. In this regard, *Lactobacillus curvatus* has advantages over any chemical preservatives, as it is also a promising microorganism as a probiotic **[27]**, with a pronounced resistance to the acidic pH-value of gastric juice, lysozyme and bile components. In 2012, it was included in the "Catalogue of Microorganisms of Technical Importance in Fermented Foods" of the Bulletin of the International Dairy Federation **[28]**, and in 2013, it was entered as a recommended biological agent in the qualification certification list of the European Food Safety Authority's **[29]**.

Scientific Hypothesis

Determination of the species composition of the spoilage microflora of the vacuum-packaged sausages while storing in a chilled state will make it possible to select the effective strains of lactic acid microorganisms, which are capable of inhibiting the growth of the spoilage bacteria. The sausage treatment with the starter culture of lactic-acid microorganisms SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging will make it possible to determine the most effective treatment option for extending their shelf life in a chilled state.

MATERIAL AND METHODOLOGY

Samples

The first-grade "Vienna sausages with chicken fillet", which are produced at a local meat-processing plant in Zakarpattia region, were used as the study material. The lactic-acid microorganisms SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*), which Chr produces. Hansen (Chr. Hansen Ukraine, LLC), were used for these studies. The polyamide casing Mini Ralen (Devro, Czech Republic) and the vacuum packaging Amilen PA/PE (Kozak+, Ukraine) were used to produce these sausages.

Chemicals

The media and diagnostic tests HiMedia (India) manufactured were used for microbiological studies. Plate count agar M091 was used for QMAFAnM to be determined. For the isolation and quantification of bacteria of the genus *Lactobacillus* - Lactobacillus MRS Agar M641, for the isolation of pathogenic and non-pathogenic staphylococci - Baird Parker, Agar M043, for Salmonella - Bismuth Sulphite Agar M027 and Xylose Lysine Deoxycholate Agar M031, for *L. monocytogenes* - Agar Palcam, Agar Oxford, for *Bacillus cereus* - Bacillus cereus (Selective agar) M833, for enterobacteria - Endo Agar, for *E. coli* and the bacteria of the Escherichia coli group (*E. coli*) - Gissa's, Kessler, Endo Agar, Simmons Agar, XLD, for the isolation of yeast and mould - Sabouraud Agar.

Animals, Plants and Biological Materials

Sausages, minced meat, SafePro BLC-48 (*Lactobacillus curvatus*) Chr. Hansen (Chr. Hansen Ukraine, LLC), Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) Chr. Hansen (Chr. Hansen Ukraine, LLC)

Instruments

Petri dishes

Disposable microbiological tubes

Pipettes and dispensers of Eppendorf (Eppendorf, Germany)

The thermostat of Binder (Binder, Germany)

Mass-spectrometer of Bruker Daltonics, Maldi Tof microflex (Bruker Daltonics, Germany)

Laboratory Methods

The sausages were produced, treated and packed at a local meat-processing plant in Zakarpattia region according to standard TU U 15.1-00419880-049-2003 **[30]**.

The microbiological studies were conducted at the Transcarpathian Regional State Laboratory of the State Service of Ukraine for Food Safety and Consumer Protection, Uzhhorod, and "Expert Centre "Biolights" LLC, Ternopil. To determine the number of microorganisms, fungi and mould, the average samples of the sausages were taken, and the washes from the sausage casings were made of 5 vacuum packages of each variant. For this purpose, serial decimal dilutions were prepared in sterile normal saline. The number of microorganisms was determined in colony-forming units (CFU), the results were expressed in lg CFU/g for minced meat and lg CFU/cm2 for the surface of the sausage casings. The genus and species-level identifications of the isolated microorganisms were performed following the current methods. Organoleptic studies of the sausages were performed following standard DSTU 4823.2:2007 **[31]**.

Description of the Experiment

"Vienna sausages with chicken fillet" were produced as per the traditional technology according to the following recipe: main raw materials, kg/100 kg: greasy pork - 20, semi-greasy pork - 32, broiler chick fillet - 45, potato starch - 3, water (ice) - 25; auxiliary raw materials, kg/100 kg: table salt - 2.30, sodium nitrate - 0.0075, Emulin food additive (guar gum thickener (E412), milk protein mixture, sodium tripolyphosphate stabiliser (E451), table salt) - 2, Ham flavour food additive (extracts of black pepper, garlic, allspice and lovage, maltodextrin, soya protein hydrolysate, yeast hydrolysate, E621) - 0.2, enhanced milk food additive (spices and spice extracts: cardamom, nutmeg, garlic, meat flavour and aroma, blood haemoglobin (colour fixer), E120, milk proteins E451, E621, E316, E301, carriers (destractose, rice flour, salt) - 1.

Sample preparation: The casing, vacuum packaging of the sausages, minced meat and sausages were sampled and their preparation for the microbiological studies was performed in accordance with the requirements of standard DSTU 4823.2:2007 **[31]**.

A total of 70 packages weighing 200 g each were used. The indicators of the control sausage variant were compared with experimental sausage variant No. 1, which was treated with starter culture SafePro BLC-48 (*Lactobacillus curvatus*) before vacuum packaging, and experimental sausage variant No. 2, which was treated with the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging, as well as experimental sausage variant No. 1 with experimental sausage variant No. 2.

Number of repeated analyses: 5 to 10 samples were used in each study.

Number of experiment replication: 1

Design of the experiment: The studies were performed in two stages: the dominant species of the spoilage microorganisms of "Vienna sausages with chicken fillet", while storing the vacuum-packaged sausages in a refrigerator at a temperature of 4±1°C, were determined at the first stage. 10 vacuum packs of "Vienna sausages with chicken fillet" weighing 200 g each, which were studied for the species composition of the dominant microflora on the first day and for spoilage signs (appearance of cloudy juice and its haziness) on the 21st day of storage, were used for this purpose.

The treatment effectiveness of "Vienna sausages with chicken fillet" with the starter lactic-acid cultures intended to inhibit the spoilage microorganisms while storing the vacuum-packaged sausages in a refrigerator at a temperature of 4±1°C was studied at the second stage. The produced batch of the sausages was divided into 3 variants, which were treated according to the scheme given in Table 1.

Table 1 Experiment scheme for influence determination of starter cultures of lactic acid microorganisms on shelf life of vacuum-packaged "Vienna sausages with chicken fillet" in a chilled state, n = 20.

Variant	Study conditions
Control	Vacuum-packaged "Vienna sausages with chicken fillet" of first grade
Experimental No. 1	"Vienna sausages with chicken fillet" of first grade treated with starter culture SafePro BLC-48 (*Lactobacillus curvatus*) at the rate of $5x10^6$ CFU/cm^2 of surface before vacuum packaging
Experimental No. 2	"Vienna sausages with chicken fillet" of first grade treated with the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) at the rate of $5x10^6$ CFU/cm^2 of surface before vacuum packaging

Chr provided the starter cultures of the microorganisms. Hansen Ukraine and comply with the general food safety requirements under Regulation No. 178/2002/EC **[32]**.

All sausage variants were stored in a refrigerator until the spoilage signs appeared. The study results were recorded on days 1, 12, 18, 25 and 30 of the sausage storage in a refrigerator.

Statistical Analysis

The obtained results were statistically processed using Microsoft Excel 2016 in combination with XLSTAT, the tabulated data are presented as x ± SD (mean ± standard deviation). One-way analysis of variance was used to compare the data. The difference between the groups was considered significant using the Tukey test at $p \leq 0.05$.

RESULTS AND DISCUSSION

One of the most important factors determining the shelf life of cooked sausage products, including "Vienna sausages with chicken fillet", is the level of microbial contamination of the raw materials from which they are produced. Greasy and semi-greasy pork, broiler chickfillets, and a mixture of spices and food additives determine the microbial contamination of the minced meat for the sausages. As can be seen from the obtained data, QMAFAnM in the fresh minced meat for the sausages was at the level of 5.64 lg CFU/g. At the same time, after cooking, this figure decreased to 1.89 lg CFU/g (Fig. 1), which indicates that a significant part of the microorganisms was neutralised after being subjected to the heat treatment and the requirements of current standard TU U 15.1-00419880-049-2003 **[30]** are complied.

The casings used to produce the cooked sausages must be resistant to high temperatures and microorganisms **[34]**. An analysis of the microbial contamination of the surface of the polyamide casing Mini Ralen revealed that the number of QMAFAnM, lactic-acid microorganisms, yeasts, and moulds on its surface did not exceed the specified requirements. Similar results were obtained during the microbiological analysis of the vacuum multiwall bags Amilen PA/PE (Table 2).

Table 2 Microbiological indicators of casing and packaging for sausages, x ± SD, n = 5, lg CFU/cm^2.

Casing	Microbiological indicators		
	QMAFAnM	Lactic-acid microorganisms	Yeasts and moulds
Polyamide casing Mini Ralen	2.1 ±0.12	<1	<1
Vacuum multiwall bags Amilen PA/PE	<1	<1	<1

The shelf life of the sausages in a chilled state is also determined by using the casings and packaging materials that can ensure their complete isolation from environmental factors and guarantee the absence of microbial contamination **[33]**.

In "Vienna sausages with chicken fillet" on the first day of vacuum-packaging storage, QMAFAnM did not exceed 3 lg CFU/g, which complied with the requirements of standard TU U 15.1-00419880-049-2003 **[30]** (Table 3); *Macrococcus caseolyticus*, which are gram-positive cocci and belong to the family Staphylococcaceae, were detected among the dominant microorganisms. These microorganisms are often isolated from dairy products **[35]**, beef, pork and sausage products, which is consistent with the obtained study results. *Macrococcus caseolyticus* is conditionally pathogenic microorganisms, which can cause animal diseases **[36]**. Besides of that, as the most studied species of the genus *Macrococcus*, it is known for its ability to ferment with flavouring and tasting to food products **[37]**, as well as its ability to synthesise the lactic acid **[38]**. It is believed that no microorganism species of the genus *Macrococcus* is considered pathogenic to humans **[39]**, so it does not pose a danger to consumers as an infection.

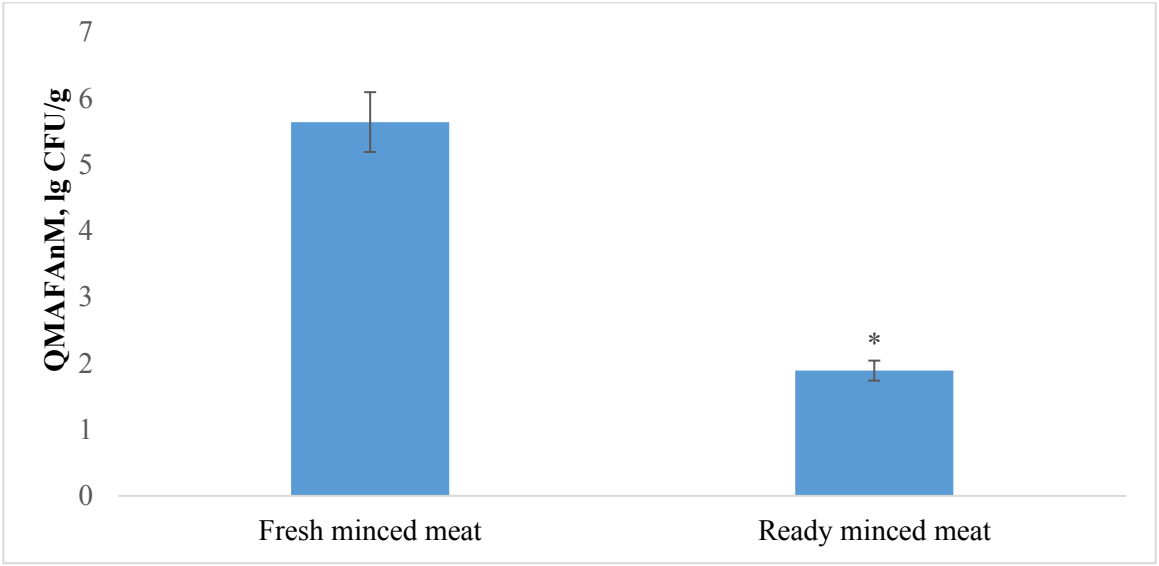


Figure 1 Microbiological indicators of minced meat for "Vienna sausages with chicken fillet".
Note: x ±SD, n=5, * p≤0.05 compared to fresh minced meat.

The shelf life of the sausages in a chilled state is also determined by using the casings and packaging materials that can ensure their complete isolation from environmental factors and guarantee the absence of microbial contamination **[33]**.

The casings used to produce the cooked sausages must be resistant not only to high temperatures but also to microorganisms **[34]**. An analysis of the microbial contamination of the surface of the polyamide casing Mini Ralen revealed that the number of QMAFAnM, lactic-acid microorganisms, yeasts, and moulds on its surface did not exceed the specified requirements. Similar results were obtained during the microbiological analysis of the vacuum multiwall bags Amilen PA/PE (Table 2).

Table 2 Microbiological indicators of casing and packaging for sausages, x ± SD, n = 5, lg CFU/cm^2.

Casing	Microbiological indicators		
	QMAFAnM	Lactic-acid microorganisms	Yeasts and moulds
Polyamide casing Mini Ralen	2.1 ±0.12	<1	<1
Vacuum multiwall bags Amilen PA/PE	<1	<1	<1

In "Vienna sausages with chicken fillet" on the first day of vacuum-packaging storage, QMAFAnM did not exceed 3 lg CFU/g, which complied with the requirements of standard TU U 15.1-00419880-049-2003 **[30]** (Table 3); *Macrococcus caseolyticus*, which are gram-positive cocci and belong to the family Staphylococcaceae, were detected among the dominant microorganisms. These microorganisms are often isolated from dairy products **[35]**, beef, pork and sausage products, which is consistent with the obtained study results. *Macrococcus caseolyticus* is

conditionally pathogenic microorganisms, which can cause animal diseases **[36]**. Besides that, as the most studied species of the genus *Micrococcus*, it is known for its ability to ferment with flavouring and tasting food products [37], as well as its ability to synthesise lactic acid **[38]**. It is believed that no microorganism species of the genus *Macrococcus* is considered pathogenic to humans **[39]**, so it does not pose a danger to consumers as an infection.

Table 3 Microbiological indicators of vacuum-packaged "Vienna sausages with chicken fillet" while storing in a chilled state, x ± SD, n = 5, lg CFU/g.

Study period	QMAFAnM	Microbiological screening with the use of MALDI-TOF
Storage within the shelf life		
1-st day	2.78 ±0.55	*Macrococcus caseolyticus, Raoultella planticola, Raoultella ornithinolytica, Morganella morganii, Citrobacter freundii*
Storage until spoilage signs appeared (cloudy juice and its haziness)		
21-st day	8.12 ±1.05	*Proteus mirabilis, Moellerella wisconsensis, Serratia liquefaciens*

Raoultella planticola and *R. ornithinolytica*, which belong to the family Enterobacteriaceae - gram-negative, non-motile, anaerobic bacteria of the genus *Raoultella*, which are most commonly found in water, soil and aquatic environments, were also detected in the sausages with the shelf life of one day. They are capable of causing septic processes of urinoexcretory ways in immunocompromised people, although little evidence of their pathogenicity has been reported **[40]**, **[41]**. Their origin in the sausages is associated with the raw materials of animal origin, particularly with poultry meat **[42]**, which, according to the recipe, was used for "Vienna sausages with chicken fillet" to be produced.

Citrobacter freundii, which was detected in the sausages on the first day of storage, belongs to the genus *Citrobacter*, family Enterobacteriaceae. These gram-negative, motile, facultatively anaerobic bacteria are isolated from chicken meat, cold-stored minced meat, and cooked-stewed sausages **[43]**, consistent with our studies.

Morganella morganii, a gram-negative, facultatively anaerobic bacterium of the family Morganellaceae, was among the microorganisms that dominated the samples of "Vienna sausages with chicken fillet" on the first day of storage. This microorganism was detected in cheese and horse meat **[44]** and in fish products, where it forms biogenic amines, the content of which indicates the product spoilage **[45]**. Since the sausages consisted of food additives containing milk proteins, they could have been the source of this bacterium, but this needs to be confirmed.

Under standard TU U 15.1-00419880-049-2003 **[30]**, the shelf life of first-grade "Vienna sausages with chicken fillet" in a polyamide casing while storing in a refrigerator at a temperature of 0 to +6°C is 12 days. Still, the use of the vacuum packaging, as expected, extended this period and the organoleptic signs of the product spoilage, such as cloudy juice and packaging delamination, were detected on the 21st day of storage.

During this period, QMAFAnM in the sausages exceeded the permissible value of 3 lg CFU/g following standard TU U 15.1-00419880-049-2003 **[30]**, (Table 3). Among the dominant microorganisms which caused the sausage spoilage, no bacteria were detected that were the main ones in the sausages on the first day of storage, which indicates that the vacuum packaging or the temperature mode created the conditions that were unsuitable for the growth and reproduction of the microflora as mentioned earlier while storing the sausages in a refrigerator. It was also found that among the main bacteria which caused the sausage spoilage were representatives of the family Enterobacteriaceae, particularly *Proteus mirabilis*, *Moellerella wisconsensis* and *Serratia liquefaciens*.

Proteus mirabilis is a facultatively anaerobic, conditionally pathogenic microorganism, which is transmitted through food. It is one of the most common histamine-producing bacteria in fermented meat products, particularly in the sausages **[46]**. *Moellerella wisconsensis*, detected in the sausages with the spoilage signs, is a gram-negative, facultatively anaerobic, nitrate-reducing and oxidase-negative rod. They are also often detected in cooked ham with spoilage signs **[47]**. *Serratia liquefaciens* is a gram-negative, facultatively anaerobic microorganism often detected in chilled pork meat **[48]**. Likely, the storage of vacuum-packaged "Vienna sausages with chicken fillet" in a refrigerator has provided the conditions for the growth and reproduction of these microorganisms.

Thus, the main microorganisms which are capable of causing sausage spoilage at the early stages are the bacteria of the families Enterobacteriaceae, Morganellaceae and Staphylococcaceae, but at the end of the shelf life - only the bacteria of the family Enterobacteriaceae, which are widely distributed in the raw materials for the sausages to be produced and in the environment. Therefore, it is possible to inhibit the growth of the bacteria, which can cause their spoilage, using the starter cultures of the lactic acid microorganisms.

When "Vienna sausages with chicken fillet" were treated with the starter culture of lactic-acid microorganisms SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging, QMAFAnM in the sausages on the first day of storage was increased by 1.09 lg CFU/g and 1.53 lg CFU/g, respectively, compared to the control ones.

When "Vienna sausages with chicken fillet" were treated with starter culture SafePro BLC-48, QMAFAnM was increased by 1.18 lg on the 12th day of storage in a chilled state; when "Vienna sausages with chicken fillet" were treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis, QMAFAnM was increased by 1.54 lg CFU/g compared to the control ones. At the same time, in the experimental variant, where the mixture of starter cultures was used, QMAFAnM exceeded by 0.36 lg CFU/g compared to the same indicator in the variant using one starter culture. A similar pattern concerning QMAFAnM in the sausages of both experimental variants was observed on the 18th day of storage.

The sausages of the control sample showed signs of spoilage on the 21st day of storage, manifested by the appearance and haziness of the juice and the delamination of the vacuum packaging, which prevented further storage and study.

The storage of "Vienna sausages with chicken fillet" of both experimental variants for up to 25 days showed that they complied with the valid standard requirements for organoleptic indicators. However, QMAFAnM in them continued to increase. At the same time, when the sausages were treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis, QMAFAnM in the sausages was 1.37 lg CFU/g higher than when the sausages were treated with starter culture SafePro BLC-48 (Table 4).

Table 4 QMAFAnM in "Vienna sausages with chicken fillet" if treated with starter cultures of lactic-acid microorganisms and stored in vacuum packaging in a chilled state.

Study period	Variant		
	Control	**Experimental No. 1 SafePro BLC-48**	**Experimental No. 2 SafePro BLC-48 + Bactoferm Rubis**
1^{-st} day	2.36 ±0.32	3.45 ±0.35*	3.89 ±0.49*
12^{-th} day	2.51 ±0.35	3.69 ±0.15*	4.05 ±0.28*,**
18^{-th} day	3.01 ±0.27	3.93 ±0.22*	4.97 ±0.65*,**
25^{-th} day	-	4.56 ±0.34	5.93 ±0.24**
30^{-th} day	-	7.78 ±0.41	8.29 ±0.60**

Note: x ± SD, n = 5, lg CFU/g, * p≤0.05 compared to the control sample, ** p≤0.05 compared to experimental variant No. 1

On the 30th day of storage, under both variants of the treatment with the starter cultures, the organoleptic indicators of "Vienna sausages with chicken fillet" complied with the valid standard requirements. Still, QMAFAnM in them continued to increase, and if the mixture of the starter cultures was used, it exceeded by 0.51 lg CFU/g, the same variant where the single culture was used. Such an increase in QMAFAnM indicates that the starter lactic acid cultures of microorganisms continue their vital activity and growth in the sausages.

The number of the lactic-acid microorganisms in "Vienna sausages with chicken fillet" on the 1st and 12th days of storage if treated with starter culture SafePro BLC-48 did not differ from the control sample, while treating with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis increased the number of the lactic-acid microorganisms by 0.3 lg CFU/g and 0.35 lg CFU/g, respectively, compared to the control sample (Table 5).

Table 5 Number of lactic-acid microorganisms in "Vienna sausages with chicken fillet" if treated with starter cultures of lactic-acid microorganisms and stored in vacuum packaging in a chilled state, x ± SD, n = 5, lg CFU/g.

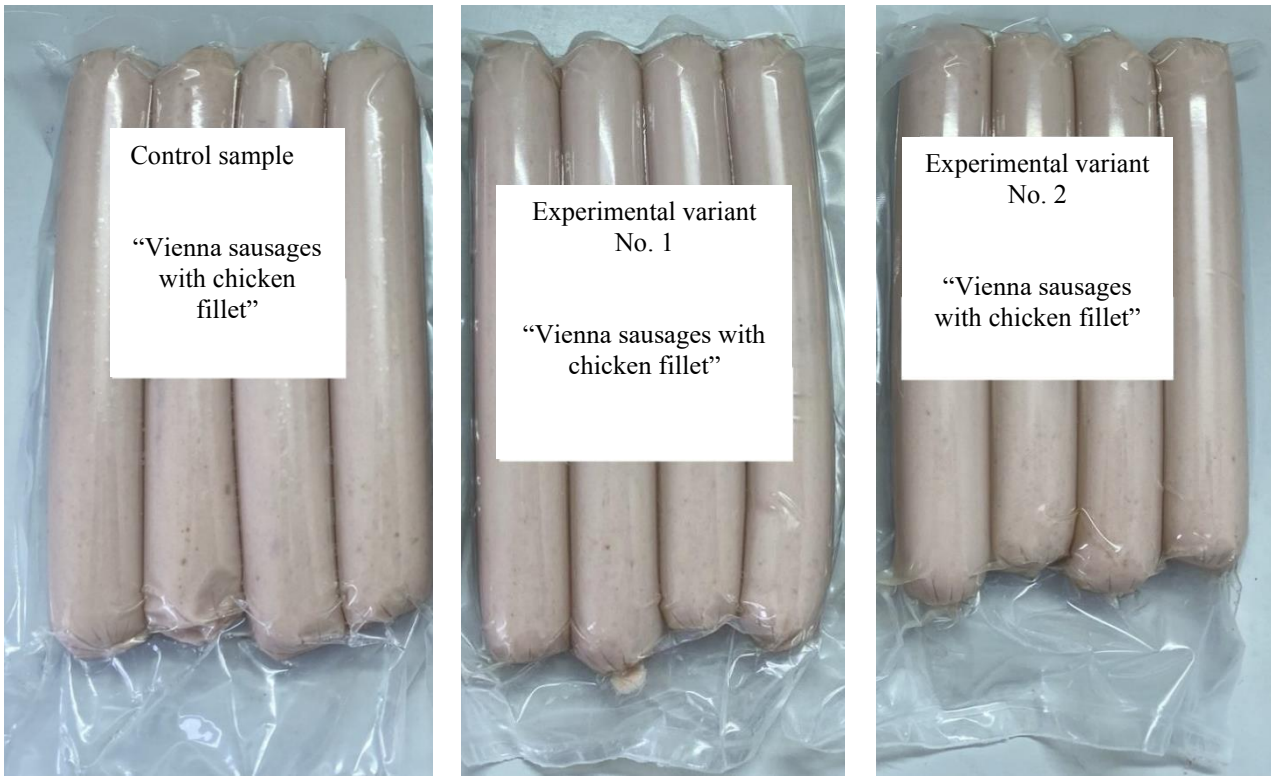
Study period	Variant		
	Control	Experimental No. 1 SafePro BLC-48	Experimental No. 2 SafePro BLC-48 + Bactoferm Rubis
1^{-st} day	1.75 ±0.13	1.88 ±0.33	2.05 ±0.29*
12^{-th} day	1.80 ±0.16	2.01 ±0.25	2.15 ±0.22*
18^{-th} day	2.03 ±0.27	2.13 ±0.41	2.37 ±0.68
25^{-th} day	-	2.56 ±0.33	3.19 ±0.33**
30^{-th} day	-	2.73 ±0.45	3.26 ±0.31**

Note: * – p≤0.05 compared to the control sample, ** – p≤0.05 compared to experimental variant No. 1

On the 18th day of sausage storage, the total number of lactic acid microorganisms did not differ significantly between the control sample and both variants of their treatment with the starter cultures.

The further storage of "Vienna sausages with chicken fillet" in a refrigerator for 25th and 30th days contributed to an increase in the number of the lactic-acid microorganisms if treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis by 0.63 lg CFU/g and 0.53 lg CFU/g, respectively, compared to the treatment with starter culture SafePro BLC-48. At the same time, the number of lactic acid microorganisms was increased in the sausages under both treatment variants during the entire shelf life (Table 5).

The sausages' spoilage signs, such as the appearance of cloudy juice, surface slime, and delamination of vacuum packaging, were observed on the 36th day of storage both when treated with starter culture SafePro BLC-48 and the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis (Fig. 3 a, b) compared to their organoleptic indicators on the 1st day of storage (Figure 2 a, b, c).



a b c

Figure 2. Sausages with a shelf life of one day: a - control sample, b - experimental variant No. 1 (treatment with starter culture SafePro BLC-48), c - experimental variant No. 2 (treatment with a mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis)

a b

Figure 3 Sausages with shelf life of 36 days.
Note: a - experimental variant No. 1 if treated with starter culture SafePro BLC-48, b - experimental variant No. 2 if treated with mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis

The lactic acid bacteria are the main microbial population found in various cooked sausages when stored in vacuum packaging. Various strains of *Lactobacillus sake* and *Lactobacillus curvatus* are also known to be common spoilage causes of cooked sausages. The growth of the lactic acid bacteria on the surface of the cooked sausages creates undesirable sensory properties such as a sour aroma and taste. Generally, during the cooking of the sausage products, high temperatures destroy the lactic acid bacteria on their surface. However, secondary contamination of the sausages with the lactic acid bacteria occurs during their cooling, prepackaging, and packing due to contact with contaminated air and technological equipment and workers **[49]**. In our experiment, the increase in the total number of the lactic-acid microorganisms in the sausages of experimental variant No. 1, which we observed throughout the entire shelf life, was probably due to the starter culture of strain SafePro BLC-48 (*Lactobacillus curvatus*), and in the sausages of experimental variant No. - due to the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) and Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*), which contributed to an increase in their shelf life, unlike the sausages of the control sample.

According to the obtained data **[50]**, the mechanism of biopreservation, prevention of pathogen growth and reduction of microbial spoilage of the cooked meat products is based on the ability of the lactic-acid microorganisms and/or their metabolic products, in particular bacteriocins, to neutralise pathogenic and opportunistic pathogenic bacteria. This study shows that the use of sacacin G, isolated from *Lactobacillus curvatus* ACU-1, for Listeria and spoilage flora to be neutralised was effective when applied to the casing both before and after stuffing of "Vienna sausages with chicken fillet". At the same time, applying the antimicrobial agent to the finished sausages inhibited the lactic acid bacteria that can spoil the meat products and the mesophilic microorganisms from the zero sampling time.

During the entire shelf life, no pathogenic and opportunistic pathogenic bacteria, including *S. aureus, L. monocytogenes, Salmonella spp., E. coli*, bacteria of the *E. coli* group, as well as yeast and mould, were detected in the sausages of both the control sample and if treated with starter culture SafePro BLC-48 or the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis (Table 6). The results of the studies are consistent with the data **[51]**, which proves the complete elimination of *L. monocytogenes* in the sausage due to the use of the bacteria's lactic acid starter cultures. The increase in the shelf life of the sausages under both treatments in our

experiment is due to the ability of *L. curvatus* to inhibit the growth of the bacteria, in particular, *Enterobacteriaceae*, *Pseudomonas fragi* and *Brochothrix thermosphacta*, which cause the spoilage of the meat products during their storage **[52]**, **[53]**.

Table 6 Number of pathogenic and opportunistic pathogenic microorganisms in "Vienna sausages with chicken fillet" if treated with starter cultures of lactic-acid microorganisms and stored in vacuum packaging in a chilled state, x ±SD, n =5, lg CFU/g.

Indicator	Variant		
	Control	Experimental No. 1 SafePro BLC-48	Experimental No. 2 SafePro BLC-48 + Bactoferm Rubis
	1st , 18th day	1st, 18th, 25th and 30th day	
S. aureus	not detected	not detected	not detected
L. monocytogenes	not detected	not detected	not detected
Salmonella spp.	not detected	not detected	not detected
bacteria of the E. coli group	not detected	not detected	not detected
E. coli	not detected	not detected	not detected
Yeast, mould	not detected	not detected	not detected

The obtained data show that the combination of starter culture Bactoferm Rubis with SafePro BLC-48 is ineffective for storing vacuum-packaged sausages, as it does not increase their shelf life. The use of starter culture Bactoferm Rubis as an aerobic microorganism, which was based on the absorption of residual oxygen, was probably not effective in this case, as the vacuum packaging of the sausages could provide a sufficient degree of oxygen removal, and the main pathogens that caused the sausage spoilage were enterobacteria, which belong to facultative anaerobes. The main effect on increasing the shelf life of the sausages was provided by starter culture SafePro BLC-48 (*L. curvatus*), which is also a facultative anaerobe, which in vacuum packaging could ensure its growth and reproduction in a refrigerator. This is confirmed by the data provided by several scientists **[27]**, which show the ability of different strains of L. curvatus to synthesize the bacteriocins, in particular curvacin A, saccacin G, saccacin P and saccacin X, curvacin 13, lactocin AL705 and curvacin 422, which tolerate a wide range of pH medium and temperature conditions, have antibacterial effects against a wide range of pathogenic bacteria and bacteria that cause the spoilage of the meat products such as *Bacillus cereus, L. monocytogenes, S. aureus* and *Enterococcus faecium*.

The idea of using oxygen absorbers for the extension of the shelf life of food products, particularly meat products, is currently realised mainly through the use of films, sachets, powders or components of packaging material in combination with chemicals such as metals and metal oxides, organic acids, antimicrobial peptides and bacteriocins, antimicrobial agents of plant origin, enzymes, lactoferrin, chitosan and bacteriophages, reduction of water activity, pH, use of multilayer composites and/or vacuum or modified atmosphere. The current demand increases their addition directly to the packaging material **[54]** rather than to the food product composition **[55]**, **[56]**. Among these preservatives, bacteriocins of lactic acid bacteria or their cultures are most often used **[57]**, **[58]**. It was proved that spraying the solution of bacteriocin *L. curvatus* on the polyethene films provided a stable antibacterial activity, and heat treatment at 70 °C did not affect the antibacterial activity of such films. Compared to the nisin-treated film, the lactocin-treated active polyethylene film inhibited Listeria more effectively, and this did not affect the functional properties of the film. At the same time, it was found that temperature and exposure time have a certain effect on the adsorption of the bacteriocin by the polyethylene film, provided that 60 minutes and 30°C are considered optimal conditions for the adsorption **[27]**.

In our experiment, the suspension spray of the starter lactic-acid cultures was applied to the polyamide casing of the sausages, which is in contact with the minced sausage on one side and the vacuum packaging on the other side, creating an additional barrier to the penetration of the microorganisms that cause the sausage spoilage. QMAFAnM on the sausage casing surface increased during the entire shelf life if treated with the suspension of starter culture SafePro BLC-48 or the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis. Under the given conditions, the treatment of the sausages with the mixture of starter cultures contributed to an increase in QMAFAnM on their surface on the 18th and 30th days of storage by 0.8 lg CFU/cm2 and 1.24 lg CFU/cm^2, respectively, compared to the treatment with the suspension of starter culture SafePro BLC-48 (Table 7).

Table 7 QMAFAnM in casing Mini Ralen of "Vienna sausages with chicken fillet" if treated with starter cultures of lactic-acid microorganisms and stored in vacuum packaging in a chilled state.

Study period	Variant	
	Experimental No. 1 SafePro BLC-48	**Experimental No.2 SafePro BLC-48 + Bactoferm Rubis**
1st day	6.78 ±1.32	7.35 ±1.21
18th day	7.12 ±0.59	7.92 ±0.84**
30th day	7.73 ±0.47	8.97 ±1.03**

Note: x ±SD, n =5, lg CFU/cm^2, ** – p≤0.05 compared to experimental variant No. 1

This makes it possible to suggest that the main species of the bacteria that colonised the polyamide casing of the sausages if treated with the spray of the starter cultures were the lactic-acid microorganisms, in experimental variant No. 1 - *Lactobacillus curvatus*, and in experimental variant No. 2 - a mixture of *Lactobacillus curvatus* and *Lactococcus lactis subsp. Lactis*.

Thus, the obtained study results show that the use of starter culture SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) for the sausage treatment makes it possible to increase the shelf life of the vacuum-packaged sausages in a chilled state by 12 days due to a complex and multicomponent inhibition mechanism of the spoilage microorganisms. The use of the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) for the sausage treatment may be promising if the development of the aerobic spoilage bacteria is confirmed. Along with this, the researchers emphasise the need to introduce effective protocols for producing meat products, which makes it possible to ensure the traceability of the production chain **[59]**.

In the future, it is necessary to determine the use effectiveness of the starter cultures of the lactic-acid bacteria in the association of different families and species of anaerobic or facultative anaerobic spoilage microorganisms of the sausages, which are produced following different recipes.

CONCLUSION

The main spoilage microorganisms of the vacuum-packed sausages on the 1st day of storage in a chilled state are the bacteria of the family Enterobacteriaceae (*Raoultella planticola, R. ornithinolytica, Citrobacter freundii*), Morganellaceae (*Morganella morganii*), and Staphylococcaceae (*Macrococcus caseolyticus*), and at the end of the shelf life for 21th day - the bacteria of the family Enterobacteriaceae (*Proteus mirabilis, Moellerella wisconsensis, Serratia liquefaciens*). The sausage spoilage is characterised by the appearance of cloudy juice, surface slime and delamination of vacuum packaging.

The treatment of "Vienna sausages with chicken fillet" with the starter culture of lactic-acid microorganisms SafePro BLC-48 (*Lactobacillus curvatus*) or the mixture of SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) before vacuum packaging contributed to an increase in QMAFAnM in the first-grade sausages on the 1st day of storage by 1.09 lg CFU/g and 1.53 lg CFU/g, on the 12th day - by 1.18 lg CFU/g and 1.54 lg CFU/g, on the 18th day - by 0.92 lg CFU/g and 1.96 lg CFU/g, respectively, compared to the control sample. In "Vienna sausages with chicken fillet", QMAFAnM was higher by 1.37 lg CFU/g on the 25th day of storage if treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis, and by 0.51 lg CFU/g on the 30th day compared to the treatment with starter culture SafePro BLC-48.

The number of the lactic-acid microorganisms in "Vienna sausages with chicken fillet" on the 1st and 12th days of storage if treated with starter culture SafePro BLC-48 did not differ from the control sample, while treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis increased the number of the lactic-acid microorganisms by 0.3 lg CFU/g and 0.35 lg CFU/g, respectively, compared to the control sample. Further storage of "Vienna sausages with chicken fillet" in a refrigerator for the 18th day did not affect, and on the 25th and 30th days it contributed to an increase in the number of the lactic-acid microorganisms if treated with the mixture of starter cultures SafePro BLC-48 + Bactoferm Rubis by 0.63 lg CFU/g and 0.53 lg CFU/g, respectively, compared to the treatment with starter culture SafePro BLC-48.

No pathogenic and opportunistic pathogenic bacteria, particularly *S. aureus, L. monocytogenes, Salmonella spp., E. coli*, bacteria of the *E. coli* group, yeast, and mould, were detected during the sausages' entire shelf life in both the control sample and if treated with starter culture SafePro BLC-48 or the mixture of starter culture SafePro BLC-48 + Bactoferm Rubis.

Thus, the obtained study results show that the use of starter culture SafePro BLC-48 (*Lactobacillus curvatus*) provides the extension of the shelf life of the vacuum-packaged sausages in a chilled state by 12 days due to the

inhibition of their spoilage microorganisms. The use of the mixture of starter cultures SafePro BLC-48 (*Lactobacillus curvatus*) + Bactoferm Rubis (*Lactococcus lactis subsp. Lactis*) for the sausage treatment does not make it possible to improve the result obtained when using SafePro BLC-48 (*Lactobacillus curvatus*). The use of the starter culture mixture of the lactic-acid microorganisms for the sausage treatment, while stored in vacuum packaging, may be promising if the development of the aerobic spoilage bacteria is confirmed.

REFERENCES

1. Bayer, O. V. Bondarets, O. V. Mykhalska V. M., Shevchenko, L. V., Stupak, O. M. Fesenko, J. V. Dovgopol, Y. V., Liniichuk, N. V., Kovalenko, V. L., Galka, I. V., Kryvenok, M. J., & Poliakovskyi, V. M. (2021). Evaluation of QuEChERS sample preparation for determination of benzimidazoles residues in meat by UPLC-MS/MS. In Methods and Objects of Chemical Analysis (Vol. 16, Issues 1, pp. 32–40). Taras Shevchenko National University. https://doi.org/10.17721/moca.2021.32-40
2. Bhattacharya, D., Nanda, P. K., Pateiro, M., Lorenzo, J. M., Dhar, P., & Das, A. K. (2022). Lactic acid bacteria and bacteriocins: novel biotechnological approach for biopreservation of meat and meat products. In Microorganisms (Vol. 10, Issue 10, p. 2058). MDPI AG. https://doi.org/10.3390/microorganisms10102058
3. Shanina, O., Minchenko, S., Gavrysh, T., Sukhenko, Y., Sukhenko, V., Vasyliv, V., Miedviedieva, N., Mushtruk, M., Stechyshyn, M., & Rozbytska, T. (2020). Substantiation of basic stages of gluten-free steamed bread production and its influence on quality of finished product. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 189–201). HACCP Consulting. https://doi.org/10.5219/1200
4. Austrich-Comas, A., Jofré, A., Gou, P., & Bover-Cid, S. (2023). Assessing the impact of different technological strategies on the fate of Salmonella in chicken dry-fermented sausages by means of challenge testing and predictive models. In Microorganisms (Vol. 11, Issues 2, p. 432). MDPI AG. https://doi.org/10.3390/microorganisms11020432
5. Pellegrini, M., Barbieri, F., Montanari, C., Iacumin, L., Bernardi, C., Gardini, F., & Comi, G. (2023). Microbial spoilage of traditional goose sausages produced in a northern region of Italy. In Microorganisms (Vol. 11, Issues 8, p. 1942). MDPI AG. https://doi.org/10.3390/microorganisms11081942
6. Julizan, N., Ishmayana, S., Zainuddin, A., Van Hung, P., & Kurnia, D. (2023). Potential of Syzygnium polyanthum as natural food preservative: a review. In Foods (Vol. 12, Issue 12, p. 2275). MDPI AG. https://doi.org/10.3390/foods12122275
7. Shevchenko, L. V., Dobrozhan, Y. V., Mykhalska, V. M., Osipova, T. Y., & Solomon, V. V. (2019). Contamination of hen manure with nine antibiotics in poultry farms in Ukraine. In Regulatory Mechanisms in Biosystems (Vol. 10, Issue 4, pp. 532–537). Oles Honchar Dnipro National University. https://doi.org/10.15421/021978
8. Beya, M. M., Netzel, M. E., Sultanbawa, Y., Smyth, H., & Hoffman, L. C. (2021). Plant-based phenolic molecules as natural preservatives in comminuted meats: a review. In Antioxidants (Basel, Switzerland) (Vol. 10, Issue 2, p. 263). MDPI AG. https://doi.org/10.3390/antiox10020263
9. Vovkotrub, V., Iakubchak, O., Horalskyi, L., Vovkotrub, N., Shevchenko, L., Shynkaruk, N., Rozbytska, T., Slyva, Y., Tupitska, O., & Shtonda, O. (2023). The microscopic structure of pork neck after cooling with showering stiving and processing by culture *Lactobacillus sakei*. (Vol. 17, pp. 759-776). Potravinarstvo Slovak Journal of Food Sciences. HACCP Consulting https://doi.org/10.5219/1905
10. Bodie, A. R., O'Bryan, C. A., Olson, E. G., & Ricke, S. C. (2023). Natural antimicrobials for *Listeria monocytogenes* in ready-to-eat meats: current challenges and future prospects. In Microorganisms (Vol. 11, Issue 5, p. 1301). MDPI AG. https://doi.org/10.3390/microorganisms11051301
11. Mihaylova-Garnizova, R., Davidova, S., Hodzhev, Y., & Satchanska, G. (2024). Antimicrobial peptides derived from bacteria: classification, sources, and mechanism of action against multidrug-resistant bacteria. In International Journal of Molecular Sciences (Vol. 25, Issue 19, p. 10788). MDPI AG. https://doi.org/10.3390/ijms251910788
12. Smaoui, S., Echegaray, N., Kumar, M., Chaari, M., D'Amore, T., Shariati, M. A., Rebezov, M., & Lorenzo, J. M. (2024). Beyond conventional meat preservation: saddling the control of bacteriocin and lactic acid bacteria for clean label and functional meat products. In Applied Biochemistry and Biotechnology (Vol. 196, Issue 6, pp. 3604–3635). Springer Science and Business Media LLC. https://doi.org/10.1007/s12010-023-04680-x
13. Dias, I., Laranjo, M., Potes, M. E., Agulheiro-Santos, A. C., Ricardo-Rodrigues, S., Fraqueza, M. J., Oliveira, M., & Elias, M. (2022). *Staphylococcus* spp. and *Lactobacillus sakei* starters with high level of inoculation and an extended fermentation step improve safety of fermented sausages. In Fermentation (Vol. 8, Issue 2, p. 49). MDPI AG. https://doi.org/10.3390/fermentation8020049

14. Dias, I., Laranjo, M., Potes, M. E., Agulheiro-Santos, A. C., Ricardo-Rodrigues, S., Fialho, A. R., Véstia, J., Fraqueza, M. J., Oliveira, M., & Elias, M. (2021). Co-inoculation with *Staphylococcus equorum* and *Lactobacillus sakei* reduces vasoactive biogenic amines in traditional dry-cured sausages. In International Journal of Environmental Research and Public Health (Vol. 18, Issue 13, p. 7100). MDPI AG. https://doi.org/10.3390/ijerph18137100
15. Segli, F., Melian, C., Muñoz, V., Vignolo, G., & Castellano, P. (2021). Bioprotective extracts from *Lactobacillus acidophilus* CRL641 and *Latilactobacillus curvatus* CRL705 inhibit a spoilage exopolysaccharide producer in a refrigerated meat system. In Food Microbiology (Vol. 97, p. 103739). Elsevier BV. https://doi.org/10.1016/j.fm.2021.103739
16. Castilho, N. P. A., Colombo, M., Oliveira, L. L., Todorov, S. D., & Nero, L. A. (2019). *Lactobacillus curvatus* UFV-NPAC1 and other lactic acid bacteria isolated from Calabresa, a fermented meat product, present high bacteriocinogenic activity against *Listeria monocytogenes*. BMC Microbiology (Vol. 19, Issue 1, p. 63). Scimago Journal & Country Rank. https://doi.org/10.1186/s12866-019-1436-4
17. Gumienna, M., & Górna, B. (2021). Antimicrobial food packaging with biodegradable polymers and bacteriocins. In Molecules (Basel, Switzerland). (Vol. 26, Issue 12, p. 3735). MDPI AG. https://doi.org/10.3390/molecules26123735
18. Hernández-Aquino, S., Miranda-Romero, L. A., Fujikawa, H., Maldonado-Simán, E. J., & Alarcón-Zuñiga, B. (2019). Antibacterial activity of lactic acid bacteria to improve shelf life of raw meat. In Biocontrol Science (Vol. 24, Issue 4, pp. 185–192). Informa UK Limited. https://doi.org/10.4265/bio.24.185
19. Sun, F., Kong, B., Chen, Q., Han, Q., & Diao, X. (2017). N-nitrosoamine inhibition and quality preservation of Harbin dry sausages by inoculated with Lactobacillus pentosus, *Lactobacillus curvatus* and *Lactobacillus sakei*. In Food Control (Vol. 73. pp. 1514-1521). Elsevier BV. https://doi.org/10.1016/j.foodcont.2016.11.018
20. Darbandi, A., Asadi, A., Mahdizade Ari, M., Ohadi, E., Talebi, M., Halaj Zadeh, M., Darb Emamie, A., Ghanavati, R., & Kakanj, M. (2022). Bacteriocins: Properties and potential use as antimicrobials. In Journal of Clinical Laboratory Analysis (Vol. 36, Issue 1, p. e24093). Wiley. https://doi.org/10.1002/jcla.24093
21. Pang, X., Song, X., Chen, M., Tian, S., Lu, Z., Sun, J., Li, X., Lu, Y., & Yuk, H. G. (2022). Combating biofilms of foodborne pathogens with bacteriocins by lactic acid bacteria in the food industry. In Comprehensive Reviews in Food Science and Food Safety (Vol. 21, Issue 2, pp. 1657–1676). Wiley. https://doi.org/10.1111/1541-4337.12922
22. Mushtruk, M., Bal-Prylypko, L., Slobodyanyuk, N., Boyko, Y., & Nikolaienko, M. (2022). Design of Reactors with Mechanical Mixers in Biodiesel Production. In Lecture Notes in Mechanical Engineering (pp. 197–207). Springer International Publishing. https://doi.org/10.1007/978-3-031-06044-1_19
23. Czerwiński, K., Rydzkowski, T., Wróblewska-Krepsztul, J., & Thakur, V. K. (2021). Towards impact of modified atmosphere packaging (MAP) on shelf-life of polymer-film-packed food products: challenges and sustainable developments. In Coatings (Vol. 11, p. 1504). MDPI AG. https://doi.org/10.3390/coatings11121504
24. Kawecki, K., Stangierski, J., & Cegielska-Radziejewska, R. (2021). The influence of packing methods and storage time of poultry sausages with liquid and microencapsulated fish oil additives on their physicochemical, microbial and sensory properties. In Sensors (Basel, Switzerland) (Vol. 21, Issue 8, p. 2653). MDPI AG. https://doi.org/10.3390/s21082653
25. Gupta, P. (2023). Role of oxygen absorbers in food as packaging material, their characterization and applications. In Journal of Food Science and Technology (Vol. 61, Issue 2, pp. 1–11). Springer Science and Business Media LLC https://doi.org/10.1007/s13197-023-05681-8
26. Bayer, O. V., Kaminska, O. V., Shevchenko, L. V., Mykhalska, V. M., Stupak, O. M., Bondarets, O. V., & Dobrozhan, Y. V. (2019). Development and evaluation of the suitability of the method for determining the content of egg coccidiostatics using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). In Methods and Objects of Chemical Analysis (Vol. 14, Issue 1, pp. 43–51). Taras Shevchenko National University. https://doi.org/10.17721/moca.2019.43-51
27. Chen, Y., Yu, L., Qiao, N., Xiao, Y., Tian, F., Zhao, J., Zhang, H., Chen, W., & Zhai, Q. (2020). *Latilactobacillus curvatus*: a candidate probiotic with excellent fermentation properties and health benefits. Foods (Basel, Switzerland) (Vol. 9, Issue 10, p. 1366). MDPI AG. https://doi.org/10.3390/foods9101366
28. Rogoskii, I., Mushtruk, M., Titova, L., Snezhko, O., Rogach, S., Blesnyuk, O., Rosamaha, Y., Zubok, T., Yeremenko, O., & Nadtochiy, O. (2020). Engineering management of starter cultures in study of temperature of fermentation of sour-milk drink with apiproducts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 1047–1054). HACCP Consulting. https://doi.org/10.5219/1437

29. EFSA Panel on Biological Hazards Scientific Opinion on the maintenance of the list of QPS biological agents intentionally added to food and feed (2013 update). In EFSA Journal (2013) (Vol. 11, p. 3449). Wiley. https://doi.org/10.2903/j.efsa.2013.3449
30. TU U 15.1-00419880-049-2003 Boiled sausage products. From 6.11.2003.
31. DSTU 4823.2:2007 Meat products. Meat products. Organoleptic assessment of quality indicators. Part 2.
32. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Retrieved from https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32002R0178
33. Cheng, H., Xu, H., Julian McClements, D., Chen, L., Jiao, A., Tian, Y., Miao, M., & Jin, Z. (2022). Recent advances in intelligent food packaging materials: Principles, preparation and applications. In Food Chemistry (Vol. 375, p. 131738). Elsevier BV. https://doi.org/10.1016/j.foodchem.2021.131738
34. Vasile, C., & Baican, M. (2021). Progresses in food packaging, food quality, and safety-controlled-release antioxidant and/or antimicrobial packaging. In Molecules (Basel, Switzerland) (Vol. 26, Issue 5, p. 1263). MDPI AG. https://doi.org/10.3390/molecules26051263
35. Zhang, Y., Min, S., Sun, Y., Ye, J., Zhou, Z., & Li, H. (2022). Characteristics of population structure, antimicrobial resistance, virulence factors, and morphology of methicillin-resistant *Macrococcus caseolyticus* in global clades. In BMC Microbiology (Vol. 22, Issue 1, p. 266). Scimago Journal & Country Rank. https://doi.org/10.1186/s12866-022-02679-8
36. Keller, J. E., Schwendener, S., Neuenschwander, J., Overesch, G., & Perreten, V. (2022). Prevalence and characterization of -methicillin-resistant Macrococcus spp. in food producing animals and meat in Switzerland in 2019. In Schweizer Archiv fur Tierheilkunde (Vol. 164, Issue 2, pp. 153–164). Gesellschaft Schweizer Tierärztinnen und Tierärzte GST. https://doi.org/10.17236/sat00343
37. Ramos, G. L. P. A., Vigoder, H. C., & Nascimento, J. S. (2021). Technological applications of *Macrococcus caseolyticus* and its impact on food safety. In Current Microbiology (Vol. 78, Issue 1). Springer Science and Business Media LLC. 11–16. https://doi.org/10.1007/s00284-020-02281-z
38. Uwamahoro, H.P., Li, F., Timilsina, A., Liu, B., Wang, X., & Tian, Y. (2022). An assessment of the lactic acid-producing potential of bacterial strains isolated from food waste. In Microbiological Research (Vol. 13, pp. 278-291). MDPI AG. https://doi.org/10.3390/microbiolres13020022
39. Mazhar, S., Kilcawley, K. N., Hill, C., & McAuliffe, O. (2020). A Systems-Wide Analysis of Proteolytic and Lipolytic Pathways Uncovers The Flavor-Forming Potential of The Gram-Positive Bacterium *Macrococcus caseolyticus* subsp. *caseolyticus*. In Frontiers in Microbiology (Vol. 11). Frontiers Media SA. https://doi.org/10.3389/fmicb.2020.01533
40. Villacís, J. E., Castelán-Sánchez, H. G., Rojas-Vargas, J., Rodríguez-Cruz, U. E., Albán, V., Reyes, J. A., Meza-Rodríguez, P. M., Dávila-Ramos, S., Villavicencio, F., Galarza, M., & Gestal, M. C. (2023). Emergence of *Raoultella ornithinolytica* in human infections from different hospitals in Ecuador with OXA-48-producing resistance. In Frontiers in Microbiology (Vol. 14). Frontiers Media SA. https://doi.org/10.3389/fmicb.2023.1216008
41. Perez, P. R. (2021). Infecciones urinarias por el género *Raoultella*. Revisión de la literatura y aportación de 1 caso por *Raoultella ornithinolytica* [Tract infections by the genus *Raoultella*. Literature review and contribution of 1 case of *Raoultella ornithinolytica*]. In Archivos Espanoles de Urologia (Vol. 74, Issue 3, pp. 276–286). Dialnet Foundation.
42. El-Shannat, S. M., El-Tawab, A. A. A., & Hassan, W. M. M. (2020). Emergence of *Raoultella ornithinolytica* isolated from chicken products in Alexandria, Egypt. In Veterinary World (Vol. 13, Issue 7, pp. 1473–1479). Scimago Journal & Country Rank. https://doi.org/10.14202/vetworld.2020.1473-1479
43. Tang, Y., Yuan, L., Chen, C., Tang, A., Zhou, W., & Yang, Z. (2023). Isolation and characterization of the new isolated bacteriophage YZU-L1 against *Citrobacter freundii* from a package-swelling of meat product. In Microbial Pathogenesis (Vol. 179, p. 106098). Elsevier BV. https://doi.org/10.1016/j.micpath.2023.106098
44. Yu, H., Huang, Y., Lu, L., Liu, Y., Tang, Z., & Lu, S. (2021). Impact of thyme microcapsules on histamine production by *Proteus bacillus* in Xinjiang smoked horsemeat sausage. In Foods (Vol. 121, Issue 10, p. 2491). MDPI AG. https://doi.org/10.3390/foods10102491
45. Ryser, L. T., Arias-Roth, E., Perreten, V., Irmler, S., & Bruggmann, R. (2021). Genetic and phenotypic diversity of *Morganella morganii* isolated from cheese. In Frontiers in Microbiology (Vol. 12, p. 738492). Frontiers Media SA. https://doi.org/10.3389/fmicb.2021.738492
46. Wang, Y., Pei, H., Liu, Y., Huang, X., Deng, L., Lan, Q., Chen, S., He, L., Liu, A., Ao, X., Liu, S., Zou, L., & Yang, Y. (2021). Inhibitory mechanism of cell-free supernatants of *Lactobacillus plantarum* on *Proteus*

mirabilis and influence of the expression of histamine synthesis-related genes. In Food Control (Vol. 125, p. 107982). Elsevier BV. https://doi.org/10.1016/j.foodcont.2021.107982

47. Spampinato, G., Candeliere, F., Amaretti, A., Licciardello, F., Rossi, M., & Raimondi, S. (2022). Microbiota survey of sliced cooked ham during the secondary shelf life. In Frontiers In Microbiology (Vol. 13, p. 842390). Frontiers Media SA. https://doi.org/10.3389/fmicb.2022.842390

48. Papatsiros, V. G., Athanasiou, L. V., Spanou, V. M., Stylianaki, I., Papakonstantinou, G., Letsios, M., Villioti, C. S., Tsekouras, N., Maragkakis, G., Papaioannou, N., & Christodoulopoulos, G. (2020). First case of *Serratia liquefaciens* isolated from urinary tract infection in sows and associated clinicopathological and pathological findings. In Letters in Applied Microbiology (Vol. 70, Issue 4, pp. 259–262). Wiley. https://doi.org/10.1111/lam.13267

49. Comi, G., Muzzin, A., Corazzin, M., & Iacumin, L. (2020). Lactic Acid Bacteria: Variability Due to Different Pork Breeds, Breeding Systems and Fermented Sausage Production Technology. In Foods (Vol. 9, Issue 3, p. 338). MDPI AG. https://doi.org/10.3390/foods9030338

50. Rivas, F. P., & Garro, O. A. (2023). Functionality of the bacteriocin sakacin G produced by *Lactobacillus curvatus* ACU-1 on cooked sausages under industrial conditions. In Letters in Applied Microbiology (Vol. 76, Issue 2, p. ovad015). Oxford University Press (OUP) https://doi.org/10.1093/lambio/ovad015

51. Tönz, A., Freimüller Leischtfeld, S., Stevens, M. J. A., Glinski-Häfeli, D., Ladner, V., Gantenbein-Demarchi, C., & Miescher Schwenninger, S. (2024). Growth control of *Listeria monocytogenes* in raw sausage via bacteriocin-producing *Leuconostoc carnosum* DH25. In Foods (Basel, Switzerland) (Vol. 13, Issue 2, p. 398). MDPI AG. https://doi.org/10.3390/foods13020298

52. Lucumi-Banguero, R. S., Ramírez-Toro, C., & Bolívar, G. A. (2021). Potential use of lactic acid bacteria with pathogen inhibitory capacity as a biopreservative agent for Chorizo. In Processes (Vol. 9, Issue 9, p. 1582). MDPI AG. https://doi.org/10.3390/pr9091582

53. Campaniello, D., Speranza, B., Bevilacqua, A., Altieri, C., Rosaria Corbo, M., & Sinigaglia, M. (2020). Industrial Validation of a Promising Functional Strain of *Lactobacillus plantarum* to Improve the Quality of Italian Sausages. In Microorganisms (Vol. 8, Issue 1, p. 116). MDPI AG. https://doi.org/10.3390/microorganisms8010116

54. Duda-Chodak, A., Tarko, T., & Petka-Poniatowska, K. (2023). Antimicrobial compounds in food packaging. In International Journal of Molecular Sciences (Vol. 24, Issue 3, p. 2457). MDPI AG. https://doi.org/10.3390/ijms24032457

55. Liu, Y., Gao, S., Cui, Y., Wang, L., Duan, J., Yang, X., Liu, X., Zhang, S., Sun, B., Yu, H., & Gao, X. (2024). Characteristics of Lactic Acid Bacteria as Potential Probiotic Starters and Their Effects on the Quality of Fermented Sausages. In Foods (Vol. 13, Issue 2, p. 198). MDPI AG. https://doi.org/10.3390/foods13020198

56. Carrión, M. G., Corripio, M. A. R., Contreras, J. V. H., Marrón, M. R., Olán, G. M., & Cázares, A. S. H. (2023). Optimization and characterization of taro starch, nisin, and sodium alginate-based biodegradable films: antimicrobial effect in chicken meat. In Poultry Science (Vol. 102, Issue 12, p. 103100). Elsevier BV. https://doi.org/10.1016/j.psj.2023.103100

57. Meloni, M. P., Piras, F., Siddi, G., Cabras, D., Comassi, E., Lai, R., McAuliffe, O., De Santis, E. P. L., & Scarano, C. (2023). Comparison of Activity of Commercial Protective Cultures and Thermophilic Lactic Acid Bacteria against Listeria monocytogenes: A New Perspective to Improve the Safety of Sardinian PDO Cheeses. In Foods (Vol. 12, Issue 6, p. 1182). MDPI AG. https://doi.org/10.3390/foods12061182

58. Nikodinoska, I., Tabanelli, G., Baffoni, L., Gardini, F., Gaggìa, F., Barbieri, F., & Di Gioia, D. (2023). Characterization of Lactic Acid Bacteria Isolated from Spontaneously Fermented Sausages: Bioprotective, Technological and Functional Properties. In Foods (Vol. 12, Issue 4, p. 727). MDPI AG. https://doi.org/10.3390/foods12040727

59. Lemos Junior, W. J. F., Marques Costa, L., Alberto Guerra, C., Sales de Oliveira, V., Gava Barreto, A., Alves de Oliveira, F., Paula, B. P. de, Esmerino, E. A., Corich, V., Giacomini, A., & Guerra, A. F. (2024). Microbial landscape of cooked meat products: evaluating quality and safety in vacuum-packaged sausages using culture-dependent and culture-independent methods over 1 year in a sustainable food chain. In Frontiers in Microbiology (Vol. 15). Frontiers Media SA. https://doi.org/10.3389/fmicb.2024.1457819

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you to Dr. Larysa Bal-Prylypko

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Snizhana Lokes National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Veterinary Hygiene, Vystavkova Str., 16, 03041, Kyiv, Ukraine
E-mail: lokes070979@icloud.com
ORCID: https://orcid.org/0009-0003-4215-0453

Larysa Shevchenko National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Veterinary Hygiene, Vystavkova Str., 16, 03041, Kyiv, Ukraine
E-mail: shevchenko_laris@ukr.net
ORCID: https://orcid.org/0000-0001-7472-4325

Kyrylo Doronin Department of Technologies and Organization of Restaurant Business, State University of Trade and Economics, building 19, Kyoto St., Kyiv, 02156, Ukraine
E-mail: k.doronin@knute.edu.ua
ORCID: https://orcid.org/0009-0001-1302-439X

Vita Mykhalska National University of Life and Environmental Sciences of Ukraine, Faculty of Veterinary Medicine, Department of Veterinary Hygiene, Vystavkova Str., 16, 03041, Kyiv, Ukraine
E-mail: mykhalska.vm@gmail.com
ORCID: https://orcid.org/0000-0003-0578-8856

***Valentyna Israelian** National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technology of meat, fish and marine products, Vystavkova Str., 16, Kyiv, 03041, Ukraine, Tel.: +38(096)724-03-99
E-mail: vs88@ukr.net
ORCID: https://orcid.org/0000-0002-7242-3227

Nataliia Holembovska National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technology of meat, fish and marine products, Vystavkova Str., 16, Kyiv, 03041, Ukraine, Tel.: +38(096)206-62-76
E-mail: natashagolembovska@gmail.com
ORCID: https://orcid.org/0000-0001-8159-4020

Nina Tverezovska National University of Life and Environmental Sciences of Ukraine, Faculty of Humanities and Pedagogy, Department of Social Work and Rehabilitation, Heroes of Defense, Str.15, 03041, Kyiv, Ukraine, Tel.: +38(044) 527-83-57
E-mail: tverezovskaya@nubip.edu.ua
ORCID: https://orcid.org/0000-0002-0672-9308

Oleksandr Savchenko National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technology of meat, Fish and marine products, Vystavkova Str., 16, Kyiv, 03041, Ukraine, Tel.: +38(050)359-62-71
E-mail: 63savchenko@gmail.com
ORCID: https://orcid.org/0000-0002-3940-6679

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 6.9.2024
Revised: 14.10.2024
Accepted: 30.10.2024
Published: 4.11.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 951-963
https://doi.org/10.5219/2033
ISSN: 1337-0960 online
www.potravinarstvo.com

The fight against illegal tobacco products

Dominik Čipka, Tereza Šalková

ABSTRACT

This paper addresses the pressing issue of combating illicit tobacco products, emphasising the legal frameworks at both the national and European levels. It explores the roles and activities of various authorities dedicated to preventing illegal tobacco trade, including the European Anti-Fraud Office (OLAF) and the Criminal Office of the Financial Administration of the Slovak Republic. The study delves into the legislative landscape governing tobacco products, assessing its effectiveness in adapting to the evolving tactics of smuggling networks. In particular, the paper scrutinises how Slovak and European regulations align to counteract the sophisticated and increasingly complex strategies employed in illegal tobacco trafficking. Statistical data highlights the prevalence of contraband tobacco in Slovakia, illustrating the financial, health, and societal ramifications of the black market. The findings also point to a correlation between high tobacco prices and an increased likelihood of exposure to illicit products. Furthermore, the research supports hypotheses regarding the adaptability of Slovak legislation to contemporary tobacco consumption trends and the critical role of inter-European collaboration in enforcement efforts. However, it also identifies potential areas for legislative improvement, particularly in streamlining tobacco regulation. This study contributes valuable insights into the challenges and successes of the Slovak Republic's ongoing fight against the illegal tobacco trade.

Keywords: Tobacco products, illegal tobacco products, fight against illegal tobacco products, European Anti-Fraud Office, Criminal Office of the Financial Administration of Slovak Republic

INTRODUCTION

One of the current trends is the fight against illicit tobacco products, which is a very delicate situation that poses risks not only in the sphere of public health but also in the sphere of public finances. The smuggling of tobacco products is becoming more sophisticated from year to year, or decade to decade, due to the adaptation of the various smuggling groups to the current legislative changes and modern techniques aimed at detecting and intercepting illegal imports. On the other hand, the question must be asked: are smugglers adapting their practices and methods to legislation and the mechanisms used to intercept smuggled goods, or are legislation and those mechanisms adapting to smuggling practices? In this paper, we will focus on the definition of tobacco products in terms of the legislation in force in the Slovak Republic, we will map the specific institutions and tools that serve to detect and intercept illegally imported tobacco products at the European level, we will describe the fight against illegal tobacco products concerning legislation and procedures in the Slovak Republic regarding the activities of the Criminal Office of the Financial Administration, as a body of the Financial Administration of the Slovak Republic, which focuses on violations of regulations in the field of public finances.

According to current statistics, up to ¼ of all cigarettes produced in the world are a commodity traded on the black market, i.e. contraband tobacco products. For the territory of the Slovak Republic, it can be stated statistically that every 16th cigarette is illegal. This statistic focuses exclusively on cigarettes; other tobacco products (the definition of which will be dealt with in Chapter 1) are not included. The level of untaxed tobacco products is currently at 4.4% . This is research from 2021, which notes that the level has increased year-on-year from 2020, when it was 4.1% in 2020. However, if we compare that level in the long term, for example, to 2008, when the level of consumption of illicit tobacco products was 8%, this is a significant improvement.

It should be noted that the Slovak Republic is not considered a country of production of illicit tobacco products, nor is it considered a destination country to which smugglers direct their primary shipments. The Slovak Republic serves as a transit country, i.e., through which illicit tobacco products mainly enter the European Union, particularly the Schengen area. The difference between the prices of tobacco products in Slovakia and in Western European countries is so great that Slovakia is not an attractive destination for the primary sale of smuggled tobacco products. From the above, it can be hypothesised that the higher the prices of tobacco products, the higher the likelihood of exposure to illicit tobacco products.

The most used transport channel for smugglers is the Slovak-Ukrainian border, and the methods of smuggling are varied. To put it bluntly, there are no limits to the imagination. There have been cases of smuggling tobacco products in cars across the Uh river—which lies on the border—artificially built underground tunnels, and, currently, a growing trend of smuggling by drones.

The importation or use of illicit tobacco products involves three main categories of threat - health, economic and societal. As the production and sale of tobacco products is subject to strict standards and criteria, which are generally not adhered to in the production of illicit tobacco products, health is the primary interest at stake. The above hypothesis can be verified by noting that the illicit tobacco products used do not have proven manufacturing processes, their content is not known (they generally contain more tar, produce more carbon monoxide or contain other toxic substances, etc.), nor are the manufacturing processes and associated storage, where there is a risk of high levels of contamination. The economic harm is mainly to public finances, since tobacco products are subject to tax as such and excise duty. This is a major drain on the national budget. Last but not least is the social factor, which consists in encouraging criminal activity. Unless society fundamentally condemns such behaviour and actions, the trade in illicit tobacco products will continue to operate.

Slovak legislation reflects modern trends in the use of tobacco products, especially concerning alternative ways of using tobacco products.

The Slovak Republic has a high level of fighting illicit tobacco products, regarding European cooperation in this area.

The Slovak Republic's legislation on tobacco products suffers from so-called hypertrophy, which weakens the law's enforceability and makes it opaque.

1. Tobacco products

„Ubi homo, ibi societas; ubi societas, ibi ius“. The above statement was made by the Roman philosopher and statesman Cicero, and can be considered timeless about the time it was made. Its content is still fully applicable today. Where there is man, there is society; where there is society, there is law. From its content we can very easily deduce the need to incorporate man, the individualist, into a larger grouping, society. For that society to function, it needs to have clearly defined structures and rules. That is to say, to have a functioning system of law. Without law there would be no order, only chaos and anarchy. Legal regulation applies to all social relations, with tobacco products being no exception.

According to European Union legislation, tobacco products are classified primarily based on their intended use and form. The classification includes the following main categories:

- Cigarettes – Rolled tobacco products for smoking.
- Cigars and Cigarillos – Larger rolled tobacco products, typically with more tobacco.
- Pipe Tobacco – Tobacco intended for use in pipes.
- Roll-your-own Tobacco – Loose tobacco for rolling into cigarettes by the consumer.
- Smokeless Tobacco Products – Products like chewing tobacco and snuff, intended for oral or nasal use without combustion.
- Novel Tobacco Products—This category includes items such as heated tobacco products, which are consumed by heating rather than burning.

These classifications and definitions are primarily found in Directive 2014/40/EU, also known as the Tobacco Products Directive (TPD), which governs the EU's manufacture, presentation, and sale of tobacco and related products. The directive lists specific requirements for each category, including packaging, labelling, maximum emission levels, and health warnings. It also sets provisions for reporting ingredients and monitoring the market for novel tobacco products.

Additionally, Regulation (EU) No 764/2008 (now incorporated into the EU Single Market rules) ensures that products legally sold in one EU country can be sold in others, provided they meet the necessary safety and labelling standards.

In the past, certain tobacco products were classified as food products in some jurisdictions, including under Slovak law. This was largely due to historical definitions and regulatory frameworks that initially grouped non-combustible items, such as chewing tobacco or snuff, with consumable items intended for ingestion or intake into

the body. However, this classification has since been revised, as tobacco products have unique health risks and require distinct regulations from food products. The key reasons for the shift away from classifying tobacco as food are:

1. **Health Risks**: Tobacco contains harmful substances, such as nicotine, tar, and other carcinogens, which are not compatible with the safety standards expected of food products. Tobacco products are now recognized for their serious health implications, which differ fundamentally from those of food.
2. **Purpose of Consumption**: Unlike food, which is consumed to provide nutritional benefits, tobacco is consumed primarily for recreational or habit-forming purposes. The effects of nicotine, an addictive component, further distinguish tobacco from food products.
3. **Regulatory Precision**: Modern regulatory frameworks, such as the EU's Tobacco Products Directive (Directive 2014/40/EU), are designed specifically for tobacco products. These frameworks address the unique risks associated with tobacco use, such as restrictions on marketing, strict labeling requirements, emission limits, and ingredient disclosure. Classifying tobacco separately from food allows for more precise and effective regulation of these risks.

Today, most countries regulate tobacco products under dedicated tobacco control laws rather than food legislation. This shift allows for more targeted measures addressing the health risks of tobacco use, including advertising restrictions, health warnings, and sales limitations. For instance, the European Union's Tobacco Products Directive (Directive 2014/40/EU) provides a comprehensive framework for regulating tobacco products across member states.

It's important to note that while the trend has moved towards specific tobacco regulation, some countries may still have remnants of older classifications, especially for traditional or culturally specific tobacco products. However, the global movement, guided by frameworks like the World Health Organization's Framework Convention on Tobacco Control (WHO FCTC), encourages nations to adopt comprehensive tobacco control measures distinct from food regulations.

In the EU, tobacco products are therefore no longer classified as food products. This change was codified through specific legislation that addresses the production, sale, and labeling of tobacco products distinctly from food, ensuring that tobacco regulation aligns with public health goals rather than dietary standards.

Since the use of tobacco products is inherent in some members of society, such regulation is necessary. Within the limits of the Slovak legal order, Act No 335/2011 Coll. on Tobacco Products and Act No 89/2016 Coll. on the Production and Sale of Tobacco Products and Related Products and on Amendments and Additions to Certain Acts play a key role in this respect.

The original legislation defining tobacco products was contained in Act No. 152/1995 Coll. on Foodstuffs, the text of the Act in force until 31.10.2011. Although the legal regulation of tobacco products was contained in the Food Act, the Act itself did not define tobacco and tobacco products as food **[1]**. Tobacco products under the Act were products intended to be smoked, chewed, snuffed and sucked if they were even partially made from tobacco, whether generically modified or not **[2]**. Thus, the legal definition was based on the product's origin, i.e., tobacco, and in light of current trends in tobacco product use, the then legal definition of tobacco products would not have been sufficient. Therefore, the legislator adopted Act No 335/2011 Coll. on Tobacco Products, which defined tobacco products as a separate category outside the scope of the legal regulation of the Food Act. The adoption of the new legislation reflected both the requirements of the European Union in terms of Regulation (EC) No 764/2008 of the European Parliament and of the Council of 09.07.2008 laying down procedures relating to the application of certain national technical rules to products lawfully placed on the market in another Member State **[3]** and **[4]**. At the same time, a legal amendment was also necessary due to the implementation of Regulation (EC) No 178/2022 of the European Parliament and of the Council of 28.01.2002 laying down procedures in food safety, according to which tobacco products cannot be classified as food.

By adopting the Tobacco Products Act, the Slovak Republic thus proclaimed compliance with the condition of alignment of national legislation with Community law. It defined a legal definition of tobacco products, which, paradoxically, remained unchanged from the original legislation. The law introduced the methodology for registering tobacco products, the manufacturing process and the associated responsibility for their quality, the marketing process, the form of packaging, the labelling of tobacco products, storage, transport and the sale itself. It can therefore be concluded that the legislation mentioned above only regulates certain concepts and institutes about tobacco products in a very general way. At the same time, it also defines certain legal offences in the field of tobacco products for which the competent public administration authority may impose a fine of between EUR 100 and EUR 2 000 **[5]**.

Act No. 89/2016 Coll. provides more specific legal regulations on producing and selling tobacco products and related products. In the authors' opinion, it can be considered the completion of the process of approximation with the Community law in the field of tobacco products, as it clearly defines individual tobacco products and other related products, as well as their components, or reflects the current trends in the use of tobacco or smokeless and smokeless tobacco products **[6]**.

The legislation also introduced maximum emission levels for tar, nicotine and carbon monoxide, with de lege ferenda considerations envisaging a gradual reduction taking into account the toxicity and addictiveness of individual tobacco products **[7]**. It can, therefore, be concluded that the law clearly and comprehensively regulates all the circumstances relating to the manufacture of tobacco products about the content of tobacco products, as well as their packaging itself, with the obligation to warn of health warnings or unambiguous identification of e-cigarettes. At the same time, the law obligates manufacturers and importers of tobacco products to submit notifications to the Slovak Trade Inspection Authority of all new categories of tobacco products they wish to place on the market no later than six months before the planned placing **[8]**. Here one can point to the legislator's willingness to adapt the standards for the marketing of new uses of tobacco products, which have escalated rapidly in recent times (e.g. e-cigarettes, vaporizers, chewing tobacco, etc.).

No legislation in the Slovak legal order negatively regulates the concept of a tobacco product. In other words, we only know the legal definition of a tobacco product. However, what can be considered an illicit tobacco product is nowhere explicitly laid down. Therefore, it is only by analogical deduction that we can conclude that an illicit tobacco product is any product, whether smoke or smokeless, as defined and named in the laws as mentioned earlier, which does not comply with the parameters laid down by those laws for the manufacture, packaging, distribution and sale of the tobacco product itself.

2. The presence of nicotine in foods and regulatory restrictions on added nicotine

In general, foods with added nicotine are not legally available due to the strict regulations surrounding nicotine as a controlled substance. Adding nicotine to foods or beverages is typically prohibited, as it would pose significant health risks and increase the potential for addiction. Regulatory bodies like the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) closely monitor and restrict the use of nicotine, allowing its inclusion only in regulated products, such as smoking cessation aids (e.g., nicotine gum, patches, lozenges) rather than in general food items.

However, there have been instances of nicotine-infused products marketed as novelties or supplements, though they are often quickly removed from the market due to regulatory concerns. Examples include:

Nicotine Beverages: Some companies have attempted to market nicotine-infused drinks, but these are generally not approved and often face regulatory action.

Nicotine Gum and Lozenges: Though not conventional food products, these items contain nicotine for smoking cessation purposes. They are classified as therapeutic products rather than food and are available only through regulated channels.

Due to the health risks associated with nicotine, especially outside controlled therapeutic uses, nicotine-infused food products are not legally permitted in most regions, and any attempt to sell such products would typically be met with strict regulatory actions.

Nicotine, commonly associated with tobacco plants, is also naturally present in trace amounts in certain edible plants belonging to the Solanaceae family, including tomatoes, potatoes, eggplants, and peppers. While nicotine in these foods occurs naturally, the concentration is exceedingly low, making typical dietary exposure harmless. However, regulatory bodies are vigilant about the addition of nicotine to food products, given its addictive properties and potential health risks.

3. Natural sources of nicotine in foods

Nicotine concentrations in edible plants vary but remain minimal compared to the levels found in tobacco. For example, tomatoes (*Solanum lycopersicum*) and potatoes (*Solanum tuberosum*) contain approximately 2–7 nanograms of nicotine per gram **[9]**. Eggplants (Solanum melongena) and bell peppers (*Capsicum annuum*) also contain trace nicotine levels, though still at non-addictive, biologically insignificant concentrations **[10]**. These naturally occurring traces do not pose a health risk, as the nicotine content is far below the threshold that could trigger dependence or adverse health effects **[11]**.

4. Health implications and comparisons to tobacco exposure

Nicotine, an alkaloid compound, is known for its stimulating and addictive properties. In tobacco, nicotine concentrations are in the milligram range per gram, vastly exceeding the nanogram quantities found in vegetables **[12]**. Consequently, the dietary intake of nicotine from vegetables is negligible and unlikely to influence health similarly to tobacco products. For instance, the amount of nicotine in a single cigarette can be thousands of times higher than what one would consume from an entire meal containing these vegetables. Thus, these dietary sources contribute to overall nicotine exposure minimally and do not carry the health risks associated with smoking or other forms of nicotine intake **[13]**.

5. Regulatory frameworks for nicotine-infused foods

In recent years, regulatory bodies such as the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have restricted the addition of nicotine to foods or beverages due to its pharmacological effects and addiction potential (FDA, 2016). Under current regulations, nicotine may only be added to certain products, specifically those intended for smoking cessation, such as nicotine gum, patches, and lozenges, which are available as over-the-counter or prescription products designed to help reduce nicotine dependence **[14]**.

Attempts to introduce nicotine-infused foods or beverages have generally been met with regulatory action. For instance, nicotine-infused drinks were briefly marketed but quickly pulled from the market due to safety concerns and the potential for abuse **[15]**. The FDA, in particular, has emphasized that any product intended to deliver nicotine to the body outside of approved cessation products is likely to be classified as either a drug or a tobacco product and thereby subject to stringent approval processes **[16]**.

6. Public health and consumer safety concerns

The restriction on nicotine addition to food products aligns with public health goals to reduce the prevalence of nicotine addiction and its associated health consequences. Nicotine, when ingested or absorbed outside regulated therapeutic contexts, can elevate heart rate and blood pressure, and has been associated with adverse cardiovascular outcomes, particularly in nicotine-naive individuals **[12]**. Moreover, easy access to nicotine-infused foods or beverages could increase the risk of accidental poisoning, particularly among children, and contribute to a rise in nicotine addiction, complicating public health efforts to reduce smoking and nicotine dependence **[11]**.

7. Emerging trends in nicotine food products

The nicotine product market has undergone significant transformation in recent years. While combustible tobacco use has seen a general decline in response to public health campaigns and regulatory action, interest in non-combustible nicotine products has risen. These alternatives range from nicotine replacement therapies (NRTs) such as gums and lozenges, to new consumer products aimed at providing a recreational nicotine experience without the harms associated with smoking. Chewing gums, chewing tobacco, and nicotine pouches have thus become areas of interest both for consumers and researchers.

7.1 Shift toward non-combustible and oral nicotine products

With increased awareness of the health risks associated with smoking, consumer interest has shifted toward oral and non-combustible nicotine products. This shift is supported by studies suggesting that smokeless and oral nicotine products may offer a less harmful alternative for nicotine intake compared to smoking **[17]**. While traditional chewing tobacco remains a staple in certain markets, new entrants like nicotine pouches, which contain synthetic nicotine without tobacco, are gaining popularity. These products are marketed as "tobacco-free" alternatives that provide nicotine satisfaction without the health risks linked to tobacco-specific nitrosamines, which are carcinogenic compounds found in tobacco **[18]**.

7.2 Growth in nicotine chewing gums and lozenges for recreational use

Traditionally, nicotine gums and lozenges have been used as part of smoking cessation programs. However, recent trends indicate a growing segment of consumers using these products recreationally rather than for quitting. This trend has prompted new formulations and flavors aimed at enhancing consumer experience, such as mint, fruit, and coffee-flavored gums and lozenges. Studies have shown that younger adults and former smokers increasingly use these nicotine products not as a means to quit smoking but as a way to manage stress, focus, and social interactions **[19]**. As these products are seen as more discreet and socially acceptable than smoking or

vaping, demand is expected to grow, especially among younger demographics seeking alternatives to traditional nicotine intake methods.

7.3 Advances in synthetic nicotine for food products

The development of synthetic nicotine, produced without tobacco leaves, is a significant innovation in the industry. Synthetic nicotine is chemically identical to tobacco-derived nicotine but is free from the contaminants associated with tobacco processing. This nicotine source has gained popularity for use in gums, lozenges, and nicotine pouches, which are often marketed as "tobacco-free" **[20]**. The appeal of synthetic nicotine products lies in their regulatory flexibility, as they often bypass tobacco-specific regulations. However, the lack of specific regulatory guidelines for synthetic nicotine has raised concerns among health authorities, prompting calls for stricter oversight **[21]**.

7.4 Regulatory challenges and public health concerns

Despite growing consumer interest, nicotine-infused food products face substantial regulatory hurdles. Most countries regulate nicotine as a controlled substance due to its addictive nature and health risks. For instance, the European Union and the U.S. Food and Drug Administration (FDA) restrict nicotine use in food products, permitting it primarily in regulated smoking cessation aids like nicotine gums and patches **[16]**. Health authorities are cautious about nicotine food products due to concerns over accidental ingestion, potential toxicity in children, and the risk of addiction **[12]**. These risks have led some countries to ban certain nicotine-infused products entirely, while others impose age restrictions and labelling requirements.

In response to rising nicotine food products, the FDA has emphasized the need for regulatory measures that address both tobacco-derived and synthetic nicotine, ensuring that any product marketed with nicotine meets safety standards **[21]**. European countries have also adopted similar policies, aiming to prevent the normalisation of nicotine use among non-smokers, especially minors.

7.5 Market expansion and innovation in flavours and product types

As nicotine consumption methods diversify, manufacturers are experimenting with new product categories and flavours to attract a broader audience. Nicotine-infused products now include flavoured gums, dissolvable lozenges, and pouches, as well as emerging categories such as nicotine mints and candy-like products. Flavours are tailored to appeal to young adults, with fruity, mint, and even dessert-inspired options available. While these innovations cater to consumer preferences, they have sparked concerns about attracting underage users. Studies indicate that flavour variety may increase the appeal of nicotine products among adolescents and young adults, potentially leading to the initiation of nicotine use **[22]**.

To counter these concerns, some jurisdictions are considering flavour restrictions on nicotine products similar to those applied to flavoured tobacco products. For instance, certain states in the U.S. have implemented bans on flavoured vaping products, and similar measures could extend to oral nicotine products if evidence of youth uptake persists **[23]**.

7.6 The role of public health campaigns and consumer education

Public health campaigns continue to play a critical role in informing consumers about the risks of nicotine use. Campaigns targeting non-smokers and young adults emphasise that while some nicotine products may reduce harm compared to smoking, they are not without risk. Education on the addictive nature of nicotine, particularly in products marketed as "tobacco-free," is essential to prevent non-smokers from initiating use **[24]**.

Additionally, researchers highlight the importance of differentiating between products designed for smoking cessation and those marketed for recreational use, as consumer awareness of these distinctions remains low **[14]**.

8. European cooperation in the investigation of illicit tobacco products

As mentioned in the introduction of the first chapter, the community needs rules to function, which will determine the limits of the behaviour of the incorporated entities. This model of functioning also reflects the functioning of the Slovak Republic and the other Member States within the European Union. The European Union is a very specific association of several states that cooperate in all spheres of life and try to raise the standard of living in each member country by taking joint steps. To this end, the so-called free flow of goods and services within the individual Member States was also established, which was achieved by establishing the Schengen area as one of the fundamental pillars. Establishing the Schengen area has virtually eliminated all border controls between Member States (and non-member states). Here, the scope for the importation of illicit tobacco products is created. If importers or smugglers manage to get illicit tobacco products into a transit country, further

interception of illicit tobacco products is difficult. We discussed the various harmful consequences in the introduction to this paper.

Based on available statistics, the overall consumption of tobacco products is on a downward trend. This is also reflected in the downward trend in the consumption of illicit tobacco products. France has the highest proportion of illicit tobacco products consumed, while the Slovak Republic has the lowest proportion **[25]**.

9. OLAF

In order to protect public finances, the European Union has set up the European Anti-Fraud Office ("OLAF"). Established in 1999, OLAF's main purpose and mission is to investigate corruption and abuse of power within the institutions of the European Union, including fraud involving the budget of the European Union. Smugglers deliberately attack the European Union's revenue to circumvent the obligation to pay administrative (including taxes) and customs duties by promoting and developing a black market in counterfeit goods. This very conduct about illicit tobacco products falls within OLAF's investigative remit **[26]**.

Eradicating tobacco smuggling is one of OLAF's core activities. According to statistics for 2023, OLAF helped seize 616 million illicit tobacco products worldwide, more than half of which were seized at the European Union's external borders. OLAF's investigative and interception activities have prevented more than €151 million in tax losses in the Member States of the European Union **[27]**.

9.1 Agreements providing for measures to combat illicit tobacco products

To streamline the interception and subsequent prevention of the smuggling of illicit tobacco products, the European Union (through the European Commission), together with the Member States, has concluded multilateral agreements with global trading companies. These agreements aim to strengthen cooperation in the fight against illicit tobacco products.

One such agreement is dated 15.07.2010 between the entities above and British-American Tabacoo (Holdings) Limited (which includes tobacco products such as Lucky Strike, Dunhill, Camel, glo, Vuse, Velo and others). This is a reciprocal agreement whereby the parties have expressed an interest in cooperating in the fight against illicit tobacco products, whereby trade in illicit tobacco products not only harms national or European economic interests but also harms the economic and other civil interests of manufacturers and distributors of tobacco products. Under the Agreement, a procedure has been agreed between the signatories according to which, if a Member State sees a tobacco product that meets the characteristics of an illicit or counterfeit tobacco product in quantities of at least 50 000, it will notify OLAF, which will then contact British-American Tobacco (BAT). BAT shall then, in cooperation with the competent authority of the Member State which has secured the seizure, inspect the seized samples to determine the origin of the cigarettes and whether they are genuine or counterfeit. BAT shall subsequently notify OLAF of the check results. Suppose it is found that BAT's tobacco products are genuine but not legally distributed. In that case, BAT undertakes to provide compensation equal to the amount of taxes and duties that would have been paid at the time of seizure in the Member State **[28]**.

Another of the agreements relating to the fight against illicit tobacco products is the Cooperation Agreement of 27.09.2010 between Imperial Tabacoo Limited ("ITL") the European Union and the Member States. The content of this agreement is very similar in principle to the agreement concluded with BAT, but what is interesting is the concept of ITL's financing of the fight against illicit tobacco products, whereby ITL sends funds annually to the other parties to the agreement to be used for the elimination of the illicit trade in tobacco products **[29]**. There is thus a clear interest, not only on the part of public law entities but also on the part of private law legal entities, in eliminating the trade in illicit tobacco products to the greatest extent possible.

9.2 Authorities "fighting" the illicit tobacco trade in the Slovak Republic

In the conditions of the Slovak Republic, the Criminal Office of the Financial Administration plays a major role in the fight against illicit tobacco. The Financial Administration of the Slovak Republic is a body of the State administration of the Slovak Republic in the field of taxes, fees and customs. According to Act No 35/2019 Coll. on Financial Administration and Amendments and Additions to Certain Acts, the Financial Administration of the Slovak Republic consists of the Financial Directorate of the Slovak Republic, customs offices, tax offices and the Criminal Office of the Financial Administration. The Criminal Office of the Financial Administration shall exercise its jurisdiction throughout the territory of the Slovak Republic. The Criminal Office carries out a number of important tasks, but in the fight against illicit tobacco products the most important is in particular that it carries out customs surveillance in agreement with the authorities of other States by secret escort of a delivery or other covert means of surveillance if there is a reasonable presumption that the consignment contains narcotic drugs,

psychotropic substances, their precursors, substances with anabolic or other hormonal effect, tobacco, tobacco products, protected plant species, protected animals and specimens of species of wild fauna and flora for which the relevant authorisation has not been issued, or any other item for the possession of which an authorisation is required, goods suspected of being in breach of tax or customs legislation, items intended for the commission of a criminal offence or items derived from a criminal offence, or where an international treaty so provides, in order to identify persons involved in the handling of the consignment; where the information thus obtained is intended to serve as evidence in criminal proceedings, the procedure shall be based on the legislation on international cooperation between judicial authorities in criminal matters **[30]**.

OLAF was mentioned earlier in this article. In connection with the implementation of the obligations arising from Article 325 of the Treaty on the Functioning of the European Union, the protection of the financial interests of the European Union in the Slovak Republic is ensured and coordinated in accordance with Section 24(4) of Act No. 575/2001 Coll. on the organisation of government activities and the organisation of central state administration, Act No. 528/2008 Coll. on assistance and support from European Community funds, as amended, Act No. 292/2014 Coll. on assistance and support from European Community funds, and Act No. z. on the contribution from the European Structural and Investment Funds and on amendment and supplementation of certain acts and Act No. 121/2022 Z. z. on contributions from the European Union funds and on amendment and supplementation of certain acts, as amended, the Department of the National Office for OLAF (hereinafter referred to as "ONÚ OLAF") **[31]** was established as a coordinating office for the fight against fraud, which is an organisational unit of the Control Section of the Office of the Government of the Slovak Republic. The role of the OLAF National Office is to coordinate legislative, administrative and operational activities to ensure the protection of the EU's financial interests and, to this end, to cooperate closely with the authorities and institutions of the Slovak Republic involved in the system of such protection **[32]**.

International treaties and, not least, national legislation play an important role in the fight against the illicit tobacco trade. To streamline action to intercept and prevent the smuggling of illicit tobacco products, the European Union (through the European Commission), together with the Member States, has concluded agreements with global trading companies aimed at strengthening cooperation at the level of the fight against illicit tobacco products, which have already been described above, but only two are currently in force, namely BAT and ITL. The aim of these agreements is that the Slovak Republic, through its authorities, primarily the Criminal Office of the Financial Administration, carries out seizures of cigarettes from a particular tobacco company, and if it discovers that the seizures are not genuine, it informs the company, which then sends money to the Member States, and therefore also to the Slovak Republic, at regular intervals.

As an example, in 2022, three vehicles were donated by Philip Morris Slovakia s.r.o. Slovak Republic. Cooperation with the tobacco company has already proved useful in 2019, when the Criminal office of the Financial Administration received five new vehicles from the tobacco company **[33]**. The value of these vehicles was for EUR 100.000,-. For the Slovak Republic, this cooperation is beneficial because, thanks to such partnerships, the Financial Administration of the Slovak Republic annually increases border controls and, last but not least, the supervision of the production and movement of tobacco products on the territory of the Slovak Republic, while the efforts of our authorities supervising the movement of tobacco products are precisely to eliminate their illegal movement. The Slovak Republic contributes not only to the protection of the interests of the Slovak Republic as such, but also to the protection of the interests of the European Union. In the case of the donation of new motor vehicles as a result of cooperation between the Slovak authorities and international tobacco companies, it can be said that the new motor vehicles can contribute even more effectively and quickly to the detection of illegal activities with tobacco products, and therefore to the prevention of leakages, whether from the state budget or the European budget. As mentioned in the introduction, the illicit trade in tobacco products is not only a threat (whether security, economic or health) to the country itself but also to the European Union.

The Annual Report 2023, prepared by the OLAF National Office in cooperation with the Working Group on Communication under the Steering Committee for the Protection of the Financial Interests of the European Union in the Slovak Republic, provides information on the cooperation of the OLAF National Office with OLAF, on the cooperation of network partners, on the activities of the PLAF National Office and other network partners in the field of the financial interests of the European Union, on the training activities of OLAF and other network partners, on information and awareness-raising activities on the protection of the European Union's financial interests, on the issue of press releases and on examples from the practice of network partners, and thus in particular on cases handled by the National Criminal Agency, the Financial Directorate of the Slovak Republic, the Antimonopoly Office of the Slovak Republic and the Supreme Audit Office of the Slovak Republic.

The report above for 2023 **[34]** informs that the Financial Administration of the Slovak Republic participated in the following joint customs operations BELLEROPHON, NOXIA, EMPACT IPCCGC OA 3.8, DEMETER IX and EMPACT/EUROPOL, in which OLAF played an important role. Operation NOXIA, coordinated by

OLAF, took place in spring 2023 and targeted deep sea containers in EU and Asian ports to prevent the smuggling of hazardous substances such as illegal waste, illegal pesticides, cigarettes, tobacco and other illegal products. In 2023, a grant application was successful, which resulted in implementing the International Training for Customs Officers project to combat excise fraud - mineral oils, tobacco (cigarettes) and traceability of tobacco products using the Track & Trace system. The training in question is aimed at combating excise fraud in the field of cigarettes, tobacco and mineral oils and traceability of tobacco products using the Track & Trace system to contribute to increasing the effectiveness of mobile customs surveillance and tax surveillance and to respond to new trends in the perpetration of excise fraud (tobacco, cigarettes, mineral oils). The report concludes with a description of the cases the Directorate General of Finance deals with.

Two cases were described in the annual report regarding the fight against illicit tobacco products. The first case concerned an illegal cigarette business worth more than EUR 782.000,-. In May 2023, armed officers of the Criminal Office of the Financial Administration carried out several house searches and searches of other premises, including motor vehicles, in the Trnava Region and in the Košice Region under the name of "Fešák", uncovering an organised group of dealers in illegal cigarettes. These interventions prevented further possible evasion of excise duties on tobacco and tobacco products. More than 6 million cigarettes from various brands such as Marble, Winston, Kent, Kent Core, Compliment Violet, Marlboro Red, Richmond, Austin, Marlboro Gold, and Rothmans were seized. Consumer packs of 20 cigarettes were not marked with a valid Slovak control mark. It was also possible to seize cash from criminal activity of approximately EUR 312.000,-, 5 cars and two trucks and a cigarette filler. Many properties, including land, with an estimated value of approximately EUR 715.000,- were seized. A total of 6 persons were detained. The documented and preliminarily assessed damage amounted to at least EUR 782.416,-.

The second case involved a successful cross-border cooperation that brought about the dismantling of a cigarette gang. As in the first case, armed officers of the Criminal Office of the Financial Administration detected an organised group with cross-border reach while operating in eastern Slovakia in May 2023, codenamed "The List". Several searches of premises were carried out, and two cigarette production lines were discovered in an agricultural building, one active and the other ready for assembly and further production of illegal cigarettes. In addition, they seized 7.3 million illegal cigarettes, 791 kilograms of tobacco, 152 kilograms of cut tobacco, mobile phones, cigarette manufacturing components and photo tapes used to protect the building and the entrance to the premises. Seven persons (6 citizens of Belarus and 1 citizen of Poland) were arrested directly during the commission of the offence. The preliminary estimate of the damage that should have been caused to the State by this illegal activity is EUR 1 million. As the organised group was also active on the Czech Republic, Poland and Austria territory, the Criminal Office of the Financial Administration carried out the case in cooperation with Czech and Polish colleagues. Czech customs officers managed to detain 13 persons, with the estimated damage amounting to EUR 4.6 million. In Poland, 17 suspects were detained, and the estimated damage amounted to EUR 16.7 million. This brings the total number of detained persons to 37.

As for 2022, an interesting case is the discovery of the illegal production of cigarettes for EUR 6.200.000,- **[35]** and **[36]**. Armed officers of the Criminal Office of the Financial Administration detected it in an action codenamed Plavec, in which they carried out several searches of non-residential premises in various industrial areas and a house search in Košice. Nearly 100 armed officers of the Financial Administration were involved in the action, and police forces were also involved. Under the code name "Swimmer", the armed officers arrested 20 persons directly at the scene of the crime. This is a historic seizure in terms of the amount of damage to the state. Excise duty evasion on tobacco and tobacco products was estimated at up to EUR 6.2 million, the largest seizure since 2018. In terms of the amount of damage, this is the largest ever seizure since 2018. The total amount of tobacco and tobacco products excise duty evaded amounts to EUR 6.2 million.

Action "Garage" **[37]** revealed more illegal cigarettes in 2024. This action targeted illegal cigarettes in the east of Slovakia and resulted in almost 520 thousand pieces of seized cigarettes. The total damage quantified in excise duty on tobacco amounted to EUR 84.515,87.

The above cases are only one of many actions taken to detect illegal trade in tobacco products in the Slovak Republic. The published results of the raids undoubtedly show that the Slovak Republic is effectively combating the illicit trade in tobacco products.

We mentioned at the outset that in the case of illicit trade in tobacco products, i.e. when it is detected, it goes hand in hand with criminal liability for the offences committed. Related to this is the Protocol for the Eradication of Illicit Trade in Tobacco Products **[38]**, which entered into force on 25 September 2018. For the Slovak Republic, under the Protocol in force, they shall, in accordance with Article 4, in particular, adopt and implement effective measures aimed at controlling or regulating the supply chain of goods covered by this Protocol in order to prevent, deter, detect, investigate and prosecute illicit trade in such goods and, to this end, shall cooperate with each other, adopt any necessary measures, in accordance with their national legislation, to enhance the

effectiveness of their competent authorities and services, including customs and police authorities responsible for the prevention, deterrence, detection, investigation, prosecution and eradication of all forms of illicit trade in goods covered by this Protocol, cooperate closely with each other in accordance with their respective national legal and administrative systems in order to enhance the effectiveness of law enforcement in combating illicit conduct, including the offences provided for under Article 14 of this Protocol. Following the adopted Protocol, each Party shall ensure that any violation is subject to appropriate criminal, civil or administrative proceedings and to effective, proportionate and dissuasive penalties. Each Party shall take such legislative and other measures as are necessary to render the conduct in question unlawful under its domestic law. Possible breaches may, in the conditions of Slovak law, be seen mainly in the form of committing administrative offences and incurring administrative liability or criminal offences and criminal liability. However, it should not be forgotten that the qualification of an act as a criminal offence takes precedence over the qualification of an act as a misdemeanour (administrative offence).

The transposition of Article 14 of the Protocol for the Eradication of Illicit Trade in Tobacco Products introduced the offence of manufacturing tobacco and tobacco products into the Criminal Code. Given the nature of the offence, the legislator chose to incorporate it into the pre-existing offence of manufacture of alcohol. According to Section 253 of the Criminal Code, this offence is committed by whoever, without a permit, produces alcohol, tobacco or tobacco products in large quantities or whoever, without a permit, possesses or puts into circulation alcohol, tobacco or tobacco products produced in large quantities, shall be punished, unless it is an act more severely punishable. At the same time, it is equally punishable to unlawfully make or possess a device for the production of alcohol, tobacco or tobacco products.

In addition to the offence mentioned above, the Slovak Criminal Code **[39]** also regulates the offence of violating the regulations on state technical measures for marking goods. This offence is committed by a person who, in contravention of a generally binding legal regulation, handles control marks, control tapes or other control technical measures for marking goods for tax purposes or for other purposes provided for by law to cause damage to another or obtain an unjustified benefit for himself or another, or who imports in contravention of a generally binding legal regulation, exports, transports, has transported, puts into circulation or possesses goods without control marks, control strips or other technical control measures to mark them for tax purposes or for other purposes provided for by law. However, the above only describes the basic facts of the offence. It is the second indent of the offence related to handling such goods (in our case, tobacco products). The object of the criminal offence of violation of the regulations on state technical measures for the marking of goods under Section 279 of the Criminal Code is the interest of the State in controlling the movement of goods subject to excise duty, and value added tax and, if the goods have been imported from a third country outside the European Union, customs duty, and on the other hand, the interest of the State in the revenue from these taxes and customs duties. An amount equal to the sum of the tax mentioned above and customs duties, which the tax subject would otherwise have been obliged to pay under the law, constitutes, within the meaning of the first sentence of Article 124(1) of the Criminal Code, damage to the revenue part of the State budget of the Slovak Republic **[40]**. The control measures shall designate alcoholic beverages (spirits) and tobacco products. It should be noted, however, that the concurrence of this offence with the offence of tax and insurance fraud under Section 276 of the Criminal Code, as well as the concurrence of this offence with the offence of damaging the financial interests of the European Union under Sections 261 to 263 of the Criminal Code, is excluded.

CONCLUSION

The illicit trade in tobacco products poses a significant threat to the global economy, undermining the financial and social stability of nations while compromising public health and security. This illegal market deprives governments of essential tax revenues and funds transnational criminal networks that destabilise regions and harm the collective interests of European Union (EU) member states and the EU. The proliferation of illicit tobacco products conflicts with public health objectives, placing added strain on healthcare systems and obstructing efforts to reduce smoking rates. In light of the evidence presented, Slovak legislation demonstrates an appropriate and adaptive response to the evolving challenges in combating the illicit tobacco trade. This conclusion is supported by the proactive role of the Criminal Office of the Financial Administration, which effectively collaborates with international bodies such as the European Anti-Fraud Office (OLAF). The office's international engagement and strategic interventions highlight Slovakia's commitment to upholding EU-wide standards in this critical area of public and economic interest. The paper also confirms that the Criminal Office's cooperation with OLAF significantly enhances the enforcement framework against illicit tobacco products. However, findings indicate that Slovakia's dual regulation of tobacco products introduces complexities, creating an impression of inconsistency that may hinder effective enforcement and regulatory clarity. To streamline operations and improve legislative transparency, it is recommended that the Slovak government consider consolidating the regulations

governing the definition, manufacture, distribution, and sale of tobacco products into a unified legal framework. This approach would likely strengthen the enforceability of laws, reduce bureaucratic redundancy, and facilitate a more coordinated response to the illicit tobacco trade, benefiting both Slovakia and the broader EU community.

REFERENCES

1. Act. 152/1995 Coll. of the Slovak Republic on foodstuffs. Retrieved from https://www.slov-lex.sk/ezbierky/pravne-predpisy/SK/ZZ/1995/152/20220721
2. Act. 152/1995 Coll. of the Slovak Republic on foodstuffs. Retrieved from https://www.slov-lex.sk/ezbierky/pravne-predpisy/SK/ZZ/1995/152/20220721
3. Explanatory report on the draft law on tobacco products. (2011, June 8). In National Council of the Slovak Republic. https://www.nrsr.sk/web/Dynamic/DocumentPreview.aspx?DocID=356748
4. Ševcová, K., Zajác, P., Čapla, J., & Čurlej, J. (2021). Development of the food act of the Slovak Republic from 1995 to 2021. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 15, pp. 982–994). HACCP Consulting. https://doi.org/10.5219/1689
5. Act. 335/2011 Coll. of the Slovak Republic on tobacco products. Retrieved from https://www.slov-lex.sk/ezbierky/pravne-predpisy/SK/ZZ/2011/335/20160520
6. Act. 89/2016 Coll. of the Slovak Republic on the manufacture and sale of tobacco products and related products and on amending and supplementing certain acts. Retrieved from https://www.slov-lex.sk/ezbierky/pravne-predpisy/SK/ZZ/2016/89/20230101
7. Explanatory Report on the Draft Law on the Production, Labeling, and Sale of Tobacco Products and Related Products (2015, August 27). In National Council of the Slovak Republic. https://www.nrsr.sk/web/Dynamic/DocumentPreview.aspx?DocID=417827
8. Act. 89/2016 Coll. of the Slovak Republic, the Production, Labeling, and Sale of Tobacco Products and Related Products and on Amending and Supplementing Certain Acts. Retrieved from https://www.slov-lex.sk/ezbierky/pravne-predpisy/SK/ZZ/2016/89/20230101.html
9. Domino, E. F., Hornbach, E., & Demana, T. (1993). The Nicotine Content of Common Vegetables. In New England Journal of Medicine (Vol. 329, Issue 6, pp. 437–437). Massachusetts Medical Society. https://doi.org/10.1056/nejm199308053290619
10. Siegmund, B., Leitner, E., & Pfannhauser, W. (1999). Determination of the Nicotine Content of Various Edible Nightshades (Solanaceae) and Their Products and Estimation of the Associated Dietary Nicotine Intake. In Journal of Agricultural and Food Chemistry (Vol. 47, Issue 8, pp. 3113–3120). American Chemical Society (ACS). https://doi.org/10.1021/jf990089w
11. Miller, M. B., & Picciotto, M. R. (2016). Nicotine Pharmacology, Abuse, and Addiction. In Neuroscience in the 21st Century (pp. 3659–3677). Springer New York. https://doi.org/10.1007/978-1-4939-3474-4_157
12. Benowitz, N. L., & Burbank, A. D. (2016). Cardiovascular toxicity of nicotine: Implications for electronic cigarette use. In Trends in Cardiovascular Medicine (Vol. 26, Issue 6, pp. 515–523). Elsevier BV. https://doi.org/10.1016/j.tcm.2016.03.001
13. Hecht, S. S. (2003). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. In Nature Reviews Cancer (Vol. 3, Issue 10, pp. 733–744). Springer Science and Business Media LLC. https://doi.org/10.1038/nrc1190
14. Levett, J. Y., Filion, K. B., Reynier, P., Prell, C., & Eisenberg, M. J. (2023). Efficacy and Safety of E-Cigarette Use for Smoking Cessation: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. In The American Journal of Medicine (Vol. 136, Issue 8, pp. 804-813.e4). Elsevier BV. https://doi.org/10.1016/j.amjmed.2023.04.014
15. Foulds, J., Veldheer, S., & Berg, A. (2011). Electronic cigarettes (e-cigs): views of aficionados and clinical/public health perspectives. In International Journal of Clinical Practice (Vol. 65, Issue 10, pp. 1037–1042). Hindawi Limited. https://doi.org/10.1111/j.1742-1241.2011.02751.x
16. FDA. (2016). Nicotine-containing products classified as tobacco products. U.S. Food and Drug Administration. Retrieved from https://www.fda.gov
17. Farsalinos, K., Romagna, G., Tsiapras, D., Kyrzopoulos, S., & Voudris, V. (2014). Characteristics, Perceived Side Effects and Benefits of Electronic Cigarette Use: A Worldwide Survey of More than 19,000 Consumers. In International Journal of Environmental Research and Public Health (Vol. 11, Issue 4, pp. 4356–4373). MDPI AG. https://doi.org/10.3390/ijerph110404356
18. Rostron, B. L., Chang, C. M., & Pechacek, T. F. (2014). Estimation of Cigarette Smoking–Attributable Morbidity in the United States. In JAMA Internal Medicine (Vol. 174, Issue 12, p. 1922). American Medical Association (AMA). https://doi.org/10.1001/jamainternmed.2014.5219

19. Collins, B. N., Lepore, S. J., Winickoff, J. P., & Sosnowski, D. W. (2019). Parents' Self-efficacy for Tobacco Exposure Protection and Smoking Abstinence Mediate Treatment Effects on Child Cotinine at 12-Month Follow-up: Mediation Results from the Kids Safe and Smokefree Trial. In Nicotine & Tobacco Research (Vol. 22, Issue 11, pp. 1981–1988). Oxford University Press (OUP). https://doi.org/10.1093/ntr/ntz175
20. Bell, K., & Keane, H. (2012). Nicotine control: E-cigarettes, smoking and addiction. In International Journal of Drug Policy (Vol. 23, Issue 3, pp. 242–247). Elsevier BV. https://doi.org/10.1016/j.drugpo.2012.01.006
21. FDA. (2021). Regulation of synthetic nicotine products. U.S. Food and Drug Administration. Retrieved from https://www.fda.gov
22. Soneji, S., Barrington-Trimis, J. L., Wills, T. A., Leventhal, A. M., Unger, J. B., Gibson, L. A., Yang, J., Primack, B. A., Andrews, J. A., Miech, R. A., Spindle, T. R., Dick, D. M., Eissenberg, T., Hornik, R. C., Dang, R., & Sargent, J. D. (2017). Association Between Initial Use of e-Cigarettes and Subsequent Cigarette Smoking Among Adolescents and Young Adults. In JAMA Pediatrics (Vol. 171, Issue 8, p. 788). American Medical Association (AMA). https://doi.org/10.1001/jamapediatrics.2017.1488
23. Meernik, C., Baker, H. M., Kowitt, S. D., Ranney, L. M., & Goldstein, A. O. (2019). Impact of non-menthol flavours in e-cigarettes on perceptions and use: an updated systematic review. In BMJ Open (Vol. 9, Issue 10, p. e031598). BMJ. https://doi.org/10.1136/bmjopen-2019-031598
24. MacMonegle, A. J., Smith, A. A., Duke, J., Bennett, M., Siegel-Reamer, L. R., Pitzer, L., Speer, J. L., & Zhao, X. (2022). Effects of a National Campaign on Youth Beliefs and Perceptions About Electronic Cigarettes and Smoking. In Preventing Chronic Disease (Vol. 19). Centers for Disease Control and Prevention (CDC). https://doi.org/10.5888/pcd19.210332
25. Illicit cigarette consumption in the EU, UK, Norway, Switzerland, Moldova and Ukraine. (2023, June 28). In KPMG. https://www.pmi.com/resources/docs/default-source/itp/kpmg-report---illicit-cigarette-consumption-in-the-eu-uk-norway-switzerland-moldova-and-ukraine---2022-results.pdf
26. OLAF in 2021: Detection and Prevention of Fraud. (2022, June 8). In OLAF https://anti-fraud.ec.europa.eu/media-corner/news/olaf-2021-exposing-and-preventing-fraud-2022-06-08_sk
27. OLAF Report 2023. (2024). In OLAF. https://anti-fraud.ec.europa.eu/document/download/b6881acf-3d38-4021-802b-47ee45e44752_en?filename=or-2023-short_en.pdf
28. Agreement on Cooperation between British-American Tobacco (Holdings) Limited and the European Union represented by the European Commission and the signatory member states of the European Union (2010, July 15). In Ministry of Finance of the Slovak Republic. https://www.mfsr.sk/files/archiv/priloha-stranky/3628/62/BAT_dohoda.pdf
29. Agreement on Cooperation dated September 27, 2010 between IMPERIAL TOBACCO LIMITED and the EUROPEAN UNION represented by the EUROPEAN COMMISSION and the individual MEMBER STATES listed on the signature pages of this agreement. (2010, September 27). In Ministry of Finance of the Slovak Republic. https://www.mfsr.sk/files/archiv/priloha-stranky/3628/62/ITL_dohoda.pdf
30. Act No. 35/2019 Coll. of the Slovak Republic on Financial Administration and on Amendments and Additions to Certain Acts. Retrieved from https://www.slov-lex.sk/ezbierky/pravne-predpisy/SK/ZZ/2019/35/20230101
31. Cooperation with OLAF. In Financial Administration of the Slovak Republic. https://www.financnasprava.sk/sk/infoservis/eu-olaf
32. Department of the National Office for OLAF. (2013) In the National Office for OLAF. https://www.olaf.vlada.gov.sk/
33. Financial Administration Criminalists Received New Vehicles. (2022, April 26). In the Financial Administration of the Slovak Republic. https://www.financnasprava.sk/sk/pre-media/novinky/archiv-noviniek/detail-novinky/_kufs-auta-ts/bc
34. Annual Report on the Activities of Network Partners in the Area of Protection of the Financial Interests of the EU in the Slovak Republic for 2023. (2024). In the Financial Administration of the Slovak Republic. https://www.financnasprava.sk/_img/pfsedit/Dokumenty_PFS/Infoservis/olaf/2024/2024.04.30_Vyr_spr_OFZ_EU_final.pdf
35. Instead of Poultry, Illegal Cigarettes Worth 6.2 Million Euros. (November 28, 2022). In the Financial Administration of the Slovak Republic. https://www.financnasprava.sk/sk/pre-media/novinky/archiv-noviniek/detail-novinky/_neleg-cigare-ts/bc
36. Ševcová, K. (2022). Binding of a civil court by a decision in criminal prodeedings. In: Naděje právní vědy 2021 : právní věda v praxi Plzeň : Západočeská univerzita v Plzni, 2022. ISBN 978-80-261-1048-4. pp. 674-685.

37. Operation 'Garage' Uncovered More Illegal Cigarettes. (July 26, 2024). In the Financial Administration of the Slovak Republic. https://www.financnasprava.sk/sk/pre-media/novinky/archiv-noviniek/detail-novinky/_garaz-ts/bc

38. PROTOCOL on the Elimination of Illegal Trade in Tobacco Products. (2012, November 12). Retrieved from https://eur-lex.europa.eu/legal-content/SK/TXT/PDF/?uri=CELEX:22016A1001(01)&from=SL

39. Act. 300/2005 Coll. of the Slovak Republic, Criminal Code. Retrieved from https://www.slov-lex.sk/ezbierky/pravne-predpisy/SK/ZZ/2005/300/20240806

40. Strémy, T., & Kurilovská, L. (2022). Criminal Code - Commentary. [ASPI System]. Wolters Kluwer ASPI_ID KO300_a2005SK. https://www.aspi.sk/products/lawText/13/404/1/2

Conflict of Interest:

No potential conflict of interest was reported by the authors.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Dominik Čipka**, Matej Bel University in Banská Bystrica, Faculty of Law, Department of Civil and Labor Law, Komenského 20, 974 01, Banská Bystrica, Slovak Republic,
E-mail: dcipka@umb.sk
ORCID: https://orcid.org/0009-0004-3462-2083

Tereza Šalková, Matej Bel University in Banská Bystrica, Faculty of Law, Department of Criminal Law, Criminology, Forensic Science, and Forensic Disciplines, Komenského 20, 974 01, Banská Bystrica, Slovak Republic,
E-mail: salkova.tereza@gmail.com
ORCID: https://orcid.org/0009-0008-4336-0387

Corresponding author:

potravinárstvo®
OPEN ACCESS
Received: 20.9.2024
Revised: 30.10.2024
Accepted: 4.11.2024
Published: 4.11.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 964-976
https://doi.org/10.5219/2026
ISSN: 1337-0960 online
www.potravinarstvo.com

Influence of stage lactation on quality and protein compositions of Kazakh mare milk and koumiss

Togzhan Boranbayeva, Zhanna Dossimova, Dulat Zhalelov, Aruzhan Zhunisbek, Ayazhan Bolat, Aida Abzhaliyeva, Maxat Toishimanov

ABSTRACT

Limited studies have examined the effects of geography, climate, and lactation on mare's milk in Kazakhstan. The study aimed to assess the protein components and quality of mare's milk and koumiss from 24 mares in southern Kazakhstan. Milk samples were collected monthly between July and December 2023. The soluble protein fraction was analysed via SDS-PAGE. Casein fractions were examined using SDS-PAGE polyacrylamide electrophoresis. The results indicated the presence of α-, β-, and κ-caseins, along with whey proteins such as α-lactalbumin and β-lactoglobulin in the milk and fermented products. The milk contained 9.02% total solids, 1.62% protein and 1.22% casein on average. The mare milk fat content was 0.71% in Almaty, and in the Zhambyl region, it was significantly higher - 1.24%. The fermented koumiss products had a fat content of 1.22% in Almaty, while in Zhambyl, it was significantly higher at 1.94%. Similarly, the casein content in the Zhambyl region was 1.38%, compared to 0.81% in Almaty. The results indicate that different zones significantly affect mare's milk's fat and protein composition.

Keywords: mare milk, koumiss, protein profile, fat, electrophoresis, SDS-PAGE

INTRODUCTION

Mare's milk represents a highly esteemed alimentary product attributable to its chemical composition, which resembles human milk **[1]**, **[2]**. It is distinguished by its elevated biological value and digestibility, a characteristic with a longstanding historical precedent, particularly in the Central Asian regions where it has been ingested for centuries **[3]**, **[4]**. Recently, a global trend has emerged regarding the consumption of mare milk **[5]**. This increasing demand can be ascribed to its advantageous health properties **[6]**. Mare's milk is highly regarded for its nutritional value and distinctive attributes **[7]**. Furthermore, it is frequently consumed in its fermented variant, known as koumiss (or kumis). This beverage is characterised by its low alcohol content (below 5%) and slightly acidic profile derived from mare's milk **[8]**. Historically, this drink was predominantly consumed by nomadic populations. Traditionally, its production entails the fed-batch fermentation of raw milk **[9]**.

Mare's milk exhibits a markedly lower concentration of casein than cow's milk. Furthermore, including mare's milk in the dietary regimen of individuals afflicted with inflammatory bowel diseases has been shown to mitigate discomfort **[10]**, **[11]**. The therapeutic advantages associated with mare's milk within the realm of gastroenterology may be attributed to the presence of lactoferrin, lysozyme, and additional proteins that possess bactericidal characteristics and enhance the proliferation of bifidobacteria **[12]**, **[13]**. Notably, lactoferrin assumes a pivotal function in the attenuation of inflammation and the stimulation of humoral immunity **[14]**, **[15]**. Owing to its elevated whey protein composition, mare's milk, akin to human milk, is categorised as an albumin type **[16]**.

The lacteal secretion of the mare is often utilised in conventional medicinal practices to aid in mitigating gastrointestinal disorders and lactose intolerance, owing to its enzymatic components that promote the

hydrolysis of lactose **[17]**. Moreover, its immunomodulatory properties may confer therapeutic advantages for chronic conditions, infectious illnesses, and immunological deficiencies, as well as in the therapeutic management of various pathologies, including chronic hepatitis and tuberculosis **[18]**. An array of scholarly investigations suggests that mare's milk may exhibit anti-inflammatory characteristics and be advantageous in treating conditions such as eczema and other dermatological issues **[19]**.

This study aimed to determine Kazakh mare's milk's physicochemical characteristics and protein fractions during lactation.

Scientific Hypothesis

This study focused on changes in contents of physical and chemical quality contents of mare milk and fermented koumiss. In this study, an analysis was undertaken regarding the physicochemical characteristics of mare's milk and koumiss throughout the lactation period. Consequently, it was determined that the chemical compositions and whey protein of the milk and koumiss can be affected by several variables: breed, stage of lactation, and seasonal variations. Furthermore, in addition to these determinants, the composition of mare's milk, particularly the whey protein content, can be altered, notably concerning protein and fat concentrations.

MATERIAL AND METHODOLOGY

Samples

Mare's milk was obtained from 24 Kazakh mares of ages ranging from 4 to 7 years, which were housed at a horse dairy farm located in the Almaty (n=12) and Zhambyl (n=12) regions (Southern Kazakhstan). These mares had given birth between March and June. The milk collection took place from July to December. Over six months, each month 24 samples of mare's milk were collected from each mare at two farms in the Almaty and Zhambyl regions of Kazakhstan. Each sample was at least 500 ml. The milk was collected by hand milking after cleaning the udder and stored in sterilised plastic bottles. The milk products were frozen at -20°C post-harvest for further processing. Before use, samples were thawed overnight at 4°C, heated in a water bath at 37°C, under mixing and cooled at room temperature.

Instruments

FOSS Milkoscan FT1 (FOSS, Denmark), Electrophoresis (Mini-PROTEAN® Tetra Cell (Bio-Rad) USA)

Laboratory Methods

Mare milk quality analysis

The samples were analysed using the reference infrared absorption method on the FOSS Milkoscan FT1 equipment (Denmark) to determine their chemical composition. Each sample was placed in a 50 cm^3 laboratory beaker, heated to (40±2)°C, thoroughly mixed, and immediately measured. This quantitative instrumental express method was used to determine the mass fractions of milk protein, fat, moisture, dry matter, and casein content, utilising an infrared analyser and infrared spectroscopy. All analyses were conducted following ISO standards: ISO 9622 IDF 141: 201, ISO 8196 IDF 128-3:2009.

Determination of mare milk protein fraction by SDS-PAGE (Sodium dodecyl-sulfate polyacrylamide gel electrophoresis)

Acrylamide Solution 30%: Exactly 29 g of acrylamide (Sigma-Aldrich) and 1 g of bisacrylamide (Sigma-Aldrich) were dissolved in double distilled water, and the volume was 100 mL. It was stored at 4°C in the amber colour bottle. Electrode (running) buffer: Exactly 3.03 g of Tris base (Sigma-Aldrich), 1 g of SDS (Sigma-Aldrich) and 14.4 g of Glycine (Sigma-Aldrich) were added to water in 1000 mL. 10% SDS (Sigma-Aldrich) Solution: Exactly 10 g of SDS (Sigma-Aldrich) was dissolved in 75 mL double distilled water, and a volume made up to 100 mL. It was stored at room temperature in a plastic container till further use. 0.5M Tris (Sigma-Aldrich) HCl (standard 31118-77 RU) (pH6.8): Exactly 6.05g of Tris base (Sigma-Aldrich) was dissolved in 60 mL double distilled water, and pH was adjusted to 6.8 with concentrated HCl (standard 31118-77 RU). Volume was made to 100 mL and stored at 4ºC. 2M Tris (Sigma-Aldrich) HCl (standard 31118-77 RU (pH8.8): Exactly 24.2g of Tris base (Sigma-Aldrich) was dissolved in 80 mL double distilled water, and pH was adjusted to 8.8 with concentrated HCl (standard 31118-77 RU). Volume was made to 100 mL and stored at 4ºC. Laemmli buffer solution adds 1 ml of 1 % bromophenol blue (Sigma-Aldrich) to 4 ml of 1.5 M tris (Sigma-Aldrich) HCI (standard 31118-77 RU) pH=6.8 add 10 of glycerol (standard 6259-75 RU) and 2 g of SDS (Sigma-Aldrich) and 5 ml of β- mercaptoethanol (Sigma-Aldrich) mix and store at -20 °C. 10% W/V Ammonium Per Sulphate (standard 20478-75 RU) (APS): APS solution was always prepared fresh by dissolving 100 mg of APS (standard 20478-75 RU) in 1mL double distilled water. 2X Sample Buffer: The sample buffer was prepared by dissolving the substances given below, and the final volume was made to 20 mL with double distilled water and stored at 4 °C till further use.

Table 1 Composition of Sample buffer.

Sl No	Components	Resolving gel 15%	Stacking gel 6%
1	Distilled water (mL)	3.09	2.4
2	Acrylamide: 30% (mL)	6	0.75
3	Tris HCl: 0.5 M 6.8 pH (µl)	-	495 µl
4	Tris HCl: 2M, 8.8 pH, (mL)	2.7 ml	-
5	SDS:10% (µL)	75	42
6	APS:10% (µL)	75	38
7	TEMED (µL)	9	5

Staining Solution: Exactly 0.2 g of Coomassie Brilliant Blue – G250 250 (Sigma-Aldrich) (0.2%) was dissolved in a solvent mixture containing ethanol, acetic acid (Sigma-Aldrich) and water in the ratio of 5:1:5. The staining solution was filtered and stored at room temperature. Destaining Solution – 10% Acetic Acid (Sigma-Aldrich): Exactly 10 mL of glacial acetic acid (Sigma-Aldrich) was made up to 100 mL with double distilled water just before use.

Gel Preparation: The separating gel was 15%, and the stacking gel was 6%. All solutions were stored at 4°C. The electrophoresis was performed in Bio-Rad Mini-PROTEAN electrophoresis system gel electrophoresis unit. The gel mixtures were gently poured into the casting modules. After filling, the separating gel (8 cm deep) was carefully overlaid with a 1-2 mm deep layer of distilled water to allow a flat surface and protect the top of the gel mixture from atmospheric oxygen. After polymerisation, the distilled water was replaced by the spacer gel (2 cm deep). The stacking gel was added to about 3 cm deep, and soon after adding, the combs were inserted. After the polymerisation of the stacking gel, the comb was removed, and wells were rinsed with cathode buffer.

The molecular weight distribution of proteins was evaluated using protein electrophoresis following the Laemmli method **[20]**. Proteins were separated in a denaturing polyacrylamide gel (15% separating gel and 6% stacking gel) using SDS-PAGE. Electrophoresis was conducted in a single buffer system with SDS-PAGE at 15 mA. The gel was stained with 0.2% Coomassie R-250 dye (Sigma-Aldrich) (prepared with 10% glacial acetic acid) and washed thrice with distilled water. Before electrophoresis, milk and fermented products (mare's milk and kumiss) were incubated with sodium dodecyl sulfate to form negatively charged complexes with milk proteins. Additionally, treating milk proteins with mercaptan, which reduces disulfide bonds, resulted in the complete dissociation of protein complexes. The mobility of a protein in polyacrylamide gel is influenced by its molecular weight.

The gel was calibrated with protein markers consisting of seven highly purified recombinant proteins, ranging in molecular weights from 10 to 250 kDa Thermo Scientific. These markers form distinct bands after electrophoresis in polyacrylamide gel and subsequent dye fixation. The resulting data were then processed using standard statistical methods.

The Quantitative determination of protein was then performed using ImageJ and Imagelab software, which digitally processed the band images.

Description of the Experiment

Sample preparation: Samples were diluted by adding an equal volume of 2X sample buffer (1:1), heated at 100°C for 3 min, centrifuged, and immediately stored at 4°C. Wide range molecular weight standards from 10 kDa to 250 kDa Thermo Scientific were used as standards. Samples and standards were applied under the cathode buffer.

Run Conditions: Electrophoresis was performed at room temperature using constant voltage. Voltage was kept constant at 100V until the samples completely left the stacking gel, and in the separating gel, it was increased to 150V, and then voltage was maintained constant (100-150V) until the tracking dye reached the bottom of the gel. Staining with Coomassie Brilliant Blue R-250 (Sigma-Aldrich): Immediately after the end of electrophoresis, gels were removed from the plates, and the gel containing the marker and samples was cut and placed in a fixative solution containing 50% ethanol and 10% acetic acid (Sigma-Aldrich). After 30 minutes, the fixative solution was replaced by a staining solution containing 0.2% Coomassie Brilliant Blue R-250 (Sigma-Aldrich), where the gels were left for 30 min. After staining, the gels were transferred to the de-staining solute ion at room temperature. De-staining was done till the bands appeared and the background became clear.

Statistical Analysis

The influence of physical-chemical indicators on the milk was evaluated by one-way ANOVA followed by Tukey's HSD post hoc test for multiple comparisons when significant differences ($p < 0.05$) between the mean values were found. All statistical analyses were done using JMP 17 Pro (JMP Statistical Discovery LLC, Cary, NC, USA).

RESULTS AND DISCUSSION

Our task was to evaluate the chemical composition of mare's milk from different regions of Kazakhstan. To achieve this, we studied mare milk samples from various climatic regions, specifically Almaty and Zhambyl.

Our analysis revealed several distinctive features in the physicochemical parameters of mare's milk, which varied significantly based on the region, season, and animal husbandry conditions. It is well-known that milk production and quality are influenced by breed, lactation stage, age, feeding level, season, and animal care conditions.

Mare's milk can be consumed fresh and is particularly beneficial for young children, serving as an excellent substitute for mother's milk. However, preserving fresh mare's milk is challenging due to its high sugar content and lack of a fat film, which cause it to sour quickly.

Table 2 Chemical composition of Almaty and Zhambyl regions Kazakh mare milk during lactation.

	Almaty mare milk samples by month						p-values by regions
	July (n=12)	August (n=12)	September (n=12)	October (n=12)	November (n=12)	December (n=12)	
Fat, %	1.23±0.2	0.95±0.24	0.56±0.18	0.49±0.23	0.50±0.22	0.51±0.04	0.0011
Protein, %	1.73±0.11	1.69±0.13	1.63±0.11	1.67±0.13	1.59±0.15	1.65±0.03	0.0027
Casein, %	1.44±0.09	1.51±0.07	1.08±0.08	1.04±0.09	1.24±0.08	1.17±0.02	0.0019
SNF, %	8.99±0.14	8.06±0.11	9.45±0.21	8.72±0.88	8.78±0.84	8.74±0.03	0.0222
TS, %	10.21±0.18	9.22±0.23	8.72±0.17	8.71±0.71	8.75±0.7	8.64±0.02	0.0001

	Zhambyl mare milk samples by month					
	July (n=12)	August (n=12)	September (n=12)	October (n=12)	November (n=12)	December (n=12)
Fat, %	1.71±0.31	1.69±0.16	1.32±0.14	1.00±0.36	0.84±0.25	0.89±0.02
Protein, %	1.61±0.13	1.72±0.11	1.48±0.07	1.57±0.08	1.51±0.07	1.58±0.01
Casein, %	1.36±0.13	1.53±0.12	0.95±0.03	1.00±0.11	1.17±0.1	1.11±0.08
SNF, %	8.41±0.36	8.63±0.31	8.43±0.47	8.54±0.23	8.65±0.54	8.50±0.11
TS, %	10.57±0.47	9.24±0.33	9.02±0.51	8.79±0.48	8.24±0.66	8.15±0.05

Note: SNF – solid not fat, TS – total solid.

Table 3 Chemical composition of Almaty and Zhambyl regions koumiss during lactation.

	Almaty koumiss samples by month						p-values by regions
	July (n=12)	August (n=12)	September (n=12)	October (n=12)	November (n=12)	December (n=12)	
Fat, %	1.28±0.02	0.0011	1.17±0.01	1.18±0.42	1.21±0.17	1.14±0.02	0.0011
Protein, %	1.72±0.01	0.0027	1.50±0.02	1.65±0.06	1.64±0.03	1.34±0.02	0.0027
Casein, %	1.50±0.02	0.0019	0.43±0.03	0.62±0.03	0.64±0.08	0.38±0.04	0.0019
SNF, %	7.70±0.01	0.0222	9.31±0.05	7.45±0.21	8.09±0.06	8.2±0.03	0.0222
TS, %	8.58±0.04	0.0001	8.17±0.12	6.26±0.13	9.87±0.11	9.67±0.27	0.0001

	Zhambyl koumiss samples by month					
	July (n=12)	August (n=12)	September (n=12)	October (n=12)	November (n=12)	December (n=12)
Fat, %	2.02±0.02	2.01±0.01	1.99±0.01	1.78±0.05	1.89±0.02	1.69±0.41
Protein, %	1.71±0.02	1.72±0.02	1.12±0.01	1.61±0.02	1.58±0.01	0.72±0.02
Casein, %	1.46±0.05	1.51±0.04	1.02±0.01	1.65±0.11	1.27±0.01	1.23±0.05
SNF, %	7.07±0.13	7.28±0.07	7.70±0.01	10.77±0.03	8.58±0.08	8.51±0.11
TS, %	6.43±0.07	6.60±0.05	5.13±0.01	8.22±0.05	7.38±0.24	7.13±0.04

Note: SNF – solid not fat, TS – total solid.

When examining the dynamics of individual components in mare's milk during lactation, we observed changes in its chemical composition. Notably, the fat content initially increased and then slightly decreased, with the highest fat content occurring in the early months of lactation. Specifically, the fat content was 1.23% and 1.17% in the first months at both regions (p=0.0011), respectively, and decreased to 0.51% and 0.84% by the end of lactation, as shown in Table 2. The peak lactation period for mares is typically in the second to third months, after which milk yield gradually declines due to the physiological characteristics of horses. The fat content of koumiss in the Almaty region ranged from 1.14 to 1.35% during lactation (Table 3). Concurrently, the fat percentage in the Zhambyl region exhibited higher values, recorded between 1.69 and 2.02%. In comparing the two regions concerning the fat content in koumiss, it is evident that the steppe Zhambyl region demonstrates significantly elevated levels, particularly noted during the summer months when increased fat concentrations in koumiss. The mean fat content of mare milk (Table 2) was similar to that of mares' milk **[21]**, especially steppe region fat contents demonstrated reflecting the differences in pasture botanical diversity. We observed a significant regional variation in fat content. The present study revealed variations in fat content, which aligns with previous research findings **[22]**. Chen et al. reported high-fat content in koumiss in their research, similar to our findings, where fat content varied from 1.14% to 2.02% depending on the lactation period **[23]**.

The transformation of mare's milk into koumiss involves significant changes in its protein content and composition, influenced by fermentation. The protein content also varied month to month, influenced by the lactation period (p=0.0027). The protein content of mare's milk ranged from 1.51% to 1.73% (p=0.0019), with a high coefficient of variation in casein content (52.47%). The protein content in koumiss was similar to mare milk protein percentage during the lactation. The content showed statistically significant results for the region, where Mariani et al. also observed the same trend **[24]**. Other researchers have also reported a decrease in protein during this fermentation process **[25]**.

In the analysis of mare's milk, it was determined that, on average, the concentration of TS exhibited values of 10.57% and 10.21%, reflecting a notable quantitative presence of these components within the fluid. The measurement of TS revealed its lowest recorded percentages, specifically 8.75% and 8.24%, during the fifth month of lactation. In contrast, the peak concentrations were observed to occur in the 1th and 2th month of lactation, where they reached maximum values of 10.57% and 10.21%. Furthermore, it was noted that in the initial month of lactation, there were instances where the appearance of an average greater quantity of substances was recorded compared to the following lactation period. The substance of interest, SNF, presented values ranging from 8.99% to 8.78%. In the Almaty region, this percentage experienced a decline by the conclusion of the lactation period. Conversely, in the Zhambyl region, the SNF content increased, manifesting values between 8.41% and 8.65%. After the lactation period, the availability of pasture grasses diminishes as primary nutritional sources for lactating organisms, subsequently leading to a reduction in both SNF and TS **[26]**. Another study observed that the TS content of mare milk decreased from 12.5% to 10.2% at the end of lactation, which aligns with the results of our study **[27]**. Access to pasture significantly impacted the quality parameters profile in the milk of Kyrgyz mares. In contrast to our study, the climatic conditions in the Almaty region closely resemble those described by Mazhitova et al. **[28]**, with similar results in fat (2.20%) and dry matter (10.93%) indices.

The SDS-PAGE analysis of the whey protein fraction revealed the most abundant whey proteins, as shown in the representative results of Figure 1 and Figure 2. Electrophoresis in SDS-PAGE allowed us to clearly identify the heterogeneity of casein and detect fluctuations in casein fractions in the studied samples of milk and koumiss.

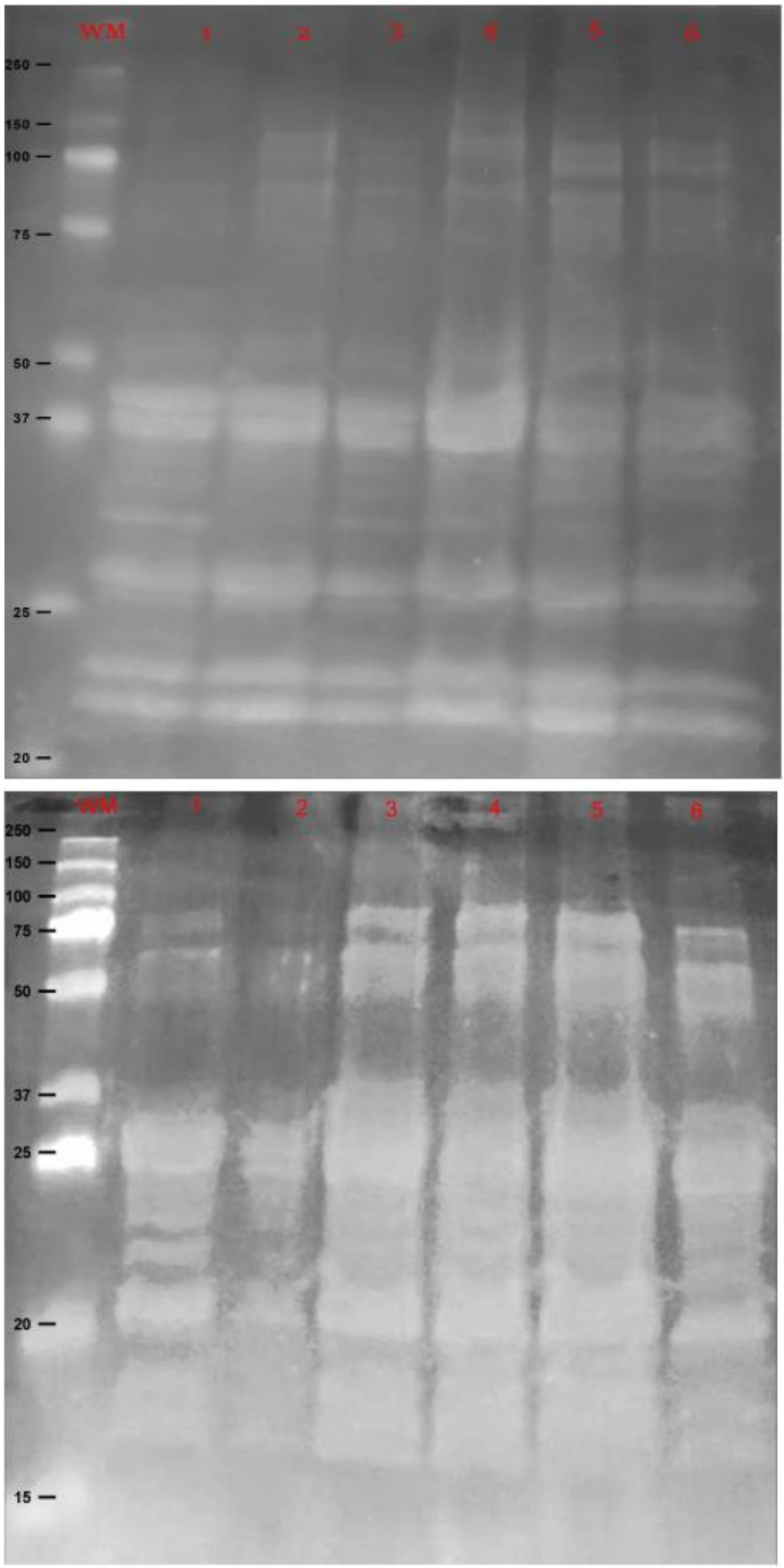


Figure 1 Electrogram mare's milk Zhambyl (upper) and Almaty (lower) region.
Note: Electrophoretic separation of mares' milk protein by month (July – December).

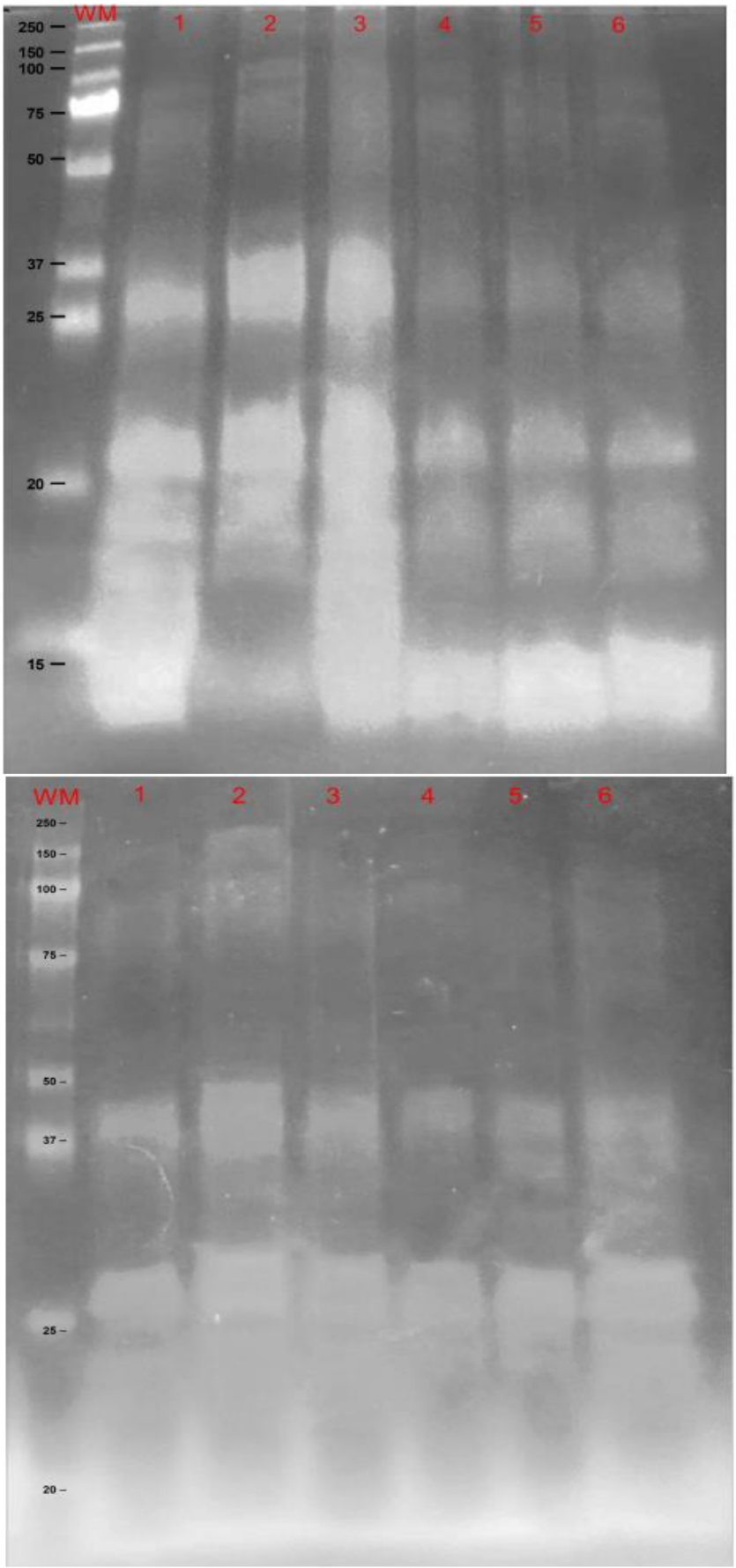


Figure 2 Electrogram koumiss Zhambyl (upper) and Almaty (lower) region.
Note: Electrophoretic separation of koumiss protein by month.

Electrophoresis results indicated that the casein content in mare's milk from the Zhambyl region increased slightly in July, August, and September but decreased in October, November, and December. Specifically, the amount of αS1-casein was 21.1 kDa in July, 21.0 kDa in August, 20.1 kDa in September, 19.9 kDa in October, 20.0 kDa in November, and 19.1 kDa in December. For β-casein, the values were 24.3 kDa in July, 24.2 kDa in August, 23.2 kDa in September, 23.8 kDa in October, 22.9 kDa in November, and 22.4 kDa in December. Additionally, the presence of lactalbumin and whey proteins was noted. The amount of β-lactoglobulin was 18.9 kDa in July, 18.7 kDa in August, 18.1 kDa in September, 18.0 kDa in October, 17.8 kDa in November, and 18.0 kDa in December. The α-lactoalbumin content was 14.5 kDa in July, 14.9 kDa in August, 14.2 kDa in September, 13.7 kDa in October, 13.0 kDa in November, and 12.8 kDa in December.

The electrogram illustrates the protein fractions of mare's milk from the Almaty region. The amount of αS1-casein was 20.0 kDa in July and August, 20.5 kDa in September, 21.6 kDa in October, 20.8 kDa in November, and 19.6 kDa in December. For β-casein, the values were 21.9 kDa in July, 22.2 kDa in August, 22.6 kDa in September, 23.4 kDa in October, and 22.9 kDa in November and December. The β-lactoglobulin content was 18.7 kDa in July, 18.2 kDa in August, 18.1 kDa in September, 18.5 kDa in October, 17.8 kDa in November, and 16.9 kDa in December.

Based on protein separation using SDS-PAGE, the most abundant proteins in mare milk samples were between 10 and 50 kDa. Our research results on protein fractions align with similar trends observed in studies by other researchers **[29]**, **[30]**. Our findings align with those of other researchers **[31]**, who reported molecular weights of 28 kDa and 22.9 kDa for α- and κ-casein, respectively. In our studies, we observed molecular weights of 27.6 kDa and 22.4 kDa, with an average casein weight of 21.0 kDa depending on the lactation period. Our results also indicate a tendency for decreasing casein content in Kazakh mare milk during lactation. Doval et al. reported that casein and whey proteins in mare milk are influenced by horse breed. Their study also indicated that these proteins remain fairly stable concerning factors such as mare care and lactation stage, although some proteins exhibited alterations **[32]**.

The electrophoresis results for koumiss from the Zhambyl region showed that the amount of αS1-casein was 20.0 kDa in July and August, 20.5 kDa in September, 21.6 kDa in October, 20.8 kDa in November, and 19.6 kDa in December. For β-casein, the values were 21.9 kDa in July, 22.2 kDa in August, 22.6 kDa in September, 23.4 kDa in October, 22.9 kDa in November, and 22.4 kDa in December. Denaturing electrophoresis in polyacrylamide gel with mercaptan allowed us to separate the casein fraction into αS1 and β-caseins, as well as the fractions of α-lactalbumin and β-lactoglobulin. The amount of β-lactoglobulin was 18.9 kDa in July, 18.7 kDa in August, 18.1 kDa in September, 18.0 kDa in October, 17.8 kDa in November, and 18.0 kDa in December. The α-lactalbumin content was 14.5 kDa in July, 14.9 kDa in August, 14.2 kDa in September, 13.7 kDa in October, 13.0 kDa in November, and 12.8 kDa in December.

Electrophoresis results for koumiss from the Almaty region showed that the amount of αS1-casein was 23.0 kDa in July, 24.8 kDa in August, 24.3 kDa in September, 24.9 kDa in October, 23.3 kDa in November, and 22.9 kDa in December. For β-casein, the values were 21.5 kDa in July, 21.9 kDa in August, 21.8 kDa in September, 21.7 kDa in October, 20.9 kDa in November, and 20.5 kDa in December. Additionally, lactalbumin and whey proteins were noted in mare's milk. Denaturing mercaptan-polyacrylamide gel electrophoresis allowed us to separate the casein fraction into αS1, αS2, β-, and κ-caseins, as well as the α-lactalbumin and β-lactoglobulin fractions. The amount of β-lactoglobulin was 16.0 kDa in July, 16.7 kDa in August, 16.5 kDa in September, 16.4 kDa in October and November, and 16.1 kDa in December. The α-lactalbumin content was 13.5 kDa in July, 14.3 kDa in August, 14.5 kDa in September, 14.6 kDa in October, 13.6 kDa in November, and 13.3 kDa in December.

The electrophoresis demonstrated elevated casein protein fractions in mare's milk compared to koumiss. Methodological advancements facilitated the separation of whey protein fractions, including α-lactalbumin and β-lactoglobulin. A 60 kDa band, indicative of whey protein, was characterised as immunoglobulins. The casein fraction, with a molecular weight of 27 to 30 kDa, exhibited different sensitivities in mare's milk **[33]**.

Ochirkhuyag et al. (2000) **[34]** reported the presence of caseins in mare milk. The observed percentage of 18 kDa β-lactoglobulin was significantly lower than in cow's milk, where 19 kDa β-lactoglobulin can constitute up to 17.5% and 16.6% of the total whey protein in mares, respectively, similar to findings by Schryver et al. (1986) **[35]**. The present study showed fractions of α-lactalbumin and β-lactoglobulin, 18.9 kDa and 14.5 kDa, respectively. Uniacke-Lowe et al. noted that the major whey proteins in equine milk are β-lactoglobulin and α-lactalbumin, which, similar to present research work, where β-lactoglobulin and α-lactalbumin fraction high profile showed in summer lactation period **[36]**. The results of other researchers reported that albumin contents increased during the lactation period **[37]**, **[38]**. β-lactoglobulin is a significant allergen in infants, whereas casein predominates in adult allergies **[39]**. Studies have suggested that goats **[40]**, mare **[41]**, donkeys **[42]**, and camel milk **[43]** can be suitable alternatives to human milk due to their hypoallergenic properties. However,

other studies indicate that cow milk may not always be a suitable alternative to breast milk, as they can cause allergies **[44]**, **[45]**. Fresh mares' milk and fermented koumiss have high nutritional value, whereas unripe koumiss has lower nutritional value with a shorter fermentation time **[46]**.

CONCLUSION

This study showed that the geographical conditions significantly affected mare milk and koumiss by quality parameters, especially protein, fat and TS. Kazakh mares were characterised by producing milk with the highest level of protein, casein. In the Almaty region, the molecular weight of αS1-casein and β-casein in mare caseins ranged from 20.0 to 19.6 kDa and 21.9 to 22.9 kDa, respectively. In the Zhambul region, the molecular weight of mare caseins ranged from 24,3 to 22,4 kDa and 21,1 to 19.1 kDa. In Almaty regions, the molecular weight of αS1-casein, and β-casein in mare caseins ranged from 23,0 to 22.9 kDa, 21.5 to 20.0 kDa. According to the study results, the number of cases in the Almaty region was less than in the Zhambyl region.

This study underscores the substantial influence of geographical and climatic factors on the quality characteristics of mare's milk and koumiss, notably affecting protein, fat, and total solids composition. Kazakh mares from different regions displayed significant variation in these parameters, with Zhambyl region samples generally exhibiting higher fat and protein content than those from Almaty. Such findings reinforce the importance of environmental conditions and pasture diversity, crucial in determining the nutritional profile of mare's milk and derived products.

The electrophoresis analysis further revealed distinct molecular weight patterns for casein and whey proteins, with differences evident between regions and throughout the lactation period. These protein profiles suggest the potential for optimising koumiss production based on regional characteristics, tailoring it to meet specific nutritional demands and health benefits. The differences in protein composition and molecular weight provide valuable insights for future research on therapeutic and dietary applications of mare's milk, especially in the context of functional foods and dietary supplements.

Given the findings, this study supports the continued exploration of mare milk products as a functional food with potential benefits for gastrointestinal health, immune support, and other therapeutic applications. The results advocate for more region-specific approaches to koumiss production, emphasising leveraging natural environmental factors to enhance product quality. Additionally, regulatory bodies and producers could consider establishing quality standards for mare milk and koumiss based on regional compositions, ensuring consistent quality while preserving the unique attributes associated with each area.

Future studies may expand upon this research by examining how other environmental factors, such as seasonal forage variations, impact mare milk composition. This could contribute to a more comprehensive understanding of how regional and seasonal conditions affect the bioactive compounds in mare's milk, potentially optimising its use in specialised nutrition and medicine.

REFERENCES

1. Uniacke-Lowe, T., & Fox, P. F. (2012). Equid Milk: Chemistry, Biochemistry and Processing. In Food Biochemistry and Food Processing (pp. 491–530). Wiley. https://doi.org/10.1002/9781118308035.ch26
2. Malacarne, M., Martuzzi, F., Summer, A., & Mariani, P. (2002). Protein and fat composition of mare's milk: some nutritional remarks with reference to human and cow's milk. International Dairy Journal (Vol. 12, Issue 11, pp. 869-877) Elsevier. https://doi.org/10.1016/S0958-6946(02)00120-6
3. Kondybayev, A., Loiseau, G., Achir, N., Mestres, C., & Konuspayeva, G. (2021). Fermented mare milk product (Qymyz, Koumiss). International Dairy Journal (Vol. 119, 105065). Elsevier. https://doi.org/10.1016/j.idairyj.2021.105065
4. Hirata, M., & Hirata, M. (2020). Milk Culture of Central Asia. *Milk Culture in Eurasia: Constructing a Hypothesis of Monogenesis–Bipolarization* (pp.149-196). Springer. https://doi.org/10.1007/978-981-15-1765-5_5
5. Pieszka, M., Łuszczyński, J., Zamachowska, M., Augustyn, R., Długosz, B., & Hędrzak, M. (2016). Is mare milk an appropriate food for people?–a review. Annals of Animal Science (Vol. 16, Issue 1, pp. 33-51). Walter de Gruyter. https://doi.org/10.1515/aoas-2015-0041
6. Jastrzębska, E., Wadas, E., Daszkiewicz, T., & Pietrzak-fiećko, e. N. A. T. A. (2017). Nutritional Value and Health-Promoting Properties of Mare's Milk-a Review. Czech Journal of Animal Science (Vol. 62, Issue 12). Czech Academy of Agricultural Sciences. https://doi.org/10.17221/61/2016-CJAS
7. Musaev, A., Sadykova, S., Anambayeva, A., Saizhanova, M., Balkanay, G., & Kolbaev, M. (2021). Mare's milk: composition, properties, and application in medicine. Archives of Razi Institute (Vol. 76, Isuue 4, pp. 1125). Razi Vaccine and Serum Research Institute. https://doi.org/10.22092%2Fari.2021.355834.1725

8. Sani, A. M., Rahbar, M., & Sheikhzadeh, M. (2019). Traditional beverages in different countries: Milk-based beverages. In Milk-based beverages (pp. 239-272). Woodhead Publishing. https://doi.org/10.3168/jds.2024-24695
9. Dong, J., Zhang, Y., & Zhang, H. (2015). Health properties of traditional fermented mongolian milk foods. Beneficial microorganisms in food and nutraceuticals (pp. 37-61). Springer. https://doi.org/10.1007/978-3-319-23177-8_2
10. Pecka, E., Dobrzański, Z., Zachwieja, A., Szulc, T., & Czyż, K. (2012). Studies of composition and major protein level in milk and colostrum of mares. Animal Science Journal (Vol. 83, Issue 2, pp. 162-168). Wiley. https://doi.org/10.1111/j.1740-0929.2011.00930.x
11. Potočnik, K., Gantner, V., Kuterovac, K., & Cividini, A. (2011). Mare's milk: composition and protein fraction in comparison with different milk species. Mljekarstvo: časopis za unaprjeđenje proizvodnje i prerade mlijeka. (Vol. 61, Issue 2, pp. 107-113). Repository of the Faculty of Agrobiotechnical Sciences Osijek. https://urn.nsk.hr/urn:nbn:hr:151:958192
12. Titisari, N., Widyaputri, T., Mahfuzah, R., & Widodo, E. (2020). Sumbawa mare milk as a preventive against inflammation in the gaster of inflammatory bowel disease (IBD) animal model. Adv. Anim. Vet. Sci. (Vol. 8, Issue 11, pp. 1170-1174). ResearchersLinks Ltd. http://dx.doi.org/10.17582/journal.aavs/2020/8.11.1170.1174
13. Fotschki, J., Szyc, A., & Wróblewska, B. (2015). Immunoreactivity of lactic acid-treated mare's milk after simulated digestion. Journal of Dairy Research. (Vol. 82, Issue 1, pp. 78-85). Cambridge university press. https://doi.org/10.1017/S0022029914000612
14. Kocyigit, E., Abdurakhmanov, R., & Kocyigit, B. F. (2024). Potential role of camel, mare milk, and their products in inflammatory rheumatic diseases. Rheumatology International. (Vol. 44, Issue 3, pp 425-434). Springer. https://doi.org/10.1007/s00296-023-05516-x
15. Romaniuk, K., Majszyk-Swiatek, M., Kryszak, K., Danielewicz, A., & Andraszek, K. (2019). Alternative use of mare milk. Folia Pomeranae Universitatis Technologiae Stetinensis. (Vol. 348, Issue 1, pp. 121-130). Agricultura, Alimentaria, Piscaria et Zootechnica. http://dx.doi.org/10.21005/AAPZ2019.49.1.13
16. Sheng, Q., & Fang, X. (2009). Bioactive components in mare milk. *Bioactive components in milk and dairy products*. Wiley-Blackwell. https://doi.org/10.1002/9780813821504
17. Cais-Sokolińska, D., Wójtowski, J., & Pikul, J. (2016). Lactose hydrolysis and lactase activity in fermented mixtures containing mare's, cow's, sheep's and goat's milk. International Journal of Food Science & Technology. (Vol 51, Issue 9, pp 2140-2148). Institute of Food Science & Technology. https://doi.org/10.1111/ijfs.13202
18. Kushugulova, A., Kozhakhmetov, S., Sattybayeva, R., Nurgozhina, A., Ziyat, A., Yadav, H., & Marotta, F. (2018). Mare's milk as a prospective functional product. Functional Foods in Health and Disease, (Vol. 8, Issue 11, pp 548-554). Academic Society for Functional Foods and Bioactive Compounds. https://doi.org/10.31989/ffhd.v8i11.528
19. Foekel, C., Schubert, R., Kaatz, M., Schmidt, I., Bauer, A., Hipler, U. C., ... & Jahreis, G. (2009). Dietetic effects of oral intervention with mare's milk on the Severity Scoring of Atopic Dermatitis, on faecal microbiota and on immunological parameters in patients with atopic dermatitis. International Journal of Food Sciences and Nutrition (Vol. 60, Issue 7, pp. 41-52). Taylor and Francis. https://doi.org/10.1080/09637480802249082
20. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Vol. 227, pp. 680-685). Springer Nature. https://doi.org/10.1038/227680a0
21. Minjigdorj, N., Baldorj, O., & Austbø, D. (2012). Chemical composition of Mongolian mare milk. Acta Agriculturae Scandinavica, Section A–Animal Science (Vol. 62, Issue 2, pp. 66-72). Taylor and Francis. https://doi.org/10.1080/09064702.2012.720999
22. Sun TianSong, S. T., Menhebilige, M., Wang JunGuo, W. J., Chen YongFu, C. Y., Zhao DianBo, Z. D., & Zhang HePing, Z. H. (2005). Analysis of chemical composition and microorganism flora of traditionally home-made koumiss in Xinjiang. China Dairy Industry (Vol. 33, No. 10, pp.9-13). National Dairy Information Center.
23. Chen, Y., Wang, Z., Chen, X., Liu, Y., Zhang, H., & Sun, T. (2010). Identification of angiotensin I-converting enzyme inhibitory peptides from koumiss, a traditional fermented mare's milk. Journal of dairy science (Vol. 93, Issue 3, pp. 884-892). https://doi.org/10.3168/jds.2009-2672
24. Mariani, P., Summer, A., Martuzzi, F., Formaggioni, P., Sabbioni, A., & Catalano, A. L. (2001). Physicochemical properties, gross composition, energy value and nitrogen fractions of Haflinger nursing mare milk throughout 6 lactation months. Animal Research (Vol. 50, Issue 5, pp 415-425). EDP Sciences. https://doi.org/10.1051/animres:2001140

25. Danova, S., Petrov, K., Pavlov, P., & Petrova, P. (2005). Isolation and characterization of Lactobacillus strains involved in koumiss fermentation. International Journal of Dairy Technology (Vol. 58, Issue 2, pp. 100-105). Wiley. https://doi.org/10.1111/j.1471-0307.2005.00194.x
26. Markiewicz-Kęszycka, M., Czyżak-Runowska, G., Wójtowski, J., Jóźwik, A., Pankiewicz, R., Łęska, B., ... & Bagnicka, E. (2015). Influence of stage of lactation and year season on composition of mares' colostrum and milk and method and time of storage on vitamin C content in mares' milk. Journal of the Science of Food and Agriculture (Vol. 95, Issue 11, pp. 2279-2286). Society of Chemical Industry. https://doi.org/10.1002/jsfa.6947
27. Pavelková, A., Tkáčová, J., & Kročko, M. (2022). The evaluation of Lipizzaner mare milk selected properties during the lactation period. Journal of microbilogy, biotechnology and food sciences (Issue 12, e9420). Faculty of Biotechnology and Food Sciences. https://doi.org/10.55251/jmbfs.9420
28. Mazhitova, A. T., & Kulmyrzaev, A. A. (2016). Determination of amino acid profile of mare milk produced in the highlands of the Kyrgyz Republic during the milking season. Journal of dairy science (Vol. 99, Issue 4, pp. 2480-2487). Elsevier. https://doi.org/10.3168/jds.2015-9717
29. Metwalli, A. M., & Al-Saleh, A. A. (2010). Susceptibility of camel, buffalo and cow milk caseins to proteolysis by different proteolytic enzymes. J. King Saud Univ. Agric. Sci. (Vol 22, pp. 59-69). Elsevier.
30. El-Salam, M. A., & El-Shibiny, S. (2013). Bioactive peptides of buffalo, camel, goat, sheep, mare, and yak milks and milk products. Food Reviews International (Vol. 29, Issue 1, pp. 1-23). Taylor and Francis. https://doi.org/10.1080/87559129.2012.692137
31. Yelubaeva, M. Y., Kenenbay, S. Y., Narmuratova, M. H., Serikbayeva, A. D. & Buralkhiev, B. A. (2017). Electrophoretic Identification of Casein in Various Types of Milk. OnLine Journal of Biological Sciences (Vol 17, Issue 4, pp. 348-352). Science Publications. https://doi.org/10.3844/ojbsci.2017.348.352
32. Blanco-Doval, A., Azkargorta, M., Iloro, I., Beaskoetxea, J., Elortza, F., Barron, L. J. R., & Aldai, N. (2024). Comparative proteomic analysis of the changes in mare milk associated with different lactation stages and management systems. Food Chemistry (Vol. 445, 138766). Elsevier. https://doi.org/10.1016/j.foodchem.2024.138766
33. Curadi, M. C., Orlandi, M., Greppi, G. F., Toppino, P. M., Barzaghi, S., & Cattaneo, T. M. P. (2000). Identification of protein fractions in mare's colostrum and milk. Milchwissenschaft (Vol. 55, Issue 8, pp. 446-449). Milk Science International.
34. Ochirkhuyag, B., Chobert, J. M., Dalgalarrondo, M., & Haertlé, T. (2000). Characterization of mare caseins. Identification of Identification of alfa$_{s1}$ and alfa$_{s2}$- caseins. Le Lait (Vol. 80, Issue 2, pp. 223-235). EDP Sciences. https://doi.org/10.1051/lait:2000121
35. Schryver, H. F., Oftedal, O. T., Williams, J., Soderholm, L. V., & Hintz, H. F. (1986). Lactation in the horse: the mineral composition of mare milk. The Journal of nutrition (Vol. 116, Issue 11, pp. 2142-2147). Elsevier. https://doi.org/10.1093/jn/116.11.2142
36. Uniacke-Lowe, T., Huppertz, T., & Fox, P. F. (2010). Equine milk proteins: chemistry, structure and nutritional significance. International Dairy Journal (Vol. 20, Issue 9, pp. 609-629). Elsevier. https://doi.org/10.1016/j.idairyj.2010.02.007
37. Pagliarini, E., Solaroli, G., & Peri, C. (1993). Chemical and physical characteristics of mare's milk. Italian Journal of Food Science (Vol. 5, No. 4, pp. 323-332). CABI.
38. Csapó, J., Csapó-Kiss, Z., Stefler, J., Martin, T. G., & Némethy, S. (1995). Influence of mastitis on D-amino acid content of milk. Journal of Dairy Science (Vol. 78, Issue 11, pp. 2375-2381). Elsevier. https://doi.org/10.3168/jds.S0022-0302(95)76865-5
39. Carroccio, A., Brusca, I., Mansueto, P., Soresi, M., D'Alcamo, A., Ambrosiano, G., ... & Di Fede, G. (2011). Fecal assays detect hypersensitivity to cow's milk protein and gluten in adults with irritable bowel syndrome. Clinical Gastroenterology and Hepatology (Vol. 9, Issue 11, pp. 965-971). AGA. https://doi.org/10.1016/j.cgh.2011.07.030
40. Lad, S. S., Aparnathi, K. D., Mehta, B., & Velpula, S. (2017). Goat milk in human nutrition and health–a review. Int. J. Curr. Microbiol. Appl. Sci. (Vol. 6, Issue 6, pp. 1781-92). National Academy of Agricultural Sciences. https://doi.org/10.20546/ijcmas.2017.605.194.
41. BARRETO, Í. M. L. G., RANGEL, A. H. D. N., Urbano, S. A., Bezerra, J. D. S., & OLIVEIRA, C. A. D. A. (2019). Equine milk and its potential use in the human diet. Food science and technology (Vol. 39, pp. 1-7). SciELO Brazil. https://doi.org/10.1590/fst.11218
42. Altomonte, I., Salari, F., Licitra, R., & Martini, M. (2019). Donkey and human milk: Insights into their compositional similarities. International Dairy Journal (Vol. 89, pp. 111-118). Elsevier, https://doi.org/10.1016/j.idairyj.2018.09.005

43. Kumar, D., Verma, A. K., Chatli, M. K., Singh, R., Kumar, P., Mehta, N., & Malav, O. P. (2016). Camel milk: Alternative milk for human consumption and its health benefits. Nutrition & Food Science (Vol. 46, Issue 2, pp. 217-227). Emerald insight. https://doi.org/10.1108/NFS-07-2015-0085

44. Lajnaf, R., Feki, S., Ameur, S. B., Attia, H., Kammoun, T., Ayadi, M. A., & Masmoudi, H. (2023). Cow's milk alternatives for children with cow's milk protein allergy-Review of health benefits and risks of allergic reaction. International Dairy Journal (Vol. 141, 105624). Elsevier. https://doi.org/10.1016/j.idairyj.2023.105624

45. El-Agamy, E. I. (2007). The challenge of cow milk protein allergy. Small Ruminant Research (Vol 68, Issue 1-2, pp. 64-72). Elsevier. https://doi.org/10.1016/j.smallrumres.2006.09.016

46. Liu, W., Wang, J., Zhang, J., Mi, Z., Gesudu, Q., & Sun, T. (2019). Dynamic evaluation of the nutritional composition of homemade koumiss from Inner Mongolia during the fermentation process. Journal of Food Processing and Preservation (Vol. *43,* Issue 8, e14022). Wiley. https://doi.org/10.1111/jfpp.14022

Funds:

This work supported by Ministry of Science and Higher Education of the Republic of Kazakhstan for 2023 – 2025: AP19579056 ‘‘Development of technology for obtaining therapeutic and preventive nutrition products based on mare's milk with immunomodulatory properties‘‘.

Acknowledgments:

We thank the Kazakh National Agrarian Research University (Almaty, Republic of Kazakhstan) for providing the research base.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Togzhan Boranbayeva, Kazakh national agrarian research university, Faculty of Engineering and Technology, Department of "Technology of Food Production and Food Safety", Abay str, 8, 050010, Almaty, Kazakhstan,
Tel.: +7702 169 7035,
E-mail: togzhan.boranbayeva@kaznaru.edu.kz
ORCID: https://orcid.org/0000-0002-1159-1200

Zhanna Dossimova, Kazakh national agrarian research university, Reference Laboratory of Dairy Products, Abay str, 8, 050010, Almaty, Kazakhstan,
Tel:+77074394182,
E-mail: janna_90.18@mail.ru
ORCID: https://orcid.org/0009-0006-6447-627X

Dulat Zhalelov, Kazakh national agrarian research university, Faculty of Engineering and Technology, Department of "Technology of Food Production and Food Safety", Abay str, 8, 050010, Almaty, Kazakhstan,
Tel:+7777 188 3689,
E-mail: zhalelov.dulat@kaznaru.edu.kz
ORCID: https://orcid.org/0000-0002-9688-2639

Aruzhan Zhunisbek, Kazakh national agrarian research university, Reference Laboratory of Dairy Products, Abay str, 8, 050010, Almaty, Kazakhstan,
Tel: +7 705 5336233,
E-mail: aruka01@inbox.ru
ORCID: https://orcid.org/0009-0002-8870-1153

Ayazhan Bolat, Kazakh national agrarian research university, Reference Laboratory of Dairy Products, Abay str, 8, 050010, Almaty, Kazakhstan,
Tel:+77087583796
E-mail: aya_030396@mail.ru
ORCID: https://orcid.org0000-0001-6263-9094

Aida Abzhaliyeva, Kazakh national agrarian research university, Faculty of Veterinary Science, Department of "Veterinary Sanitary Expertise", Abay str, 8, 050010, Almaty, Kazakhstan,
Tel:+77784099470,
E-mail: aidonpompi@mail.ru
ORCID: https://orcid.org/0000-0002-5462-8261

***Maxat Toishimanov**, Kazakh national agrarian research university, Kazakhstan-Japan innovation center, Food and environment safety laboratory, Abay str, 8, 050010, Almaty, Kazakhstan,
Tel:+77079193922,
E-mail: maxat.toishimanov@gmail.com
ORCID: https://orcid.org/0000-0002-6070-4574

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 10.9.2024
Revised: 30.10.2024
Accepted: 5.11.2024
Published: 6.11.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 977-992
https://doi.org/10.5219/2021
ISSN: 1337-0960 online
www.potravinarstvo.com

The effect of the carcass fat thickness on the qualitative technological and sensory attributes of beef

Olha Kruk, Anatolii Ugnivenko, Dmytro Nosevych, Oleksandr Natalich, Mykola Gruntkovskyi, Iryna Kharsika, Oleksandr Androshchuk, Inna Stetsiuk

ABSTRACT

The established correlations between subcutaneous fat thickness and the quality attributes of carcasses and beef are relevant for producers and the processing industry. The purpose of the study is to establish the characteristics of slaughter, chemical composition, sensory physical, and technological properties of beef made of young bulls belonging to the Ukrainian black-and-white dairy breed aged 18 to 24 months, depending on the thickness of the fat on the carcass. The colour of muscle and adipose tissue, the conformation of carcasses, the development of subcutaneous fat, marbling, chemical composition, and sensory attributes of beef and broth made of it were determined in the context of different fat thicknesses on the carcass. With an increase in the thickness of subcutaneous fat, the fleshiness (conformation) of carcasses increases by 55.2% (P>0.95), the cover of carcasses with fat increases by 43.5 (P>0.99), and muscle penetration improves by 45.8% (P>0.95). With the thickening of subcutaneous fat from 0.5 to 1.1 cm or more, there is a tendency of tendons and ligaments in carcasses to increase by 53.6%, with adipose tissue increasing by 25.6%, points for the juiciness of boiled beef increasing by 20.8%, its tenderness increasing by 12.5%, and the reduction in the m. longissimus dorsi 'loin eye' area increased by 7.3%, marbling increased by 19.0%, reduction of moisture content in meat increased by 27.8%, and its boiling properties increased by 7.9%. With an increase in the thickness of subcutaneous fat in beef, there was a tendency for the reduction of its acidity (pH), the amount of dry matter, the total content of fat and minerals, deterioration of taste, aroma, residue after chewing boiled meat, flavour, and aroma, concentration, and transparency of broth made of it. The practical significance of these studies is to obtain knowledge that allows the assessment of the quality characteristics of carcasses and beef by the thickness of subcutaneous fat for their further use by producers and processing industries.

Keywords: marbling, conformation of carcasses, muscle tissue, sensory attributes, subcutaneous fat

INTRODUCTION

In cattle, adipose tissue found under the skin is an important attribute of carcass quality, significantly affecting beef and the consumer's decision to purchase it, since visible fat is a significant (36%) factor when consumers choose **[1]**. Therefore, subcutaneous fat thickness was included in the Carcass Beef Grades and Standards (USDA, 2001) **[2]** Australia (MSA, 2015) **[3]**. They are used to classify, grade, and determine the quality of carcasses and describe their value for the meat industry **[4]**. Subcutaneous fat is considered **[5]** as both positive and negative beef quality criteria. It protects the carcass from losses during evaporation **[6]**, affecting the meat's tenderness **[7]**. Adipose tissue is a less wanted part of the carcass because it increases the animals' feed costs **[8]** and reduces the slaughter yield **[9]**.

Therefore, studies to determine the parameters of subcutaneous fat thickness in 18-24-month-old young bulls belonging to the Ukrainian black-and-white dairy breed, with which beef would have optimal slaughter,

physicotechnological, chemical, and tasting properties, are relevant. There would be a rationale for including them in Ukraine's regulatory documents for the classification of cattle carcasses.

In Ukraine, a significant share of beef is produced from animals of the Ukrainian black-and-white dairy breed (UBWDB). Specific aspects of quantitative and qualitative characteristics of meat made of them with different thicknesses of subcutaneous fat have not been studied. As a factor of differences in the taste of beef, it is a more important attribute of the quality of carcasses than conformation **[10]**. According to the MSA cattle carcass assessment system (2015) **[3]**, subcutaneous fat's minimum thickness is 3 mm. In each market, this value is to meet the consumer requirements. Increased thickness of fat under the skin (by 1 mm) reduces the cost of a kilogram of a carcass by 0.018 Australian dollars **[11]**, which is a key factor affecting the cost of beef. With increased subcutaneous fat thickness, carcass weight, fleshiness index, grade, and beef marbling deteriorate **[12]**. Subcutaneous fat does not affect meat's acidity (pH), colour, and boiling properties **[13]**. In young bulls with high productivity and meat quality, the subcutaneous fat thickness should be 8.0 mm **[14]**. The quality of beef depends on the fat content in the middle part of the muscles, which varies depending on the age and live weight of the animals and **[15]** their breed **[16]**.

Therefore, the purpose of the research was to evaluate the attributes of slaughter, chemical composition, sensory and physicotechnological properties of beef made the young bulls belonging to the Ukrainian black-and-white dairy breed in the period from 18 to 24 months of their age, depending on the thickness of subcutaneous adipose tissue, and to establish its optimal parameters, with which it would be possible to provide adequate meat and fat yields and significant technological and sensory attributes of edible beef for consumers.

Scientific Hypothesis

Previous research has shown that subcutaneous fat thickness is an important aspect of cattle carcasses. It affects the forecasting of the quality and quantity of beef, including its chemical composition, as consumers' cost and perception of meat during purchasing hinges on it. However, the relationship between the different thicknesses of subcutaneous fat and beef's physical, technological, chemical, and tasting characteristics in the Ukrainian black-and-white dairy breed animals may differ from the data obtained in previous studies on beef and beef and dairy cattle.

MATERIAL AND METHODOLOGY

Samples

The study in the Zhuravushka Farm, Brovary Raion, Kyiv Oblast involved 34 carcasses of 18-24-month-old young bulls of the Ukrainian black-and-white dairy (UBWD) breed. The animals were slaughtered in the slaughterhouse of the Zhuravushka Farm in the village of Kalynivka. The body weight of bulls was determined by weighing before slaughter after a 24-hour deprivation of food with free access to water. Fresh carcasses were sawn in half and weighed. The half-carcasses were cooled down and stored at +2°C for up to 24 hours. Next, they were cut into quarters between the 12th and 13th rib. On the cross-section of *m. longissimus dorsi* between the 12th and 13th rib, at the point where the half-carcass was divided into anterior and posterior parts, the length and depth of the 'loin eye' were measured according to the diagram shown in Figure 1, and the thickness of subcutaneous fat was measured with a ruler.

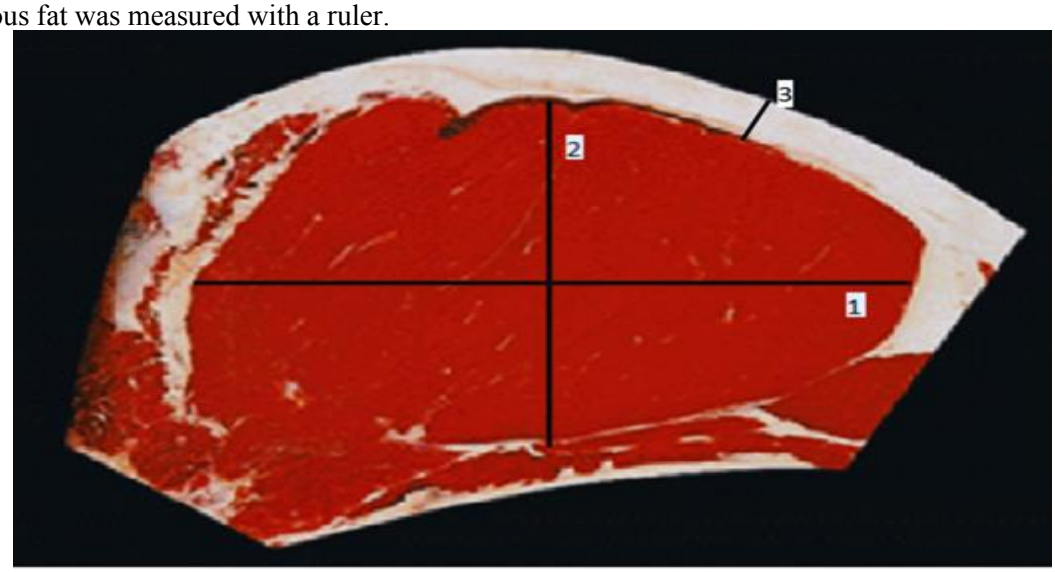


Figure 1 Length (1) and depth (2) of the 'loin eye' and measurement (3) of subcutaneous fat thickness.

The 'loin eye' area was calculated according to (formula 1) under the Order of the Ministry of Agrarian Policy and Food of Ukraine No. 290 of 6 August 2004 **[17]**:

$$S = a \times b \times 0{,}8 \quad (1)$$

Where: S is the area of the 'loin eye', cm^2; a is the length of the 'loin eye', cm;
b is the depth of the 'loin eye', cm; 0.8 is the coefficient.

On a scale of 1 to 7, according to the methods described in JMGA (2000) **[18]**, the color of the muscle (Figure 2) and fat (Figure 3) tissues was determined. According to the EUROP system (2008) **[19]**, the conformation of carcasses was visually classified into 5 classes (E, U, R, O, P) (Figure 4). For statistical analysis, we converted them into numbers on a scale of 1 (corresponds to P) to 5 (corresponds to E). The thickness of carcass fat was evaluated visually and broken down into five classes (from 1 = lean to 5 = very fat) (Figure 5). Marbling of meat was determined using a 12-point scale by the JMGA method (2000) **[18]** (Figure 6).

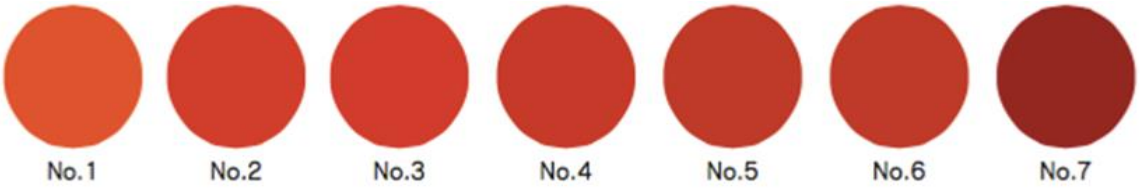


Figure 2 Muscle tissue color grading scale according to the method (JMGA, 2000) **[18]**.

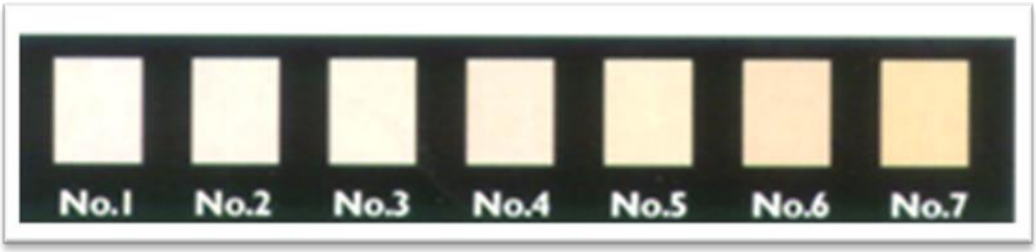


Figure 3 Adipose tissue color grading scale according to the method (JMGA, 2000) **[18]**.

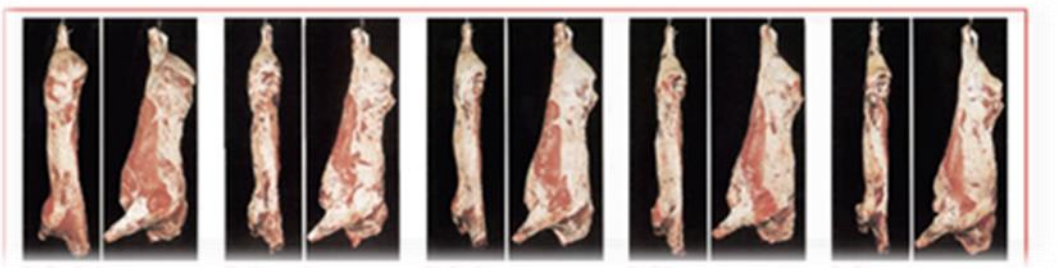


Figure 4 Carcass conformation grading scale according to the system (EUROP, 2008) **[19]**.

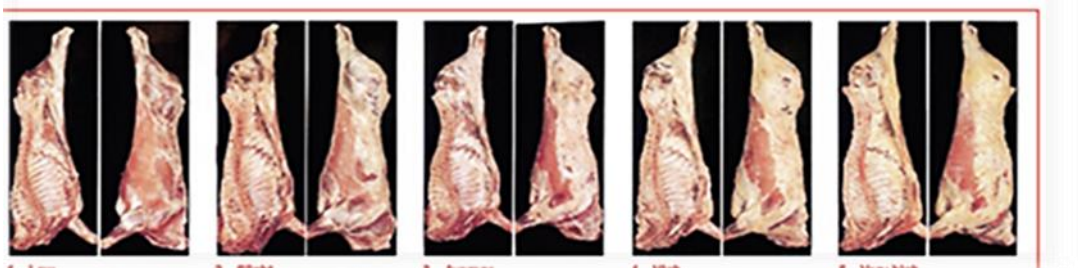


Figure 5 Carcass fat growth grading scale according to the EUROP system (2008) **[19]**.

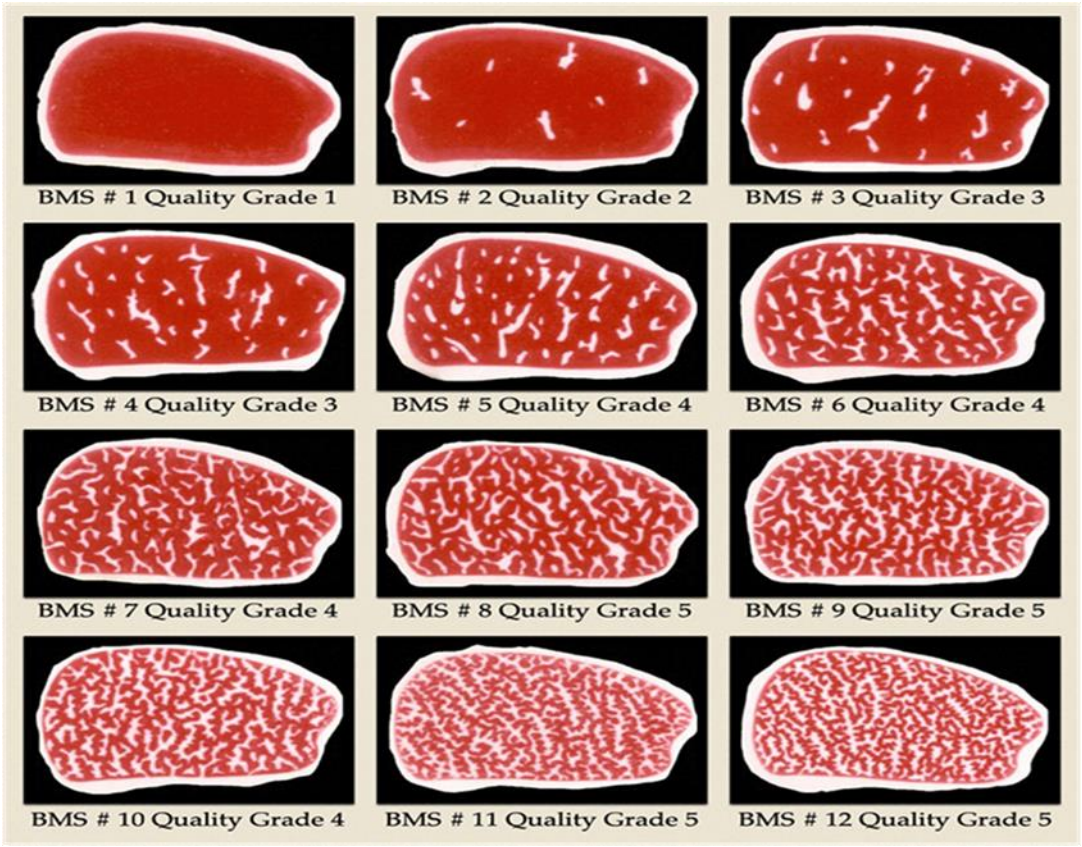


Figure 6 *M. longissimus dorsi* marbling grading scale according to the JMGA (2000) standard **[18]**.

Chemicals

Gel for ultrasound examination (Himlaborreactiv LLC, Ukraine).

Animals, Plants, and Biological Materials

Carcasses of young bulls belonging to the Ukrainian black-and-white dairy breed of Zhuravushka Farm, Brovary Raion, Kyiv Oblast.

Instruments

Static scales 4BDU-15X-P (Axis, Ukraine). Weight unit ≥0.5 kg, weighing range from 10 to 1,500 kg. Monthly weighing of young bulls and weighing before slaughter.

Gas chromatography (Kupol_55, Shimadzu Corporation, Japan).

Drying chamber (SNOL, Chemlaborreaktiv LLC, Ukraine).

Steam water distiller (Velp Scientific UDK 129, Italy).

Laboratory ionomer I-160M. Measurement of beef's pH.

Automatic penetrometer PMDP. Measurement of beef's penetration.

Ruler.

Laboratory Methods

The total fat content in beef was determined in the laboratory of the Department of Meat, Fish and Seafood Technologies of the National University of Life and Environmental Sciences of Ukraine (NULES of Ukraine) according to DSTU ISO 1443:2005 **[20]**; total ash content was determined according to DSTU ISO 936:2008 **[21]**; moisture content was determined according to DSTU ISO 1442:2005 **[22]**; and pH was determined according to DSTU ISO 2917-2001 **[23]**.

The moisture retention capacity of meat was studied using the content of bound water as a percentage of meat weight. The bound moisture content was determined by the 'press method' by the amount of water released from the meat under light pressing, and absorbed into the filter paper, forming a wet spot. The spot area's size depends on the meat's ability to retain water. The better the moisture retention capacity, the smaller the wet spot. Using a planimeter, the total area of the wet spot that forms under the compressed meat and the released moisture absorbed by the filter paper was defined. The area of the wet spot was defined by the difference between the total area of the spot and the area occupied by meat **[24]**.

The content of bound water (%) in meat was found using the formula (2) **[25]**:

$$B = \frac{(A - 8.4 \times b) \times 100}{M} \quad (2)$$

Where: B is the bound moisture content about the weight of meat, %;
A is the moisture content in the weighed average sample, mg;
b is the wet spot area, cm^2;
M is a weighted average meat sample, mg.

Eight tasters in the ' Meat Quality ' laboratory of the Department of Milk and Meat Production Technologies, NULES of Ukraine, evaluated the sensory attributes of boiled beef (aroma, juiciness, tenderness, ease of chewing) and broth made of it (color, taste, concentration) according to the requirements set out in the paper **[25]**.

Description of the Experiment

Sample preparation: Before slaughter, the animals were deprived of food for 24 hours with free access to water. The slaughtering was carried out by the European Regulation No. 1099/2009 of 24 September 2009 'On the Protection of Animals at the Time of Killing' **[26]**. After slaughtering young bulls, carcasses' conformation and subcutaneous adipose tissue development were visually examined. After 24-hour storage of carcasses at +2°C in the refrigerator, the thickness of subcutaneous fat, muscle marbling, length, and depth of the 'loin eye' were defined.

Number of samples analyzed: in the experiment, 34 left half-carcasses and *m. longissimus dorsi* samples were used for analysis.

Number of repeated analyses: all the studied attributes were determined in the samples selected for the study only once.

Number of experiment replications: Each study was carried out five times, and the number of samples was three, resulting in fifteen repeated analyses.

Design of the experiment: from birth to 4 months of age, 34 young bulls were kept in a group. Then, they had feed (produced on the farm) on the fattening site. After slaughtering, the degree of fat covering the carcasses was defined in young bulls, with the conformation (muscularity) of carcasses, the thickness of fat on the carcass, the colour of muscle and adipose tissue, and the marbling of *m. longissimus dorsi* defined. In determining these attributes, the carcasses were divided into three groups depending on the thickness of fat covering them (0.5-0.7 cm; 0.8-1.0 cm; 1.1 cm and more). In determining the chemical composition of beef and its technological and tasting properties, carcasses were divided into two groups 0.5-0.7 cm and 0.8-1.0 cm fat thickness. Experts conducted a tasting of boiled meat to study its sensory attributes. The degree of meat tenderness was defined by the ease with which teeth first penetrate the meat with which it breaks down during chewing, and the amount of residue left after it. Satisfaction with the tenderness of the meat was based on the interaction between its texture and the 'sensation in the mouth' during biting and chewing.

The juiciness of meat was defined as the perceived amount of juice and the level of lubrication when chewing. They determined the initial juiciness, which evaluates the liquid in the meat released during the first bite, associated with the water content. Besides, the persistent or general juiciness of beef during prolonged chewing was studied, and it was associated with the fat content, which stimulates saliva production in tasters. Muscle tissue samples were analysed according to sensory attributes (tenderness, juiciness, taste, residue after chewing). The same experts evaluated the nutritional quality of meat.

Statistical Analysis

Statistical analysis was used in the following aspects of the studies described above. Comparative analysis was performed: the quality of beef carcasses was compared between different groups of animals in the context of the thickness of fat under the skin.

Microsoft Exel 2016 was used to perform statistical analysis of the data obtained to determine the arithmetic mean (M), its error (±m), and the reliability criterion (td) between the groups.

The purposeful use of statistical analysis in this context was to collect objective data, analyze it to identify dependencies and patterns and support decision-making in beef production with maximum quality indicators and recommendations for further processing.

RESULTS AND DISCUSSION

Thicker fat on the carcass is the reason for the reduction in the slaughter yield, muscle tissue, in particular the first-grade muscle tissue, bone content, increase in adipose tissue, tendons and ligaments in the carcass, and the second-grade meat (Table 1). A decrease in the slaughter yield, the proportion of muscle tissue, and other attributes as specified in previous research papers associated with different thicknesses of fat on the carcass **[7]**, and its growth **[27]**, which mainly depends on related mating **[28]**, cattle breeds **[29]**, and fleshiness of animals **[30]**.

Table 1 Attributes of slaughter and morphological composition of young bull carcasses with different fat thicknesses on the carcass, M±m.

Attribute	Thickness of fat on the carcass, cm.		
	0.5 – 0.7 (n=19)	0.8 – 1.0 (n=9)	1.1 and more (n=6)
Body weight after the deprivation of food, kg	407±9.2	425±22.3	434±16.4
Slaughter output (carcass), %	45.9±0.15	45.6±0.11	45.5±0.91
Muscle tissue, %	71.1±0.80	70.6±0.74	70.8±1.21
including the top-grade meat, %	22.8±0.80	22.6±1.34	22.8±1.81
- //- first-grade meat, %	47.2±0.60	45.2±0.72	46.8±1.13
- //- second-grade meat, %	30.0±1.19	32.2±1.87	30.4±2.64
Adipose tissue, %	4.4±0.41	3.7±0.85	5.2±0.21
Tendons and ligaments, %	1.5±0.14	1.6±0.14	2.2±0.22
Bones, %	23.0±0.50	24.1±0.41	21.8±1.35

Table 2 shows the main qualitative attributes of carcasses, depending on the thickness of fat covering them. With an increase in subcutaneous fat from 0.5 – 0.7 to more than 1.1 cm, they improve by 43.5 (P>0.99), and the fleshiness (conformation) of carcasses increases by 55.2% (P>0.95) since animals have higher indicators with better growth of adipose tissue under the skin **[31]**.

Table 2 Qualitative attributes of young bull carcasses with different subcutaneous fat thickness, M±m.

Thickness of fat on the carcass, cm	Attributes					
	conformation of carcasses, points	growth of fat on the carcasses, points	'loin eye' area, cm^2	marbling of beef, points	muscle tissue color, points	adipose tissue color, points
From 0.5 to 0.7 (n=19)	2.9±0.97	2.3±0.11	83.7±4.79	5.0±0.85	5.1±0.13	4.6±0.14
From 0.8 to 1.0 (n=9)	3.7±0.25	2.6±0.26	83.0±9.73	7.1±0.74	5.4±0.18	4.9±0.12
1.1 and more (n=6)	4.5±0.37*	3.3±0.23**	78.0±4.49	4.2±1.34	5.3±0.46	5.0±0.49

Notes: *)P>0.95; **)P>0.99.

An increase in fat thickness from 1.1 cm and more is the reason for reducing the *m. longissimus dorsi* 'loin eye' area by 7.3%, directly correlating with the carcass weight and the amount of muscle tissue, including the top- and first-grade meat **[32]**. A decrease in the 'loin eye' area with the growth of adipose tissue under the skin confirms the data obtained in previous studies **[33]**. This confirms that with a larger subcutaneous fat thickness, the growth of *m. longissimus dorsi* decreases, meaning the number of valuable edible parts in the carcass also decreases. A decrease in the 'loin eye' area, the slaughter weight, and the amount of muscle tissue, including top- and first-grade meat with the growth of fat under the skin, can be explained by the fact that *m. longissimus dorsi* is located mainly in the thoracic and lumbar regions, which are the most valuable meat pieces, and their muscle tissue makes up a significant proportion of the carcass. However, there is data **[34]** that the 'loin eye' area increases with an increase in the fat thickness on the carcass from 3 to 25 mm and depends on the genotype of animals **[35]**. Due to the direct relationship between the 'loin eye' area and the output of valuable meat pieces in carcasses **[36]**, data on its value suggests **[37]** using it to forecast the amount of beef obtained and its belonging to a particular grade.

The beef marbling grade increases by 42.0%, with the thickness of adipose tissue on the carcass being in the 0.8 to 1.0 cm range. With its increase of more than 1.1 cm, the beef marbling decreases by 19.0%. The fact that the thickness of fat on the carcass does not directly correlate with the marbling of beef was also established in previous studies **[38]**. This means that it is impossible to obtain good sensory attributes of beef with thicker subcutaneous fat. Marbling is the main factor determining the sensory quality of meat **[39]**, it has a positive effect on its taste **[40]** and tenderness **[41]**.

With an increase in fat thickness on the carcass, the muscle tissue colour grade increases since its lower content is associated with a decrease in the discolouration of beef **[42]**. Deeper muscle tissue colour affects the consumer's choice **[43]**. Therefore, it is used to indicate meat freshness **[44]**. The muscle tissue colour depends on the chemical form of myoglobin **[45]**. Fresh beef contains deoxymyoglobin, which renders it red pigmentation. In the presence of oxygen, it is oxidised to oxymyoglobin. This results in a bright pink-red meat colour. When deoxymyoglobin is oxidised to methemoglobin, beef acquires a brownish shade. The beef colour improves with an increase in adipose tissue thickness on the carcass. Similar data was obtained in previous research, too **[34]**.

The results of the defined penetration strain show that with the fat thickness on the carcass ranging from 0.5 to 0.7 cm, the needle of the PMDP device penetrates the beef sample in 180 seconds ($P>0.95$) to a lower depth (by 45.8%), which speaks for its toughness, than with a larger (0.7-1.0 cm) fat thickness value (Table 3).

Table 3 Technological attributes of beef with a different fat thickness on the carcass, M±m.

Thickness of fat on the carcass, cm	Attributes		
	water binding capacity, %	boiling properties, %	penetration, mm
from 0.5 to 0.7 (n=11)	62.1±2.99	38.1±1.95	20.7±1.78
from 0.8 to 1.0 (n=4)	48.6±6.92	35.3±2.35	14.2±1.79*

Note: *)P>0.95.

This can be explained by the fact that the higher subcutaneous fat content protects the carcass kept in the refrigerator from drying out and losing its moisture, which ensures the juiciness of the finished product **[7]** associated with a reduction in the destruction and deformation of muscle fibers **[46]**. In young bulls, fat significantly affects the texture of meat **[47];** therefore, to prevent the contracting of muscle fibres, which makes the boiled beef tough, its thickness under the skin should be at least 5.00 mm. **[48]**. With a small amount of subcutaneous fat in the muscles, the temperature quickly decreases, and the fibers contract due to a decrease in the glycolysis process **[49]**. This is typical for more tough and dehydrated meat.

To a certain extent, subcutaneous adipose tissue retains water in the meat and binds it during processing. Therefore, with an increase in the thickness of subcutaneous fat, the water retention capacity reduces by 13.5 points. Retaining water in fresh meat is associated with its suitability for storage and better yield after cooking **[50]**. According to our data, a larger fat thickness on the carcass helps reduce the amount of moisture in boiled beef by 2.8 points. Other researchers have confirmed the established properties **[51]**.

Meat losses during water draining negatively affect the weight of carcasses and meat pieces and the yield and quality of processed products **[52]**. Due to beef's water loss during heating, the proteins become less elastic and tougher. The penetration strain increases because the product becomes tough **[53]**. The deterioration of the technological properties of muscle tissue with a smaller subcutaneous fat thickness can also be explained by the fact that collagen fibrils contract during heating. Denaturation of muscle proteins occurs at different temperatures.

Therefore, reducing the boiling time for beef means improving its culinary and technological properties. It is an important aspect that indicates the economic value of meat since its higher indicators increase waste during cooking. There is a suggestion to improve the water binding capacity and structural and mechanical properties of meat by grinding it fine **[54]**, by using a starter culture based on a combination of *Staphylococcus carnosus*, *L. plantarum*, *L. rhamnosus*, and *L. paracasei* (SC_2) **[55]**, centrifugal mixing of components of a multifunctional protein additive made from whey, sodium alginate, and soy fiber **[56]**. It is possible to improve the quality of meat and extend its term of preservation by treating carcasses with microorganisms of *Leuconostoc carnosum* and *Lactobacillus sakei* strains **[57]**.

The total fat content of beef reduces with an increase in the thickness of adipose tissue under the skin (Table 4).

Table 4 Chemical composition of beef with different thicknesses of fat on the carcass, M±m.

Thickness of fat on the carcass, cm	Attributes					
	acidity (pH)	moisture content, %	dry matter, %	protein, %	total fat content, %	total ash content, %
from 0.5 to 0.7 (n=11)	5.89±0.131	69.8±1.26	30.7±1.38	20.9±0.85	7.6±0.48	2.1±0.34
from 0.8 to 1.0 (n=4)	5.73±0.114	74.3±1.88	25.6±1.88	20.1±0.75	3.6±1.18	1.9±0.60

This contradicts data obtained during previous studies **[38]**. According to it, there is an apparent relationship between these attributes. Beef tends to increase in moisture content with an increase in subcutaneous fat thickness, which is explained by better protection of carcasses from crusting and losses.

In groups of carcasses with different adipose tissue thicknesses, the pH level was defined, which was compared with the classification scale (normal pH ≤5.8; atypical pH >5.8 but <6; typical DFD pH ≥6), used in **[58]**. In the range from 8 to 10 mm, the thickness of subcutaneous fat results in the normalisation of the PH (5.73) of beef, and with its smaller value, atypical acidity occurs (pH=5.89). Atypical beef has a higher level of methemoglobin **[46]**, which negatively affects meat penetration (see Table 3). With thicker subcutaneous fat and lower pH in muscle tissue, the glycolysis process occurs more intensively, with lactic acid developed. Due to this, it remains microbiologically stable.

With a smaller adipose tissue thickness on the carcass, the protein content in *m. longissimus dorsi* increased by 4.0%. With a lower degree of fatness in young bulls, the content of proteins associated with catabolic processes (glycolysis), muscle structure, and contraction increases (P≤0.05). With a higher degree of fatness, it is associated with energy metabolism **[59]**. In young bulls, with a larger thickness of adipose tissue, meat formed on the carcass with lower indicators of total ash content. Therefore, the evaluation of carcasses of young bulls belonging to the Ukrainian black-and-white dairy breed at the age of 18 to 24 months by subcutaneous fat thickness does not allow producers to forecast the chemical composition of beef depending on its size before slaughter.

The conducted tasting of boiled beef showed that with an increase in fat thickness on the carcass, there was a tendency to increase (by 12.5%) the main component of sensory evaluation of beef, that is, its tenderness (Table 5). It is affected by the amount and type of fat present in meat **[60]**, which softens the fibers and minimises their destruction **[42]**. Positive changes in experts' perception of beef tenderness are caused by the fat thickness on the carcass exceeding 6 mm. **[61]**.

Table 5 Sensory properties of boiled beef with different fat thicknesses on the carcass, M±m.

Thickness of fat on the carcass, cm	Sensory properties of cooked meat, points				
	juiciness	taste	aroma	tenderness	residue after chewing
0.5 to 0.7 (n=9)	2.4±0.10	2.6±0.13	3.1±0.15	2.4±0.07	3.3±0.16
from 0.8 to 1.0 (n=4)	2.9±0.30	2.4±0.41	2.7±0.28	2.7±0.50	2.9±0.31

With an increase in subcutaneous fat thickness, there is also a tendency to increase the rating of beef by 20.8% according to an important feature: juiciness, which affects its nutritional quality. Fat that lubricates muscles between fibres increases the degree of tenderness and juiciness of the meat. Juiciness has a positive effect on the quality of beef consumption. In the mouth, it is characterised by the amount of juice during chewing **[62]**, which closely correlates (r= 0.67) with the fat content in the middle of the muscle **[63]**. With a higher subcutaneous fat thickness, there is a tendency to reduce the values of such sensory attributes of beef as taste, aroma and residue after chewing. The taste of beef is affected by marbled fat, which contains more oleic acid and less stearic acid than subcutaneous fat. **[64]**.

Fat is also a source of aromatic compounds, which is important for meat quality. The aroma and taste of beef appear due to the reaction of non-volatile fatty acids during heat treatment and the type and concentration of volatile substances being released **[65]**. Thus, an increase in subcutaneous fat thickness leads to a decrease in the total fat content in the middle of the muscles and a deterioration in the estimated values of attributes of cooked meat aroma, taste, and residue after chewing.

After tasting boiled beef broth, the indicators were slightly higher graded, and the fat thickness on the carcass was smaller (from 0.5 to 0.7 cm) (Table 6).

Table 6 Sensory attributes of boiled beef broth with different fat thicknesses on the carcass, M±m.

Thickness of fat on the carcass, cm	Attributes of broth tasting, points		
	taste and aroma	concentration	transparency
0.5 to 0.7 (n=9)	3.1±0.13	3.1±0.23	3.0±0.25
from 0.8 to 1.0 (n=4)	2.9±0.14	2.8±0.33	2.4±0.24

With an increase in subcutaneous fat thickness, the broth concentration lowers since, with a decrease in the total fat content in the muscles, it diffuses from the endomysium and peremysium cells into boiled water to a lesser extent. Reduced dry matter, protein, and total ash content in meat (see Table 4) means less protein, extractive matter, and mineral salts enter the broth.

So, the results obtained show that with an increase in the thickness of subcutaneous fat in the young bulls of the Ukrainian black-and-white dairy breed aged 18 to 24 months, there is an apparent improvement in the conformation (fleshiness) of carcasses, the growth of adipose tissue and the penetration of meat, and a tendency to improving its tenderness and juiciness. The qualitative feature of carcasses – the thickness of subcutaneous fat – does not significantly affect the slaughter yield or the weight of muscle tissue, including the top- and first-grade meat, which correlate with the *m. longissimus dorsi* 'loin eye' area **[66]**. With an increase in subcutaneous fat, the chemical composition of muscle tissue tends to worsen. Besides, the larger thickness of adipose tissue under the skin does not affect the sensory attributes of boiled beef broth. With a larger subcutaneous fat thickness, muscle tissue's pH decreases due to glycolysis. For 18-24-month-old young bulls belonging to the Ukrainian black-and-white dairy breed, the optimal adipose tissue thickness on the carcass should range from 8 to 10 mm.

In this study, various statistical analysis methods were used to study the quality of beef carcasses, particularly the characteristics of the "muscle eye" zone. The main aspects of the analysis included the following:

Comparative analysis: The quality of beef carcasses from different groups of animals was compared. In particular, the "muscle eye" zone was evaluated according to parameters, including the average size and structure of muscle fibres. This approach made it possible to determine the difference between groups based on objective meat quality characteristics.

Correlation analysis: relationships between quality indicators of beef carcasses and parameters of the "muscle eye" zone were identified and analysed. In particular, the dependence of such characteristics as muscle mass, fat content, and moisture was investigated, which helped determine the relationships between physicochemical parameters and structural features of muscle tissue.

Analysis of changes over time: the dynamics of changes in the quality of beef carcasses and indicators of the "muscle eye" zone over time were studied. This study involved evaluating the changes that occur under the influence of various factors, such as adjusting the diet or increasing the level of physical activity of the animals. Such studies have helped to identify long-term trends and dependencies affecting meat quality.

Factor analysis: The study of the "muscle eye" zone determined the main factors significantly impacting the quality of the beef carcass. This analysis helped us better understand which parameters have the greatest influence on the structure and quality of meat and establish their importance to produce high-quality products.

Analysis of variance (ANOVA): was used to assess differences between groups of animals concerning their carcass quality and muscle eye characteristics. This method made it possible to establish which factors significantly impact beef meat quality accurately. 6. Regression analysis: was used to assess the influence of individual variables on beef carcass quality, for example, the dependence of meat quality on nutritional parameters, animal breed, or level of physical activity. Regression models helped to identify the main variables influencing product structure and quality.

Cluster analysis was used to group the studied carcasses based on similarities in the quality parameters of the muscle eye. This made it possible to single out groups of carcasses with similar quality characteristics and to identify optimal parameters for creating a high-quality product.

Principal component analysis (PCA): was used to reduce the dimensionality of the data and simplify the interpretation of the results, allowing the identification of the main factors affecting beef quality. This method made it easier to highlight the most important indicators determining carcasses' quality. The complex use of various statistical analysis methods made it possible to obtain objective and comprehensive data on the quality of beef carcasses, particularly the "muscle eye" zone.

The comparative, correlational, factorial, dispersion, and regression analyses conducted revealed significant regularities and interrelationships between carcass quality indicators and numerous factors, such as animal nutrition and physical activity. Thanks to this study, the key factors influencing the quality of beef meat were

identified, and the obtained results can serve as a basis for making decisions in the field of production optimisation to ensure high-quality standards.

Although the increased content of beef fat under the skin protects the muscles from dehydration in the refrigerator **[7]**, it has a low nutritional value, so it is considered **[67]** waste. To make human nutrition healthy, **[68]** options are developed to replace fatty raw materials with dietary ones. Health-improving properties of meat are boosted with rosemary extract **[69]**, iodine compounds **[70]**, textured wheat protein **[71]**, by grinding **[72]**, with sea salt and betanin dye made of beet juice **[73]**. Beef fat is used to develop **[74]** diesel fuel production technologies.

Beef producers and processors are focused on the quality of carcasses, determined by the thickness of adipose tissue the price in the market depends on. The thickness of subcutaneous fat, which determines the degree of fatness of cattle in Ukraine, does not positively affect the sensory and culinary attributes of beef required by consumers **[75]**. The tenderness, taste, and juiciness of meat depend **[76]** on the adipose tissue content in the middle of the muscles. Young bulls of the Ukrainian black-and-white dairy breed aged 18 to 24 months with a thickness of adipose tissue on the carcass ranging from 8 to 10 mm have a fat content in the middle of the muscles making up 3.6% only. Since the quality characteristics of carcasses compared with the quality of beef are more sensitive to changes in the management of ruminant breeding, it is possible to achieve their optimal combination under different feeding conditions **[41]** of cattle.

In this regard, in Ukraine, there is an issue raised of solving the problem of beef production, which should combine the quality of beef and carcass. In the future, researchers should focus on establishing the optimal thickness of subcutaneous fat in cattle of other breeds, as this will reduce the amount of waste from carcasses and improve their visual and sensory quality. It is necessary to research to determine the quantitative and qualitative attributes of beef and establish factors for managing the breeding of cattle of common Ukrainian breeds, to reach a compromise between the thickness of subcutaneous fat and technological and sensory attributes and chemical composition of meat.

Further scientific research is needed to study the relationship between the thickness of adipose tissue on the carcass and the quantitative and qualitative characteristics of beef and to establish factors for managing the breeding of cattle of common Ukrainian breeds to reach a compromise between the thickness of fat on the carcass and the technological and sensory characteristics of meat and its chemical composition.

CONCLUSION

With an increase in the thickness of subcutaneous fat, the fleshiness (conformation) of carcasses significantly improves by 55.2% (P>0.95), the growth of adipose tissue increases by 43.5% (P>0.99), the penetration of meat increases by 45.8% (P>0.95), the content of adipose tissue, tendons, ligaments, and bones increases, and the juiciness and tenderness of boiled meat graded increases.

With an increase in the thickness of fat on the carcass, there is a reduction in the slaughter yield (of a carcass), muscle tissue of the top- and first-grade meat, the *m. longissimus dorsi* 'loin eye' area, marbling, moisture retention capacity, boiling properties and acidity (pH) of beef, the amount of dry matter, the total content of fat, ash, and protein, the taste and aroma of boiled beef, the concentration and transparency of broth.

The optimal thickness of subcutaneous adipose tissue in young bulls of the Ukrainian black-and-white dairy breed aged 18 to 24 months should be 8 to 10 mm.

In the future, it is advisable to research the relationship between the thickness of adipose tissue on the carcass and the quantitative and qualitative characteristics of beef and establish factors for managing the breeding of cattle of common Ukrainian breeds to reach a compromise between the thickness of fat on the carcass and the technological and sensory characteristics of meat and its chemical composition.

REFERENCES

1. Rondoni, A., & Grasso, S. (2021). Consumers behavior towards carbon footprint labels on food: A review of the literature and discussion of industry implications. In Journal of Cleaner Production (Vol. 301, p. 127031). Elsevier BV. https://doi.org/10.1016/j.jclepro.2021.127031
2. Realini C. E., Kallas Z., Pérez-Juan M., Gómez I., Olleta J. L., Beriain M. J., Albertí P., Sañudo C. (2014). The relative importance of cues underlying Spanish consumers' beef choice and segmentation, and consumer liking of beef enriched with n-3 and CLA fatty acids. In Food Quality and Preference. (Vol. 33. pp. 74–85). Elsevier BV. https://doi.org/10.1016/j.foodqual.2013.11.007
3. USDA. (2001) United States Standards for Grades of Feeder Cattle.
4. Meat Standards Australia (MSA). 2015. Available at: https://www.mla.com.au/globalassets/mla-corporate/marketing-beef-and-lamb/documents/meat-standards-australia/msa-2015-16-annual-outcomes-report.pdf

5. Brito, G., Soares de Lima, J. M., Del Campo, M., Luzardo, S., Correa, D., & Montossi, F. (2024). The implementation of grading systems for beef carcass value differentiation: the Uruguayan experience. In Animal Frontiers. (Vol. 14, Issue 2, pp. 29–34). National Institutes of Health. https://doi.org/10.1093/af/vfae004
6. Hocquette, J. F., Gondret, F., Baéza, E., Médale, F., Jurie, C., & Pethick, D. W. (2010). Intramuscular fat content in meat-producing animals: development, genetic and nutritional control, and identification of putative markers. In Animal. (Vol 4, Issue 2, pp. 303–319). National Institutes of Health. https://doi.org/10.1017/S1751731109991091
7. da Silva Bernardo, A. P., Ribeiro, F. A., Calkins, C. R., & Pflanzer, S. B. (2020). Bone and subcutaneous fat influence on yield, physicochemical traits, and color stability of dry-aged loin from grass-fed nellore bulls. In Meat and Muscle Biology. (Vol. 4, Issue 1, pp. 1–8). Iowa State University Digital Press. https://doi.org/10.22175/mmb.11141.
8. Malheiros, J. M., Baldassini, W. A., Dias, V. A. D., Silva, J. A. I. V., Curi, R. A., & Chardulo, L. A. L. (2015). Chemical and sensory meat characteristics of Nellore cattle (Bos indicus) finished with different levels of backfat thickness in the longissimus thoracis muscle. In Boletim da Indústria Animal Nova Odessa. (Vol 72, Issue 4, pp. 341–348). Boletim de Indústria Animal. http://dx.doi.org/10.17523/bia.v72n4p3411
9. Ugnivenko, A., Nosevych, D., Antoniuk, T., Chumachenko, I., Ivaniuta, A., Slobodyanyuk, N., Kryzhova, Y., Rozbytska, T., Gruntovskyi, M., & Marchyshyna, Y. (2022). Manifestation of living and post-slaughter traits of productivity in inbred and outbred bull calves of Ukrainian meat cattle breed. In Potravinarstvo Slovak Journal of Food Sciences. (Vol 16, pp. 356–366). HACCP Consulting. https://doi.org/10.5219/1769
10. Ju, M. S., Jo, Y. H., Kim, Y. R., Ghassemi Nejad, J., Lee, J. G., & Lee, H. G. (2024). Supplementation of complex natural feed additive containing (C. militaris, probiotics and red ginseng by-product) on rumen-fermentation, growth performance and carcass characteristics in Korean native steers. In Frontiers in Veterinary Science. (Vol. 10, p. 1300518). National Institutes of Health https://doi.org/10.3389/fvets.2023.1300518
11. Riley, R. R., Savell, J. W., Murphey, C. E., Smith, G. C., Stiffler, D. M., & Cross, H. R. (1983). Effects of electrical stimulation, subcutaneous fat thickness and masculinity traits on palatability of beef from young bull carcasses. In Journal of Animal Science. (Vol. 56, Issue 3, pp. 584–591). Oxford Academic. https://doi.org/10.2527/jas1983.563584x
12. Polkinghorne, R., Philpott, J., Gee, A., Doljanin, A., & Innes, J. (2008). Development of a commercial system to apply the Meat Standards Australia grading model to optimise the return on eating quality in a beef supply chain. In Australian Journal of Experimental Agriculture. (Vol. 48, Issue 11, pp. 1451–1458). CSIRO PUBLISHING. https://doi.org/10.1071/EA05181
13. Palamarchuk, I., Zozulyak, O., Mushtruk, M., Petrychenko, I., Slobodyanyuk, N., Domin, O., Udodov, S., Semenova, O., Karpovych, I., & Blishch, R. (2022). The intensification of dehydration process of pectin-containing raw materials. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 15–26). HACCP Consulting. https://doi.org/10.5219/1711
14. Song, Z., & Hwang, I. (2023). Objective meat quality from quality grade and backfat thickness of Hanwoo steers. In Food Science of Animal Resources. (Vol. 43, Issue 3, p. 531–539). National Library of Medicine. https://doi.org/10.5851/kosfa.2023.e15
15. Zurbriggen, G. A., Maglietti, C. S., Pouzo, L. B., Testa, M. L., Riffel, S. L., Elizalde, J. C., & Pavan, E. (2022). Extending the feeding period beyond 8.0 mm of subcutaneous fat reduces feed efficiency without improving meat colour and tenderness of non-implanted feedlot steers. In Journal of Animal and Feed Sciences. (Vol. 31, Issue 4, pp. 1–11). Kielanowski Institute of Animal Physiology and Nutrition. https://doi.org/10.22358/jafs/151153/2022
16. Ugnivenko, A., Slobodyanyuk, N., Shtonda, O., Antoniuk, T., Pylypchuk, O., Karpovskyi, V., Kruk, O. (2021). Influence of the features of weight gain, age and direction of breed productivity on the quality parameters of beef. In Food Science & Technology. (Vol 15, Issue 1, pp. 108–116). Odesa National University of Technology. https://doi.org/10.15673/fst.v15i1.1963
17. Yu, H., Yu, S., Guo, J., Wang, J., Mei, C., Abbas Raza, S. H., Gong, C., & Zan, L. (2024). Comprehensive Analysis of Transcriptome and Metabolome Reveals Regulatory Mechanism of Intramuscular Fat Content in Beef Cattle. In Journal of Agricultural and Food Chemistry. (Vol 72, Issue 6, pp. 2911–2924). National Library of Medicine. https://doi.org/10.1021/acs.jafc.3c07844
18. JMGA. Beef carcass grading standart. Japan meat grading association. – (2000). Tokyo, Japan.

19. Commission Regulation (EC). 2008. Commission Regulation (EC) No 1249/2008 of 10 December 2008 laying down detailed rules on the implementation of the Community scales for the classification of beef, pig and sheep carcasses and the reporting of prices thereof.
20. DSTU ISO 1443:2005. Meat and meat products. General specifications. Quality management systems – Requirements.
21. DSTU ISO 936:2008. Meat and meat products. Method of determination of mass total ash. Quality management systems – Requirements.
22. DSTU ISO 1442:2005. Meat and meat products. Method of determining moisture content. Quality management systems – Requirements.
23. DSTU ISO 2917:2001. Meat and meat products. Determination of pH (control method). Quality management systems – Requirements.
24. Ugnivenko, A., Getya, A., Nosevych, D., Antoniuk, T., Kruk, O., Slobodyanyuk, N., Ivaniuta, A., Omelian, A., Gryshchenko, S., & Israelian V. (2022). The study of "muscle eye" in bulls of Ukrainian black-spotted dairy-meat breed as a factor in improving the properties of meat products. In Potravinarstvo Slovak Journal of Food Sciences. (Vol. 16. pp. 519–529). HACCP Consulting https://doi.org/10.5219/1762
25. Antoniuk, T. (2020). Technology of animal slaughter products. Methodical instruction and tasks for independent work for Bachelor Degree student of the specialty 204 – «Animal products Mamefacturing and processing technology». https://nubip.edu.ua/site/default/files/u249/tehnologiya_produktiv_zaboyu_tvarin.
26. Council Regulation (EC) No. 1099/2009 of September 24, 2009 on the protection of animals at the time of death.
27. Oler, A., Głowinska, B. & Młynek, K. (2015). Slaughter and carcass characteristics, chemical composition and physical properties of longissimus lumborum muscle of heifers as related to marbling class. In Archives Animal Breed. (Vol. 58, pp. 145–150). https://doi.org/10.5194/aab-58-145-2015
28. Ugnivenko, A., Kos, N., Nosevych, D., Mushtruk, M., Slobodyanyuk, N., Zasukha, Y., Otchenashko, V., Chumachenko, I., Gryshchenko, S. & Snizhko, O. (2022). The yield of adipose tissue and by-products in the course of the slaughter of inbred and outbred bulls of the Ukrainian beef breed. In Potravinarstvo Slovak Journal of Food Sciences. (Vol. 16, pp. 307–319). doi: https://doi.org/10.5219/1758
29. Zhou, M., Zhu, Z., Sun, H. Z., Zhao, K., Dugan, M. E., Bruce, H., Fitzsimmons, C., & Guan, L. L. (2022). Breed dependent regulatory mechanisms of beneficial and non-beneficial fatty acid profiles in subcutaneous adipose tissue in cattle with divergent feed efficiency. In Scientific Reports. (Vol. 12, Issue 1, pp. 4612–4628). https://doi.org/10.1038/s41598-022-08572-8
30. Ugnivenko, A., Kruk, O., Nosevych, D., Antoniuk, T., Kryzhova, Y., Gruntovskyi, M., Prokopenko, N., Yemtcev, V., Kharsika, I. & Nesterenko, N. The expressiveness of meat forms of cattle depending on the content of adipose tissue under the skin and between the muscles. In Potravinarstvo Slovak Journal of Food Sciences. 2023. (Vol. 17, pp. 358–370). https://doi.org/10.5219/1869
31. Kruk, O., Ugnivenko, A., Antoniuk, T., Kolisnyk, O., Nosevych, D., Drachuk, I., Kolesnikova, O., Zhurenko, V., Shtonda, O. & Vakulenko, V. (2023). Quality of bull beef of the Ukrainian black and white dairy breed in dependence on the development of subcutaneous adipose tissue. In Potravinarstvo Slovak Journal of Food Sciences. (Vol. 17, pp. 997–1008). HACCP Consulting. https://doi.org/10.5219/1917
32. Kruk, O., Ugnivenko, A., Antoniuk, T., Kolisnyk, O., Slobodyanyuk, N., Nosevych, D., Naumenko, T., & Gruntkovskyi, M. (2024). Evaluation of beef carcass quality using the muscle eye area M. longissimus dorsi. Potravinarstvo Slovak Journal of Food Sciences. (Vol. 18, pp. 619–632). HACCP Consulting. https://doi.org/10.5219/1989
33. Smith, G.C., Carpenter, J.L., Cross, H.R., Murphey, C.E., Abraham, H.C., Savell, J.W., Parrish, F.C.Jr., Davis, G.W., & Berry, B.W. (1985). Relationship of USDA marbling groups to palatability of cooked beef. In Journal of Food Quality. (Vol. 7, Issue 4, pp. 289–308). Wiley Press Room. https://doi.org/10.1111/j.1745-4557.1985.tb01061.x
34. Lee, J. M., Choe, J. H., Park, H. K., Kim, Y. H., Park, B. Y., Kim, K. T., Koh, K.-Ch., Seo, S.-Ch., & Hwang, K. S. (2011). Effect of backfat thickness on the carcass grade factors and carcass price in Hanwoo cows and steers. In Food Science of Animal Resources. (Vol 31, Issue 2, pp. 280–289). Korea Institute of Science and Technology Information. https://doi.org/10.5851/kosfa.2011.31.2.280
35. Chi, N. T. K., Hue, P. T., Hanh, T. Q. & Ngoan, L. D. (2023). Carcass characteristics and meat quality of crossbred (Brahman× Lai Sind) and (Red Angus× Lai Sind) bulls kept in small scale farms. Online Journal of Animal and Feed Research. (Vol. 13, Issue 3, pp. 153–161). https://doi.org/10.51227/ojafr.2023.24
36. Batorska, M., Wiecek, J., Kunowska-Slosarz, M., Puppel, K., Slosarz, J., Golebiewski, M. & Balcerak, M. (2018). The effect of carcass weight on chemical characteristics and fatty acid composition of Longissimus

dorsi and Semimembranosus muscles of Eropean wild boar (Sus scrofa scrofa) meat. In Canadian Journal of Animal Science. (Vol. 98, Issue 3, pp. 557–564). Canadian Science Publishing. https://doi.org/10.1139/cjas-2017-0090

37. Rogoskii, I., Mushtruk, M., Titova, L., Snezhko, O., Rogach, S., Blesnyuk, O., Rosamaha, Y., Zubok, T., Yeremenko, O., & Nadtochiy, O. (2020). Engineering management of starter cultures in study of temperature of fermentation of sour-milk drink with apiproducts. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 14, pp. 1047–1054). HACCP Consulting. https://doi.org/10.5219/1437
38. Brethour, J. R. (2004). The relationship of average backfat thickness of feedlot steers to performance and relative efficiency of fat and protein retention. In Journal of animal science, (Vol 82, Issue 11, pp. 3366–3372). Oxford University Press. https://doi.org/10.2527/2004.82113366x
39. Sakowski, T., Grodkowski, G., Gołebiewski, M., Slósarz, J., Kostusiak, P., Solarczyk, P. & Puppel, K. (2022). Genetic and environmental determinants of beef quality – A Review. Frontiers in Veterinary Science. (Vol. 9, pp. 819605–819613). Frontiers. https://doi.org/10.3389/fvets.2022.819605
40. O'Quinn, T. G., Legako, J. F., Woerner, D. R., Kerth, C. R., Nair, M. N., Brooks, J. C., Lancaster J. M. & Miller, R. K. (2024). A current review of US beef flavor II: Managing beef flavor. In Meat Science. (Vol. 209. pp. 109403). Elsevier BV. https://doi.org/10.1016/j.meatsci.2023.109403
41. Beck, P.A., Beck, M.R., & Apple, J.K. (2022). Production systems and nutrition. Reference Module in Food Science. U.S. department of agriculture. https://doi.org/10.1016/B978-0-323-85125-1.00029-6
42. Im, C., Song, S., Cheng, H., Park, J., & Kim, G. D. (2024). Changes in meat quality and muscle fiber characteristics of beef striploin (M. longissimus lumborum) with different intramuscular fat contents following freeze-thawing. In LWT. (Vol 198, pp. 1–8). Elsevier BV. https://doi.org/10.1016/j.lwt.2024.116081
43. Hughes, J., Clarke, F., Li, Y., Purslow, P. & Warner, R. (2019). Differences in light scattering between pale and dark beef longissimus thoracis muscles are primarily caused by differences in the myofilament lattice, myofibril and muscle fiber transverse spacings. In Meat Science. (Vol. 149, pp. 96–106). Elsevier BV. https://doi.org/10.1016/j.meatsci.2018.11.006
44. Liu, T., Wu, J-P., Lei, Z-M., Zhang, M., Gong, X-Y., Cheng, S-R., Liang, Y., & Wang, J-F. (2020). Fatty Acid Profile of Muscles from Crossbred Angus-Simmental, Wagyu-Simmental, and Chinese Simmental Cattles. In Food Science Animal Resour. (Vol. 40, Issue 4, pp. 563–577). PubMed Central. https://doi.org/10.5851/kosfa.2020.e33
45. Raza, S. H. A., Khan, R., Abdelnour, S. A., Abd El-Hack, M. E., Khafaga, A. F., Taha, A., Ohran, H., Mei, C., Schreurs, N. M., & Zan, L. (2019). Advances of molecular markers and their application for body variables and carcass traits in qinchuan cattle. In genes. In Genes (Basel). (Vol. 10, Issue 9, pp. 717). MDPI. https://doi.org/10.3390/genes10090717
46. Ijaz, M., Li, X., Zhang, D., Hussain, Z., Ren, C., Bai, Y., & Zheng, X. (2020). Association between meat color of DFD beef and other quality attributes. In Meat Science. (Vol 161, pp. 107954). Elsevier BV. https://doi.org/10.1016/j.meatsci.2019.10795
47. Ozdemir, V. F., Kocyigit, R., Yanar, M., Aydin, R., Diler, A., Palangi, V., & Lackner, M. (2024). An investigation of slaughter weight and muscle type effects on carcass fatty acid profiles and meat textural characteristics of young Holstein Friesian bulls. In Heliyon. (Vol 10, Issue 6, pp. 1–8). By Elsevier. https://doi.org/10.1016/j.heliyon.2024.e27
48. Baldassini, W. A., Chardulo, L. A. L., Silva, J. A. V., Malheiros, J. M., Dias, V. A. D., Espigolan, R., Baldi. F., Albuquerque, L. G., Fernandes. T., & Padilha, P. M. (2016). Meat quality traits of Nellore bulls according to different degrees of backfat thickness: a multivariate approach. In Animal Production Science. (Vol. 57, Issue 2, pp. 363–370). CSIRO Publishing. https://doi.org/10.1071/AN15120
49. Santana, M. H. A., Rossi Jr, P., Almeida, R., & Cucco, D. C. (2012). Feed efficiency and its correlations with carcass traits measured by ultrasound in Nellore bulls. In Livestock Science. (Vol. 145, Issue 1–3, pp. 252–257). Elsevier BV. https://doi.org/10.1016/j.livsci.2012.02.012
50. Warner, R. D. (2023). The eating quality of meat: IV – Water holding capacity and juiciness. In Lawrie's meat science. (pp. 457–508). Woodhead Publishing.
51. Bureš, D., Needham, T., Bartoň, L., Lebedová, N., Kotrba, R., Řehák, D., Kučerová, I., Klouček, P., & Hoffman, L. C. (2024). Consumer acceptance and quality of game meat "droëwors" sausages with different levels of added fat. In Meat Science. (Vol 210, pp. 109424). Elsevier. https://doi.org/10.1016/j.meatsci.2024.109424
52. Aaslyng, M. D. (2002). Quality indicators for raw meat. In Meat processing: Improving quality. 157–174. Book chapter.

53. Rahman, M. F., Iqbal, A., Hashem, M. A., & Adedeji, A. A. (2020). Quality assessment of beef using Computer Vision Technology. In Food Science of Animal Resources, (Vol 40, Issue 6, pp. 896–907). National Institutes of Health. https://doi.org/10.5851/kosfa.2020.e57

54. Vasyliv, V., Mushtruk, M., Zheplinska, M., Mukoid, R., & Tkachenko, S. (2021). Method of Electrohydraulic Activation of Water-Lime Suspension in Sugar Production. In Lecture Notes in Mechanical Engineering (pp. 664–673). Springer International Publishing. https://doi.org/10.1007/978-3-030-91327-4_64

55. Bal-Prylypko, L., Danylenko, S., Mykhailova, O., Nedorizanyuk, L., Bovkun, A., Slobodyanyuk, N., Omelian, A. & Ivaniuta, A. (2024). Influence ofstarter cultures on microbiological and physical-chemical parameters of dry-cured products. In Potravinarstvo Slovak Journal of Food Sciences. (Vol. 18, pp. 313–330). HACCP Consulting. https://doi.org/10.5219/1960

56. Palamarchuk, I., Mushtruk, M., Vasyliv, V., Stefan, E., Priss, O., Babych, I., Karpovych, I., & Pushanko, N. (2024). Modelling the centrifugal mixing process of minced meat to optimise the production of chopped meat semi-finished products. In Potravinarstvo Slovak Journal of Food Sciences. (Vol. 18, pp. 297–312). HACCP Consulting. https://doi.org/10.5219/1959

57. Vovkotrub, V., Iakubchak, O., Vovkotrub, N., Shevchenko, L., Lebedenko, T., Holembovska, N., Pylypchuk, O., & Omelian, A. (2024). Quality and safety of pork meat after cooling and treatment with lactic starters. In Potravinarstvo Slovak Journal of Food Science. (Vol 18, pp. 439–452). HACCP Consulting. https://doi.org/10.5219/1954

58. Ferreira, G. A., Barro, A. G., Terto, D. K., Bosso, E. B., Dos Santos, É. R., Ogawa, N. N., & Bridi, A. M. (2024). Sensory quality of beef with different ultimate pH values Brazilian perspective. In Meat Science. (Vol 209, 109415). National Institutes of Health. https://doi.org/10.1016/j.meatsci.2023.109415

59. Santiago, B., Baldassini, W., Neto, O. M., Chardulo, L. A., Torres, R., Pereira, G., Curi, R., Chiaratti, M.R., Padilha, P., Alessandroni, L., & Gagaoua, M. (2023). Post-mortem muscle proteome of crossbred bulls and steers: Relationships with carcass and meat quality. In Journal of Proteomics. (Vol 278, pp. 104871). By Elsevier. https://doi.org/10.1016/j.jprot.2023.104871

60. Mushtruk, M., Palamarchuk, I., Palamarchuk, V., Gudzenko, M., Slobodyanyuk, N., Zhuravel, D., Petrychenko, I., & Pylypchuk, O. (2023). Mathematical modelling of quality assessment of cooked sausages with the addition of vegetable additives. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 242–255). HACCP Consulting. https://doi.org/10.5219/1845

61. Pacheco, R. F., Machado, D. S., Restle, J., Sartori, D. B. S., Costa, P. T., & Vaz, R. Z. (2024). Metanálise da qualidade da carne de bovinos abatidos com diferentes espessuras de gordura subcutânea. In Pesquisa Agropecuária Brasileira. (Vol. 58, e03110, pp. 1–9). SciELO Brasil. https://doi.org/10.1590/S1678-3921.pab2023.v58.03110

62. Listrat, A., Gagaoua, M., Normand, J., Andueza, D. J., Gruffat, D., Mairesse, G., Chesneau, G., Mourot, B.-P., Gobert, C., & Picard, B. (2020). Are there consistent relationships between major connective tissue components, intramuscular fat content, and muscle fiber types in cattle muscle? In Animal. (Vol. 14, Issue 6, pp. 1204–1212). Springer International Publishing. https://doi.org/10.1017/s1751731119003422

63. Eun-Young Jung, Young-Hwa Hwang & Seon-Tea Joo. (2016). The Relationship between Chemical Compositions, Meat Quality, and Palability of the 10 Primal Cuts from Hanwoo Steer. In Korean journal for food science of animal resources. (Vol. 36, Issue 2, pp. 145–151). PubMed Central. https://doi.org/10.5851/kosfa.2016.36.2.145

64. Troy, D. J., Tiwari, B. K., & Joo, S. T. (2016). Health implications of beef intramuscular fat consumption. In Korean Journal for Food Science of Animal Resources. (Vol 36, Issue 5, pp. 577–582). PubMed Central. https://doi.org/10.5851/kosfa.2016.36.5.577

65. Ponnampalam, E. N., Priyashantha, H., Vidanarachchi, J. K., Kiani, A., & Holman, B. W. B. (2024). Effects of Nutritional Factors on Fat Content, Fatty Acid Composition, and Sensorial Properties of Meat and Milk from Domesticated Ruminants: An Overview. In Animals. (Vol 14, Issue 6, pp. 840–879). Springer International Publishing. https://doi.org/10.3390/ani14060840

66. Kruk, O., & Ugnivenko, A. (2024). The area of loin eye in bulls of the Ukrainian Black-and-White dairy breed and its relationship with beef characteristics. In Animal Science and Food Technology. (Vol. 15, Issue 2, pp. 93–106). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.2.2024.93

67. Li, X., Fu, X., Yang, G., & Du, M. (2020). Review: Enhancing intramuscular fat development via targeting fibro-adipogenic progenitor cells in meat animals. In Animal. (Vol. 14, Issue 2, pp. 312–321). Elsevier BV. https://doi.org/10.1017/s175173111900209x

68. Kryzhova, Y., Slobodianiuk, N., & Moskalenko, I. (2023). Application of modern technologies to improve the quality of sausage products. In Animal Science and Food Technology. (Vol. 14, Issue 1, pp. 49–64). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.1.2023.43

69. Riabovol, M. V. & Bal-Prylypko, L. V. (2021). Justification and development of sausage technology with health properties. In Animal Science and Food Technology. (Vol. 12, Issue 1, pp. 39–47). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal2021.01.039

70. Nikolaenko, M.S. (2021). Optimization of the technology of production of functional semi-finished meat products covered in pastry. In Animal Science and Food Technology. (Vol. 12, Issue 1, pp. 19–27). National University of Life and Environmental Sciences of Ukraine. https://dx.doi.org/10.31548/animal/2021.01.019

71. Filin, S., Bal-Prylypko, L., Nikolaenko, M., Holembovska, N., & Kushnir, Yu. (2023). Development of technology for plant-based minced semi-finished products. In Animal Science and Food Technology. (Vol. 14, Issue 2, pp. 100–112). National University of Life and Environmental Sciences of Ukraine. https://doi.org/10.31548/animal.2.2023.100

72. Mushtruk, M., Palamarchuk, I., Palamarchuk, V., Gudzenko, M., Slobodyanyuk, N., Zhuravel, D., Petrychenko, I., & Pylypchuk O. (2023). Mathematical modelling of quality assessment of cooked sausages with the addition of vegetable additives. In Potravinarstvo Slovak Journal of Food Sciences. (Vol 17, pp. 242–255). HACCP Consulting. https://doi.org/10.5219/1845

73. Bal-Prylypko, L., Nikolaenko, M., Kanishchev, O., Beyko, L., & Holembovska, N. (2023). Improving the technology for the production of raw dried beef products. In Animal Science and Food Technology. (Vol. 14, Issue 4, pp. 26–39). ResearchGate BV. https://doi.org/10.31548/animal.4.2023.26

74. Mushtruk, M., Mushtruk, N., Slobodyanyuk, N., Vasyliv, V. & Zheplinska, M. (2024). Enhanced energy independence: converting animal fat into biodiesel. In International Journal of Environmental Studies. (Vol. 81, Issue 1, p. 1–11). ResearchGate BV. https://doi.org/10.1080/00207233.2024.2314860

75. Randhawa, I. A. S., McGowan, M. R., Porto-Neto, L. R., Hayes, B. J., & Lyons, R. E. (2021). Comparison of Genetic Merit for Weight and Meat Traits between the Polled and Horned Cattle in Multiple Beef Breeds. In Animals. (Vol. 11, Issue 3, p. 870). Springer International Publishing. https://doi.org/10.3390/ani11030870

76. Park, S. J., Beak, S.-H., Jung, D. J. S., Kim, S. Y., Jeong, I. H., Piao, M. Y., Kang, H. J., Fassah, D. M., Na, S. W., Yoo, S. P., & Baik, M. (2018). Genetic, management, and nutritional factors affecting intramuscular fat deposition in beef cattle – A review. In Asian-Australasian Journal of Animal Sciences. (Vol. 31, Issue 7, pp. 1043–1061). Asian Australasian Association of Animal Production Societies. https://doi.org/10.5713/ajas.18.0310

Funds:

The study was conducted within the framework of the academic research work “To substantiate systems of production and monitoring of safe animal husbandry products” funded by the state budget of Ukraine (state registration number: 0121U110192).

Acknowledgements:

We would like to thank you to Dr. for Anatolii Ugnivenko.

Conflict of Interest:

The authors have no conflicts of interest.

Ethical Statement:

According to Protocol No. 10 of 18.04.2020 at the meeting of the Ethics Commission of the Faculty of Livestock Raising and Water Bioresources, National University of Life and Environmental Sciences of Ukraine, Act No. 3 and 4 were signed during the experimental research, i.e. in the process of the slaughter of cattle "all the rules of the current legislation of Ukraine were observed, following DSTU 4673: 2006.

Contact Address:

Olha Kruk, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Milk and Meat Production Technologies, Heroiv Oborony St., 15, 03041, Kyiv, Ukraine

E-mail: olgakruk2016@ukr.net

ORCID: https://orcid.org/0000-0001-9975-8994

Anatolii Ugnivenko, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Milk and Meat Production Technologies, Heroiv Oborony St., 15, 03041, Kyiv, Ukraine
E-mail:ugnivenko@nubip.edu.ua
ORCID: https://orcid.org/0000-0001-6278-8399

Dmytro Nosevych, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Milk and Meat Production Technologies, Heroiv Oborony St., 15, 03041, Kyiv, Ukraine
E-mail: dknosevvich@i.ua
ORCID: https://orcid.org/0000-0003-2495-2084

Oleksandr Natalich, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Milk and Meat Production Technologies
Heroiv Oborony St., 15, 03041, Kyiv, Ukraine
E-mail: oleksandrnatalich@gmail.com
ORCID: https://orcid.org/0000-0003-3245-6179

Mykola Gruntkovskyi, National University of Life and Environmental Sciences of Ukraine, Faculty of Livestock Raising and Water Bioresources, Department of Technologies in Poultry, Pig and Sheep Breeding, Heroiv Oborony str., 15, Kyiv, 03041, Ukraine
E-mail: kolya_gr26@ukr.net
ORCID: https://orcid.org/0000-0002-6969-2987

Iryna Kharsika, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technologies of Meat, Fish and Seafood, Polkovnyka Potiekhina Str., 16, 03121, Kyiv, Ukraine
E-mail: veretynska23@ukr.net
ORCID: https://orcid.org/0000-0002-2789-7757

Oleksandr Androshchuk, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Management of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Heroiv Oborony Str., 15, 03041 Kyiv, Ukraine
E-mail: testoo@ukr.net
ORCID: https://orcid.org/0009-0002-3713-5278

***Inna Stetsiuk**, National University of Life and Environmental Sciences of Ukraine, Faculty of Food Technology and Quality Control of Agricultural Products, Department of Technologies of Meat, Fish and Marine Products, Vystavkova Str., 16, studying building No 12, Kyiv, 03041, Ukraine
E-mail: Stetsyukinna8513@ukr.net
ORCID**:** https://orcid.org/0000-0001-8392-6527

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 31.7.2024
Revised: 24.10.2024
Accepted: 30.10.2024
Published: 6.11.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 993-1005
https://doi.org/10.5219/2006
ISSN: 1337-0960 online
www.potravinarstvo.com

Enhancing the quality of wholemeal bread with chia, sesame, and rosehip: mathematical modelling and organoleptic analysis

Liliya Alashbayeva, Makhamedkali Kenzhekhojayev, Assel Borankulova, Bayan Muldabekova, Madina Yakiyayeva, Sholpan Tursunbayeva, Zhanerke Dyusembaeva

ABSTRACT

In this paper, the research was conducted using mathematical modelling methods to improve the quality of the product. This study aimed to determine the optimum composite mixture for producing whole wheat flour by adding sesame seeds, chia seeds, and crushed rosehip. Following the mathematical matrix, 20 different samples have been baked. The basic criteria were porosity and specific volume. The results were entered into Exel to draw up a graph. According to the graphic analysis, the most optimal mixture in terms of the dry matter mass in the dough was as follows in %: rosehip - 1.1%, chia seeds - 1.5%, and sesame - 2.2%. The organoleptic and physicochemical properties of the resulting samples were later analysed according to the recipes based on the selected composition of seeds. By swelling the protein shells of chia and sesame in a humid environment, amino acids in the flour combine into a chain to form a skeleton. At the same time, the ascorbic acid in the rosehip binds with the carbon atoms in the chain, strengthening the framework. As a result, large amounts of gases formed in whole grain flours are trapped in these frames, increasing the porosity of bread by 21.8%, increasing the volume of production by 29.5%, absorbing proteolytic enzymes under the influence of globulins in chia grain, slowing down amino acid degradation, reducing moisture content by 3%.

Keywords: whole-wheat flour, organoleptic and physicochemical properties, chia seeds, sesame rosehip.

INTRODUCTION

The strategy of creating conditions for producing a new generation of food products with specified quality characteristics, including specialised functional and enriched foods, includes research to develop their formulations and new technological methods **[1]**.

Most customers choose products made from the best-pureed varieties, as the texture of whole-grain cereal products is unappealing. Whole-meal bran flour reduces the quality of bread. However, lately, due to the focus on healthy eating, considerable attention is given not to the organoleptic properties but to the nutritional value of the bread. The assortment of bread products with various food additives used to increase their nutritional value has been increased **[2]**, **[3]**.

Whole-grain foods are well known for their nutritional benefits **[4]**. Whole-grain flour (WGF) is the flour. That has produced no peeled grain from the outer layers of the endosperm, germ, and membrane. Without them, the grain is a varietal product with high carbohydrate content, causing difficulty in the functioning of the gastrointestinal tract, obesity, and other diseases. The choice of food additive to be added in the production of floury culinary products is related to the chemical composition of whole-meal flour and the degree of its effect on the human body **[5]**, **[6]**.

To maintain good health, people should also consume foods high in bran and fiber daily, within the limits of the daily allowance. Baking with whole-meal bread products requires continuous improvement in quality and organoleptic properties to meet customers' demands. The addition of sesame seeds, chia seeds, and crushed rosehip seeds to the dough not only improves the nutritional value of the flour **[7]**, but it also significantly raises the quality of the baked bread.

Due to wholemeal flour's high carbon dioxide content, its gas-forming capacity was considerably high. However, the dough's lifting power was low because the quantity and quality of the gluten-forming particles were low. Therefore, vitamin C (300-4000 mg/100 g) in-ground rosehips improves the gelling ability.

Furthermore, the concentrations of high molecular weight thiols in the glutenins of flours from different wheat cultivars were determined. The values ranged from 5.6 to 8.2 micromol/kg of protein and showed a correlation between flour quality and SH concentration. In addition to AA and mixing of dough, the concentrations of the protein thiols in the glutenins isolated from the dough increased to a maximum when 100 mg of AA/kg of flour was added. Higher concentrations of AA led to a decrease in the SH concentration **[8]**. The protein in chia seeds and rosehips increases gelling **[9]**, **[10]**. The combination of additive combinations enhances the rheological properties of bread.

The model recipe and the technological parameter obtained a whole-meal wheat bread recipe as a control in the research. Mathematical modelling was used for this purpose. The organoleptic properties of the control sample of bread were pleasant. Nevertheless, it did not have a specific taste and aroma. Additionally, the peculiar porosity of the product was not enough. The composition of rosehip powder, chia seeds, and sesame seeds was created for whole-meal wheat flour. This composition was rich in all the vitamins and minerals that boost immunity and had high antioxidant properties **[11]**.

Plants play an essential role in human health maintenance. Sesame seeds are reservoirs of many nutritional components; the major bioactive components include minerals, vitamins, polyunsaturated fatty acids (PUFA), and unique natural antioxidants such as lignans (sesamin and sesamolin). Moreover, the seeds are an excellent source of the sulfur-containing amino acid methionine, which is rare in other plant proteins **[12]**.

Sesame has high health benefits but should not be consumed in large quantities. The problem is its high-calorie content potential for allergies and its ability to affect the human body. It is, therefore, best not to use sesame if you have poor blood clotting and kidney stones. The optimal amount of sesame per day is 1 or 2 tablespoons **[13]**, **[14]**.

Chia contains an appreciable amount of proteins and phytochemicals. The nutritional value of chia was the reason why it was used in prophylaxis of several non-infectious diseases such as obesity, hypertension, cardiovascular diseases (CVDs), cancer, and diabetes **[15]**, **[16]**. In the meantime, the consumption of chia seeds reduces weight and improves metabolism. However, the optimal daily intake is 25 g. Stabilisation of bread quality, the expansion of the range, and other factors lead to the correction and optimisation of the ratio of the recipe components. In addition, evaluation of the effectiveness of the baking enhancers used to influence the dough preparation process under the conditions of different properties of the main and supplementary raw materials.

Scientific hypothesis

The increased nutritional value of bread will depend on the beneficial properties of grain mixtures.

MATERIAL AND METHODOLOGY

Samples

The whole-wheat flour from winter wheat crops "Daulet" ("Research Institute of Agriculture and Crop Production" Almaty region, Republic of Kazakhstan) was used as a raw material. Additionally, sesame, chia seeds, and rosehip seeds were used as plant raw materials. Based on the calculation of daily consumption of sesame and chia seeds, they were used in an amount of 7% per 100 g of flour and, based on organoleptic characteristics of rosehip fruit, rosehip 3% per 100 g of flour, based on their optimal effect on the organism **[17]**, **[18]**, **[19]**. Various types of experimental bread were considered experiments, considering the use of these raw materials. Samples of whole-meal wheat flour bread were taken as a control.

Chemicals

All reagents were of analytical grade and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

Animals, Plants and Biological Materials

The study used beef, horse meat, and pork for analysis.

Instruments

We used Drying cabinet SESH-3M (V-KIP, RF), Zhuravlev device (RosPromMash, RF), Bread volume meter series BVM-L370LC (TexVol, Sweden).

Laboratory Methods

The built-in surface diagram made it possible to select the best combination of a mixture of constituent components, which were difficult to find by other means except for known data.

The following indicators of whole grain bread were analysed: porosity according to GOST 5669-96 **[20]**, humidity according to GOST 21094-2022 **[21]**, acidity according to GOST 5670-96 **[22]**, volume and organoleptic indicators according to GOST 5667-65 **[23]**.

The standard method (electric dry cabinet) and the accelerated method were used to determine humidity. All laboratory studies used standard methods.

The acidity of bread was measured in degrees of acidity. The degree of acidity is the amount of 0.1 N sodium hydroxide or caustic soda solution (ml) required to neutralise the acids in 100 g of breadcrumbs.

Bread porosity is the ratio of the pore volume of the bread to the total volume of breadcrumbs. The porosity of bread was determined by the Zavyanov method using a Zhuravlev device.

Other bread's physical properties as volume, specific volume, height, width, length, density, weight, maximum diameter) were determined with a BVM-L370LC (TexVol Sweden), measured with a fully digital laser sensor and a "Bread Volume Meter" device.

Description of the Experiment

Sample preparation: Dissolve pressed baking sourdough in warm water at 40 °C. Separately sift whole grain flour, previously finely ground with a dispersion of 140 microns, and composite flour from rosehip, chia and sesame seeds in small portions. Pour half of the whole grain flour and composite flour into a bowl and mix. The kneaded batter is placed in a proofer at 33-35 °C. The autolysis (autolysis) begins; that is, gluten develops. This stage can last 45-60 minutes. After this, the second part of whole grain flour and salt are added to the dough. Kneads the dough on a dough mixer. It should turn out soft and not too clogged with flour. The total number of test samples is 20 pieces, according to Table 2, and for comparison, a control sample was baked (the recipe is listed in Table 3). Also, place it in the proofer for 20 minutes. During this time, gluten is activated. Afterwards, the dough will be completely ready for further work. Form the dough into round balls and shape it into bread. Rolling a dense ball with good surface tension is necessary without squeezing the air when moulding. Send the dough to the proofer for 2 hours, after which we bake in a preheated baking oven at a temperature of 180 degrees for 15-20 minutes, or you can start baking at a temperature of 240-260 degrees (10 minutes), then set it to 220 degrees (20 minutes) , and finally - bake for 30-50 minutes at 150 degrees.

Number of samples analyzed: we analysed twenty-one samples.

Number of repeated analyses: All measurements of instrument readings were performed two times.

Number of experiment replications: The number of repetitions of each experiment to determine one value was two times.

Design of the experiment: Optimal amounts of recipe components, including the different volumes of sesame, chia, and rosehip, were prepared by single-phase baking, including yeast-free baking.

A predictive analysis of the quality of whole-meal wheat flour bakery products based on raw vegetable materials was conducted. A mathematical analysis determined their compatible optimum.

Using a central composite rotatable uniform planning (CCRUP), rational dosages of the components of a biologically active composition were calculated in the preparation of dough, and the effect of these dosages on the quality indicators of the finished bread was studied.

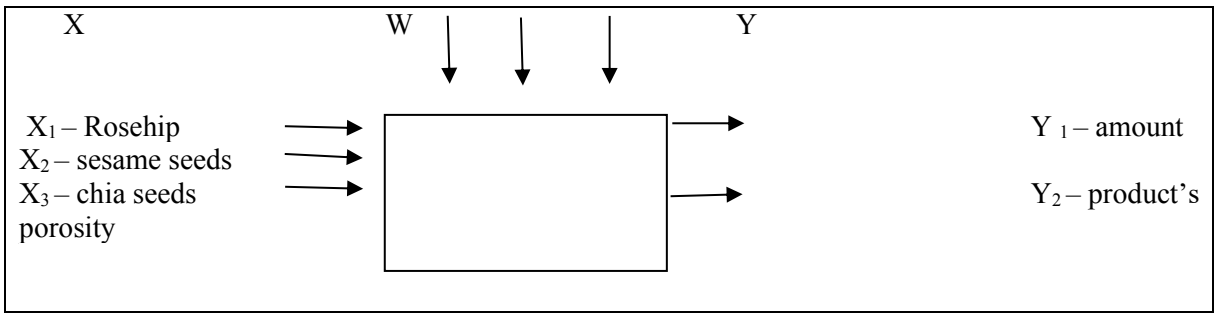


Figure 1 The model for determining the effect of the number of additives on bread quality.

The operating mechanism is complex and unknown, and a "black box" phrase has been used to illustrate the process in question (Figure 1). W - "Input" is entering information about the recipe size of the functional bread in the accidental exposure process. "Output" controls the results described by the optimisation criterion.

The output's Y functionally depends on the state X:Y = f (X) state. However, the dependence of the input data is unknown **[24]**.

The main factors were X1—rosehip powder %, X2—sesame seeds %, and X3—chia seeds. The amount of functional raw materials was chosen as the criteria for assessing the influence: product volume—Y1%, product porosity—Y2%. The regression equation was obtained from the results of mathematical processing with CCRUP.

Methods of search optimisation, planning of multifactorial experiments and statistical processing of experimental data were used to solve the tasks. For this purpose, optimisation parameters and factors that affect the technical indicators of the quality and safety of bread (the most important ones) were selected. The experimental research plan was determined, and a mathematical model was developed based on the obtained experimental data. The influence of controlled factors on the parameters of the process "output" in the stationary zone of the factor space was researched **[24]**, **[25]**.

Table 1 gives the influence parameters of the new functional bread additives on the finished bread's technological parameters and selected conversion levels.

Table 1 Research on the parameters for the effect of recipe doses on bread quality parameters and their degree of variability in the laboratory.

Controllable parameters: coded (natural)	Coded levels			Variation interval
	-1	0	1	
X_1 – rosehip, g	2	3	4	1
X_2 – sesame, g	6	7	8	1
X_3 – chia seeds, g	6	7	8	1

Table 2 Statistical description of the effect on the quality of the bread. According to the quantity of the recipe of raw materials in its composition.

Experiment №	Coding factor indicators			Natural factor indicators			Evaluation criteria indicators	
	X_1	X_2	X_3	X_1, %	X_2, %	X_3, %	Y_1, cm^3/100g	Y_2, cm^3/100 g
1	1	1	1	4	8	8	247	54.7
2	1	1	-1	4	8	6	247	55.1
3	1	-1	1	4	6	8	234	56.2
4	1	-1	-1	4	6	6	239	53.6
5	-1	1	1	2	8	8	230	52.8
6	-1	1	-1	2	8	6	232	52.2
7	-1	-1	1	2	6	8	227	51.1
8	-1	-1	-1	2	6	6	225	51.2
9	-1.68	0	0	1.32	7	7	227	54.8
10	1.68	0	0	4.68	7	7	244	61.5
11	0	-1.68	0	3	5.32	7	229	51.2
12	0	1.68	0	3	8.68	7	243	51.3
13	0	0	-1.68	3	7	5.32	238	52.2
14	0	0	1.68	3	7	8.68	240	67.1
15	0	0	0	3	7	7	250	67.3
16	0	0	0	3	7	7	250	67.3
17	0	0	0	3	7	7	250	67.3
18	0	0	0	3	7	7	250	67.3
19	0	0	0	3	7	7	250	67.3
20	0	0	0	3	7	7	250	67.3

A mathematical model of the process was created. That allowed us to calculate the parameters of the internally selected interval transition. Table 2 shows the dependence of the "Output" parameter on the "Input" parameters.

The method had required to draw of two-dimensional sections $Y=f(X_1, X_2)$, $Y=f(X_1, X_3)$ and $Y=f(X_2, X_3)$ at $X_3=0$, $X_2=0$ and $X_1=0$, accordingly.

Searching for multivariate dependencies was conducted under a priori uncertainty about the limited experimental data and type of regression functions for bread quality indicators.

Statistical Analysis

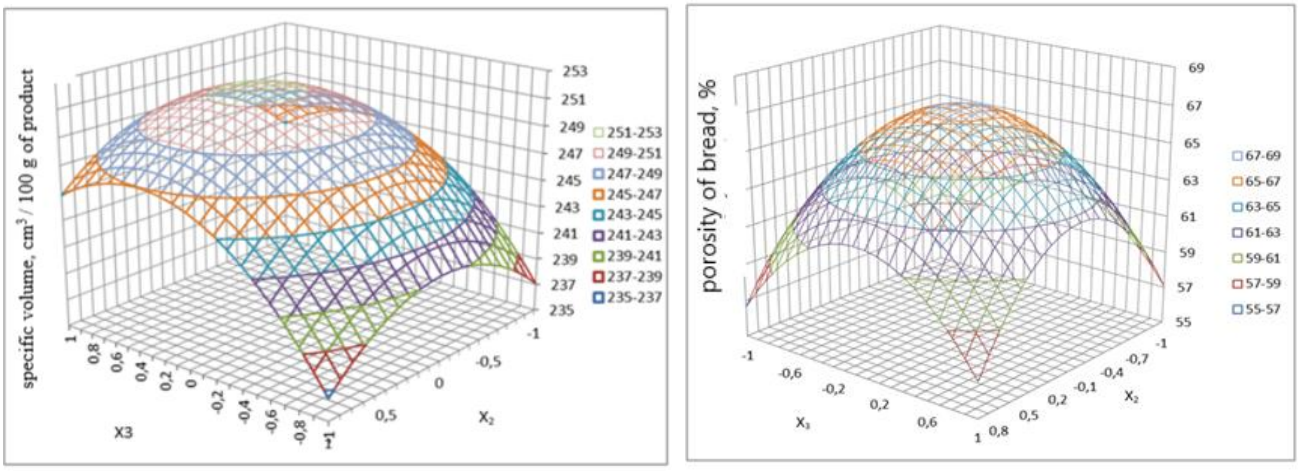
The data obtained during the experiments were processed using the mathematical method of variation statistics using the Statistika 10.0 developer StatSoft, USA. The data were also analysed using MS Excel for Windows 10 Pro, 2010. The data collected during the study were subjected to independent testing, and questionnaires were conducted to assess the organoleptic characteristics of control and test samples. The analysis used absolute and relative statistical indicators and tabular and graphical methods to present the results. Values were estimated using mean and standard deviations. The experiment was conducted with three repetitions. Considering the presence of significant coefficients of pair interaction (i.e., the nonlinearity of the objective function and quality assessment criteria) in numerous equations, the search for optimal processing modes was performed using nonlinear programming methods. This Newton method is part of the 'Find a solution' procedure in the MS Office Excel package.

RESULTS AND DISCUSSION

Consumers in many developed countries have recently started to choose bakery products made without preservatives, sprouted grains, and whole-wheat flour **[26]**. This shows that the focus is not on the bread's physical and organoleptic qualities but on its nutritional value. Bread products made from whole wheat flour have low organoleptic values **[27]**. They contain bran and germ in addition to endosperm **[28]**. Whole grain products contain more fiber, so you feel full faster **[29]**. The fiber content of whole grain spelt flour is slightly lower than wheat flour, but they have similar amounts of soluble fiber **[30]**. Whole grain spelt flour has a moderate effect on blood sugar levels when ranked by glycemic index **[31]**

Whole-wheat flour is chemically rich in minerals and vitamins, but the gluten's low amino-acid content deteriorates the bread's rheological properties **[32]**. Research was carried out to prevent such deficiencies with raw vegetable materials and determine the necessary raw materials per 100 g of flour.

Figure 2 shows a graph of the porosity and volume of 20 bakery samples based on the matrix sample obtained from the research.



a) **b)**

Figure 2 Graph of whole-meal wheat bread with vegetable raw materials.
Note: a) Effect of the ratio of sesame, chia and rosehip on the specific volume of the bread; b) Effect of the ratio of sesame, chia and rosehip on the porosity of the bread.

Graphical analysis and mathematical calculations showed that the optimal wheat flour in the dough in terms of % weight is rosehip powder - 2.86 %, sesame - 6.9 %, chia - 7.13 %.

Conducted studies have shown the effectiveness of using rosehip, sesame, chia and sesame raw materials as an additive to whole grain flour in the preparation of bread **[33]**. Today, calcium deficiency causes many negative

consequences for patients **[34]**. Rose hips, sesame and chia seeds were selected as additives for the production of bread products enriched with calcium and highly digestible by the body in order to solve such urgent problems **[35]**. Sesame and chia seeds are one of the highest among food production raw materials in terms of calcium content **[36]**. In addition to calcium, the selected grains contain a large amount of minerals and vitamins, which play an important role in the body.

The dough was prepared according to a new recipe based on the positive effect (organic and physicochemical properties) of chia and sesame seeds on whole-meal bread (Table 3).

Table 3 Recipe for wholemeal bread dough.

Name of the raw material	Dry matter content, %	Count of 100 g raw materials added to the flour, %
Whole-meal grain	83.2	100
Salt	96.5	1.5
Pressed baking yeast	25.00	2.5
Rosehip	-	2.86
Chia seeds	-	7.13
Sesame	-	6.9
Water	49% calculated based on humidity	
Total	-	104

The organoleptic properties of wholemeal wheat bread with the addition of rosehip, chia, and sesame were studied according to a recipe based on graphic analysis. The research data are shown below in Figure 3.

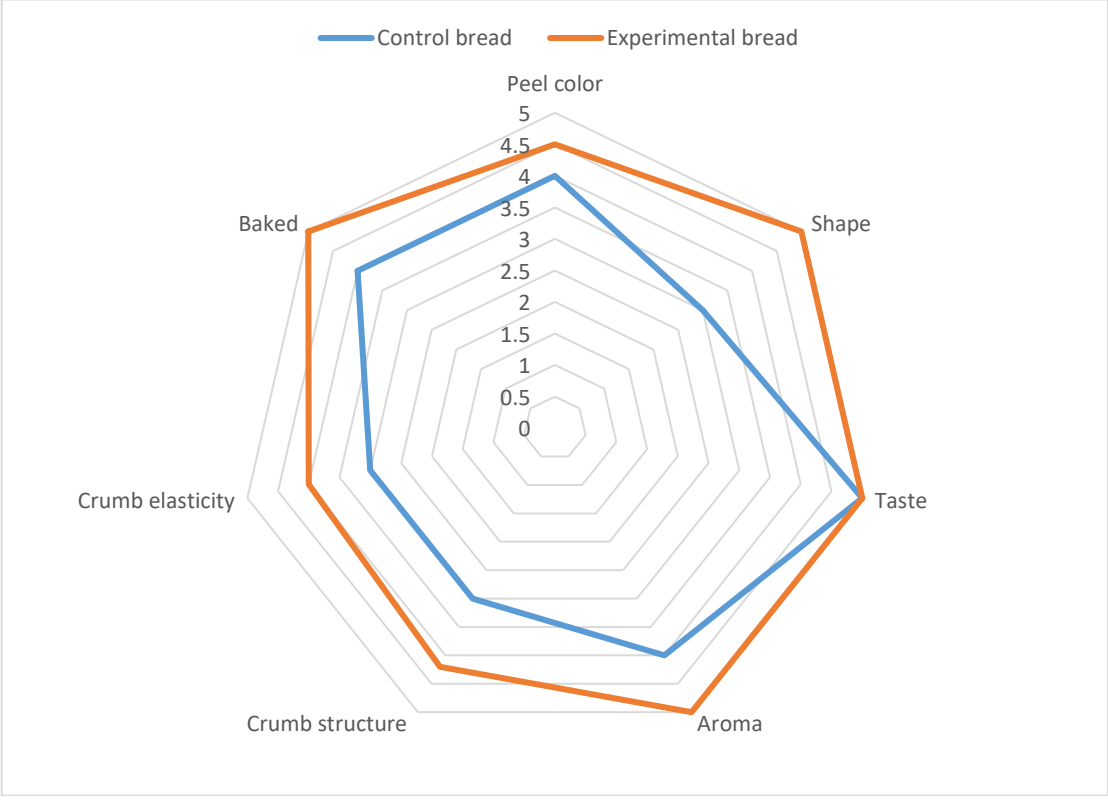


Figure 3 The effect of raw vegetable materials on wholemeal wheat bread's organoleptic characteristics.

According to organoleptic properties, the control sample of whole-meal wheat bread had an inconsistent shape, slightly convex, an outer crust, and a rough and lumpy surface, unbroken and hollow. The colour was brownish-black with light spots. Baked, slightly moist to the touch, elastic, and dense, it did not return to its former shape when lightly pressed with the finger, conformed to the product type, was sweeter, and had no extraneous smell.

The experimental bread's organoleptic properties were much better than the control sample's. The surface was rough but without cracks or concavities. The bread was fully baked, slightly moist, elastic, and recovered when lightly pressed with the finger. It was traceable with a light brown colour, pleasant to the taste, and aromatic.

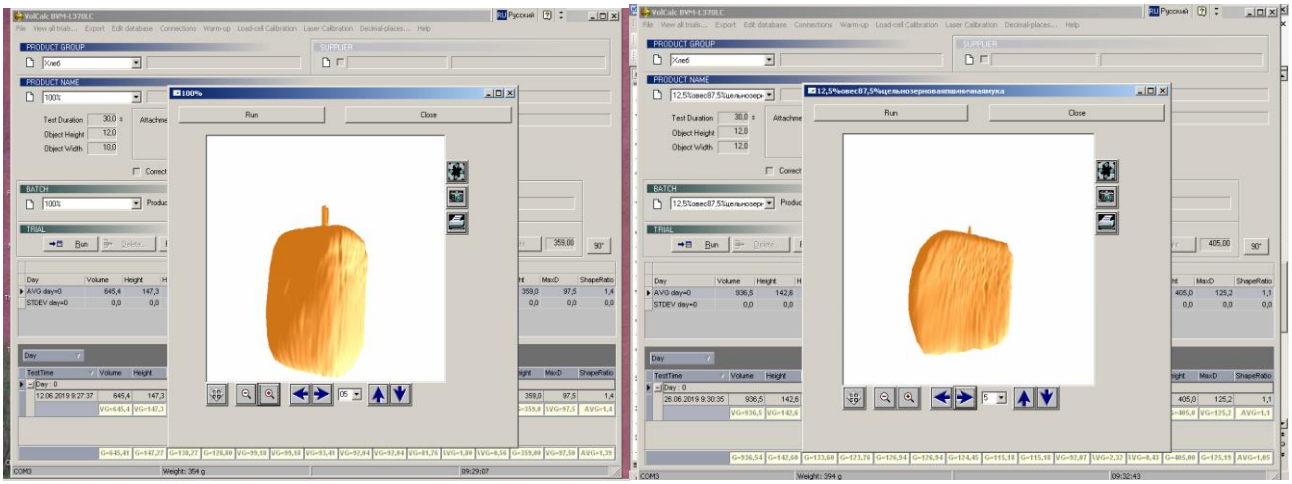
Research data for the physicochemical properties of the bread samples are presented in Table 4.

Table 4 Physicochemical properties of bread samples.

Name of the parameter	Control sample of bread	Experimental bread
Moisture content of crumb, %	48	46.5
Acidity, deg	6.5	6.4
Porosity, %	55	67
Size, cm	645	916.5

The physical and chemical properties of the products compared with the control sample in the experimental bread product were as follows: Moisture decreased by 3%; there was no significant change in acidity; porosity increased by 21.8%; and volume increased by 29.5%.

Research on different physical properties (volume, specific volume, height, width, length, density, mass, and the maximum diameter of the product) of whole-meal bread measured with the device "BVM—L370LC Bread volume meter." The results are shown in Figure 4.



A) **B)**

Figure 4 3D view of whole-meal bread created with the "BVM - L370LC Bread Volume Meter".
Note: A) Whole-meal wheat bread; B) Whole-meal wheat bread with the addition of vegetable raw materials.

The physical parameters of the prototype compared to the control were as follows: specific volume increased by 28 %, length 9.3 %, width 7.1 %, height 15 %, maximum diameter 4.6 %, and density 7.5 %.

In the production of wheat bread, the possibilities of using improvers based on the principle of different effects are based on the improvement of their functional properties, which indicates the interaction of the main components included in their composition with the structural components of the dough made from whole grain flour **[37]**. The functional properties of improvers affect the formation of bread properties, directly related to the intensity and microbiological purity of the processes occurring during dough fermentation **[38]**.

In recent years, the frequency of spoilage of wheat bread by thermophilic microorganisms is increasing, because in the current situation, according to the demand of the consumer, in the process of production of dietary and healthy products, the addition of bran and seeds as raw materials and the production of products without preservatives are increasing **[39]**. Traces of microbiological contamination are observed during the preparation of high value whole wheat bread **[40]**, because the endosperm of the grain in whole wheat flour is not separated. Therefore, there is a risk of contamination with spore-forming bacteria and microscopic fungi that produce toxic substances. It is very effective to use white cabbage containing lysozyme as an inhibitor of disease-causing pathogens in bread **[41]**.

Calculating rational dosages of the components of the composition of plant materials during the dough preparation and studying the effect of these dosages on the quality indicators of the finished bread was carried out using a central composite rotatable uniform planning **[42]**. Mathematical modelling was the convenient and accurate method for determining the rate of different vegetable raw materials according to defined criteria **[43]**.

A marked improvement in the bread products was observed during the physicochemical analysis of the recipe developed based on the graphical analysis **[44]**.

If a higher grade of flour had been used, the more the moisture content of the bread decreases, the higher the percentage of dry matter would be **[45]**. Due to the high fibre and ash content of wholemeal bread, its moisture content was high, and the moisture content of the bread product with the addition of vegetable raw materials decreased by 3 % **[46]**. Due to the colloidal structure of the dough, the moist phase was reduced because the presence of globulins in chia and sesame seeds inhibits the activity of proteolytic enzymes, which have autolytic properties **[47]**. The breakdown of amino acids limits their transfer to the wet phase **[48]**.

Due to the high content of mono- and disaccharides in the pores **[49]** and fibres of the flour, the acidity of wholemeal bread is 2.5 times higher than that of higher-grade flour because fermentation is more intense even in the first phase **[50]**.

Furthermore, no matter how intense the carbon dioxide was, carbon dioxide retention was low due to the weakness of the amino acids that made up gluten **[51]**.

Bread made from low-grade flour is sour and has low porosity and specific gravity. The bottom of the product was very dense. The appearance of the crust deteriorates to become brown **[52]**.

Adding vegetable raw materials did not significantly change the bread products compared to the control sample **[53]**. The bread's sourness was characterised by its defined acceptability and use of raw materials **[54]**.

The porosity of the control sample increased by 21.8%, and the sample with the addition of raw vegetable materials had a higher porosity. Bread porosity shows the percentage of the total number of pores. The porosity of the bread depends on its absorbency. The uniformly thin-walled porosity of well-loosened bread was easily rinsed out and absorbed by the digestive juices, so it was fully absorbed.

Based on scientific research, while porosity and volume were reduced, chia and sesame seeds were added to the dough unmilled. If they had been added in milled form, it would have increased acidity w. Chinese scientist Lin observed an increase in the specific gravity of bread when a protein shell was added. So, in this research, chia seeds and sesame were added as grains. Based on these data, chia seeds and sesame were added without grinding. When exposed to a moist environment, the outer protein shell moistened, forming a gelatinous layer 12 times larger than chia. The protein shell of sesame in a moist environment also binds well to flour proteins **[55]**.

The addition of rosehip, as a plant raw material, was in the form of a powder, as only a large amount of ascorbic acid included in its composition was required, and its amino acid content was low **[56]**.

The daily allowance of sesame and chia seeds should not exceed two teaspoons. The research added 3% rosehip, 7% chia, and sesame to the dough. Considering the above, only 7% of chia and sesame seeds had been added to the dough. There is a reason for adding 3% rosehip fruit: if this amount has been exceeded, the acidity of the bread will increase, and the whole-meal bread will turn brown due to its high sugar content, which affects its colour.

We believe that vegetable raw materials depend on colloidal and biochemical processes, which occur in close contact with each other inside the dough. The reason for this is the high gluten content of the vegetable raw material, i.e. the very high gel-forming protein layers in the outer layer of sesame and chia seeds, in which the amino acid chains bind with the amino acid chains of the flour, resulting in porosity. Rosehip powder rich in vitamin C was used to enhance it. Because rosehip powder contains 650 mg of vitamin C in 100 grams, it forms a strong frame, binding carbon atoms in the amino acid chain's outer and inner side layers by disulfide bonds. It was determined that the organoleptic and physicochemical properties of the product were improved.

CONCLUSION

Due to the weak bond in the proteins that make up the gluten in wholemeal flour and its high proteolytic enzyme content, the moisture content of the product increases. Generally, the bread quality is deteriorating due to the breakdown of amino acids and their transfer from the liquid phase to the solid phase. Therefore, producers of wholemeal bread need to introduce new ways of improving product quality. The vegetable raw materials were added to the whole-meal flour during this research. The porosity and volume values were considered the highest, following the two main criteria presented to the whole-wheat flour. Suppose the protein shells of chia seeds and sesame swell in a humid environment. In that case, they combine with the amino acid chains in the flour to form a framework in the rosehip fruit that binds to the carbon atoms in the side groups of the amino acid chain. The results were obtained from the graphical analysis of the raw materials and reported using specific mathematical modelling. When sesame seeds, chia seeds, and rosehip powder were added to the dough, the organoleptic and physicochemical properties of whole-meal flour were determined to be significantly enhanced.

The bread's porosity increased by 21.8%, reaching 67% in the experimental samples compared to 55% in the control sample. The bread volume increased by 29.5%, from 645 cm^3 in the control sample to 916.5 cm^3 in the experimental samples. The optimal mixture for wholemeal bread dough was 2.86% rosehip powder, 7.13% chia

seeds, and 6.9% sesame seeds, which showed the best performance regarding specific volume and porosity. The moisture content of the experimental bread decreased by 3%, from 48% in the control to 46.5% in the experimental samples, improving the bread's texture and shelf life. The specific volume of the bread increased by 28%. Other physical dimensions, such as length, width, and height, improved by 9.3%, 7.1%, and 15%, respectively. These results demonstrate the positive effects of adding chia, sesame, and rosehip to wholemeal bread, significantly improving its quality, structure, and nutritional value.

REFERENCES

1. Misteneva, S.Yu., Soldatova, E.A., & Mizinchikova I.I. (2019). Improving the technology of flour confectionery products using whole grain wheat flour. Digest of articles. International scientific and technical conference dedicated to the 90th anniversary of the Faculty of Technology VSUIT (pp. 81-89). http://old.vsuet.ru/science/conference2019/mat_28-03-2019.pdf.
2. Iztayev, A., Urazaliev, R., Yakiyayeva, M., Maemerov, M., Shaimerdenova, D., Iztayev, B., Toxanbayeva, B., & Dauletkeldi, Y. (2018). The investigation of the impact of dynamic deterioration of ozone on grass growth and the consequence of ion-ozone cavitation treatment. Journal of Advanced Research in Dynamical and Control Systems (Vol. 10, Issue 13, pp. 663–671.
3. Iztayev, B., Yakiyayeva, M., Magomedov, M., Iztayev, A., Kenzhekhojayev, M., & Spandiyarov, Y. (2021). Devising technology of the accelerated method for making yeast-free bakery products from wheat flour. In Eastern-European Journal of Enterprise Technologies (Vol. 4, Issue 11(112), pp. 50–57). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2021.238148
4. Navrotskyi, S., Guo, G., Baenziger, P. S., Xu, L., & Rose, D. J. (2019). Impact of wheat bran physical properties and chemical composition on whole grain flour mixing and baking properties. In Journal of Cereal Science (Vol. 89, p. 102790). Elsevier BV. https://doi.org/10.1016/j.jcs.2019.102790
5. Alashbayeva, L., Shansharova, D., Mynbayeva, A., Borankulova, A., & Soltybayeva, B. (2021). Development of technology for bakery products. In Food Science and Technology (Vol. 41, Issue 3, pp. 775–781). FapUNIFESP (SciELO). https://doi.org/10.1590/fst.61120
6. Muldabekova, B., Zhazykbayeva, G., Maliktayeva, P., Izteliyeva, R., & Alashbayeva, L. (2023). Preparation and examination of the quality of gingerbread made with composite flour and sugar beet. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 17, pp. 514–528). HACCP Consulting. https://doi.org/10.5219/1880
7. Nkurikiye, E., Lou, H., Churchill, F., Watt, J., Blodgett, P., Karkle, E., Siliveru, K., & Li, Y. (2023). Physicochemical, rheological, and baking properties of wheat flours from different mill streams. In Cereal Chemistry (Vol. 101, Issue 3, pp. 468–479). Wiley. https://doi.org/10.1002/cche.10744
8. Koehler, P. (2003). Concentrations of Low and High Molecular Weight Thiols in Wheat Dough As Affected by Different Concentrations of Ascorbic Acid. In Journal of Agricultural and Food Chemistry (Vol. 51, Issue 17, pp. 4948–4953). American Chemical Society (ACS). https://doi.org/10.1021/jf0260601
9. Kononenko, L. M., Yevchuk, Ya. V., Voitovska, V. I., & Tretiakova, S. O. (2021). The use of a sesame flour in a special bread technology. In Collected Works of Uman National University of Horticulture (Vol. 1, Issue 98, pp. 299–306). Uman National University of Horticulture. https://doi.org/10.31395/2415-8240-2021-98-1-299-306
10. Bilyk, O., Bondarenko, Y., Hryshchenko, A., Drobot, V., Kovbasa, V., & Shutyuk, V. (2018). Studying the effect of sesame flour on the technological properties of dough and bread quality. In Eastern-European Journal of Enterprise Technologies (Vol. 3, Issue 11 (93), pp. 6–16). Private Company Technology Center. https://doi.org/10.15587/1729-4061.2018.133233
11. Kamar, M., Evans, C., & Hugh-Jones, S. (2016). Factors influencing adolescent whole grain intake: A theory-based qualitative study. In Appetite (Vol. 101, pp. 125–133). Elsevier BV. https://doi.org/10.1016/j.appet.2016.02.154
12. Yr Mykolenko, S., Aliiev, E. B., & Yr Dudin, V. (2023). Composite wheat-amaranth flour baking properties and bread freshness. In IOP Conference Series: Materials Science and Engineering (Vol. 1277, Issue 1, p. 012021). IOP Publishing. https://doi.org/10.1088/1757-899x/1277/1/012021
13. Iztayev, A., Baibatyrov, T., Mukasheva, T., Muldabekova, B., & Yakiyayeva, M. (2020). Experimental studies of the baisheshek barley grain processed by the ion-ozone mixture. In Periódico Tchê Química (Vol. 17, Issue 35, pp. 239–258). Dr. D. Scientific Consulting. https://doi.org/10.52571/ptq.v17.n35.2020.22_iztayev_pgs_239_258.pdf
14. Tursunbayeva, S., Iztayev, A., Mynbayeva, A., Alimardanova, M., Iztayev, B., & Yakiyayeva, M. (2021). Development of a highly efficient ion-ozone cavitation technology for accelerated bread production. In Scientific Reports (Vol. 11, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-021-98341-w

15. Szopa, A., Motyka, S., & Ekeirt, H. (2023). Chia sprouts and microgreens as a new nutraceutical raw materials and their health-promoting impact in modern dietetics. In Current Issues in Pharmacy and Medical Sciences (Vol. 36, Issue 1, pp. 33–44). Walter de Gruyter GmbH. https://doi.org/10.2478/cipms-2023-0008
16. Mburu, M. (2021). The Role of Chia Seeds Oil in Human Health: A Critical Review. In European Journal of Agriculture and Food Sciences (Vol. 3, Issue 4, pp. 1–4). European Open Science Publishing. https://doi.org/10.24018/ejfood.2021.3.4.270
17. Alekhina, N. N., Ponomareva, E. I., & Bakaeva, I. A. (2023). Development of Functional Bread Technology Based on a Baking Mixture of Bioactivated Wheat and Rye. In Food Metaengineering (Vol. 1, Issue 2). All-Russian Dairy Research Institute. https://doi.org/10.37442/fme.2023.2.16
18. Nurgozhina, Z., Shansharova, D., Umirzakova, G., Maliktayeva, P., & Yakiyayeva, M. (2022). The influence of grain mixtures on the quality and nutritional value of bread. In Potravinarstvo Slovak Journal of Food Sciences (Vol. 16, pp. 320–340). HACCP Consulting. https://doi.org/10.5219/1767
19. Iztayev, A., Yakiyayeva, M., Kulazhanov, T., Kizatova, M.,, Maemerov, M.,, Stankevych, G., Toxanbayeva, B., & Chakanova, Z. (2018). Efficient mathematical models of ion-ozon cavitation treatment for long-term storage of grain legume crops. Acta Technica CSAV (Ceskoslovensk Akademie Ved). (Vol. 63, Issue 1, pp. 1-8).
20. GOST 5669-96. (1997). Bakery products. Method for determining porosity. Minsk: Interstate Council for Standardization, Metrology and Certification, 2. Retrieved from: https://files.stroyinf.ru/Data2/1/4294823/4294823423.pdf?ysclid=ltoaeedqdy440625942
21. GOST 21094-2022. (2022). Bakery products. Methods of the moisture determination. Moscow: Russian Institute of Standardization, 13. Retrieved from: https://files.stroyinf.ru/Data/789/78939.pdf?ysclid=ltoaqiawqh65115428 3
22. GOST 5670-96, (1997). Bread, rolls and buns. Methods for determination of acidity. Minsk: Interstate Council for Standardization, Metrology and Certification, 5. Retrieved from: https://files.stroyinf.ru/Data2/1/4294823/4294823423.pdf?ysclid=ltoaeedqdy440625942
23. GOST 5667-65. (2006). Bread and bakery products. Rules of acceptance, methods of sampling, methods for determination of organoleptic characteristics and mass. Moscow: Standardinform, 5. Retrieved from: https://files.stroyinf.ru/Data2/1/4294823/4294823426.pdf?ysclid=ltoawpofui981965047
24. Ostapchuk, N.V. (1977). Mathematical modeling of technological processes of grain storage and processing. M.: Kolos (240 p.).
25. Ostapchuk, M.V., & Stankevich H.M. (2006). Mathematical modeling on a computer. Textbook, Odesa: Druk (313 p.).
26. Tauferová, A., & Hellingerová, H. (2023). Effect of whole-grain flour addition on bread sensory quality. In Maso international – journal of food science and technology (Vol. 13, Issue 1, pp. 3–13). Walter de Gruyter GmbH. https://doi.org/10.2478/mjfst-2023-0001
27. Alekhina, N., Ponomareva, E., Zharkova, I., & Grebenshchikov, A. (2021). Assessment of Functional Properties and Safety Indicators of Amaranth Flour Grain Bread. In Food Processing: Techniques and Technology (Vol. 51, Issue 2, pp. 323–332). Kemerovo State University. https://doi.org/10.21603/2074-9414-2021-2-323-332
28. Burchenko, L., Bilyk, O., Bondarenko, Y., Perederii, I., & Kochubei-Lytvynenko, O. (2018). Intestigation of the effect of a mixture of sprouted grains on the quality and nutritional value of bakery products. In Technology audit and production reserves (Vol. 6, Issue 3(44), pp. 42–47). Private Company Technology Center. https://doi.org/10.15587/2312-8372.2018.150602
29. Dapčević-Hadnađev, T., Tomić, J., Škrobot, D., Šarić, B., & Hadnađev, M. (2022). Processing strategies to improve the breadmaking potential of whole-grain wheat and non-wheat flours. In Discover Food (Vol. 2, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1007/s44187-022-00012-w
30. Li, L., Houghton, D., Lietz, G., Watson, A., Stewart, C. J., Bal, W., & Seal, C. J. (2022). Impact of Daily Consumption of Whole-Grain Quinoa-Enriched Bread on Gut Microbiome in Males. In Nutrients (Vol. 14, Issue 22, p. 4888). MDPI AG. https://doi.org/10.3390/nu14224888
31. Johnston, R., Martin, J. M., Vetch, J. M., Byker-Shanks, C., Finnie, S., & Giroux, M. J. (2019). Controlled sprouting in wheat increases quality and consumer acceptability of whole-wheat bread. In Cereal Chemistry (Vol. 96, Issue 5, pp. 866–877). Wiley. https://doi.org/10.1002/cche.10187
32. Cao, H., Gao, F., Shen, H., Su, Q., Guan, X., Sun, Z., & Yu, Z. (2023). Influence of partial substitution of wheat flour with sprouted oat flours on physicochemical and textural characteristics of wheat bread. In Journal of Cereal Science (Vol. 110, p. 103649). Elsevier BV. https://doi.org/10.1016/j.jcs.2023.103649

33. Ferreira, S. P. L., Jardim, F. B. B., Fonseca, C. R. da, & Costa, L. L. (2020). Whole-grain pan bread with the addition of jabuticaba peel flour. In Ciência Rural (Vol. 50, Issue 8). FapUNIFESP (SciELO). https://doi.org/10.1590/0103-8478cr20190623
34. Nevzorov, V., Matskevich, I., Koh, Z., Teplyashin, V., & Kireev, V. (2024). Method for producing wheat and barley grain bread from hulled grain. In Bulletin of KSAU (Vol. 0, Issue 1, pp. 215–221). Krasnoyarsk State Agrarian University. https://doi.org/10.36718/1819-4036-2024-1-215-221
35. Adegoke, A. S., Banji, A. F., Adetayo, A. O., & Ugochi, O. H. (2023). Nutritional and Sensory Quality Attributes of Bread Produced from Wheat-Sprouted Finger Millet Flour. In International Journal of Science for Global Sustainability (Vol. 9, Issue 2, pp. 35–44). Federal University Gusau. https://doi.org/10.57233/ijsgs.v9i2.456
36. Marti, A., Cardone, G., Pagani, M. A., & Casiraghi, M. C. (2018). Flour from sprouted wheat as a new ingredient in bread-making. In LWT (Vol. 89, pp. 237–243). Elsevier BV. https://doi.org/10.1016/j.lwt.2017.10.052
37. Saranraj, P., & Sivasakthivelan, P. (2016). Microorganisms involved in spoilage of bread and its control measures. In book: Bread and Its Fortification Nutrition and Health Benefits. Publisher: CRC Press, Taylor & Fransis Group, New York, USA. (Vol. 13, Issue 7, pp. 132-149).
38. Venturi, M., Galli, V., Pini, N., Guerrini, S., Sodi, C., & Granchi, L. (2021). Influence of different leavening agents on technological and nutritional characteristics of whole grain breads obtained from ancient and modern flour varieties. In European Food Research and Technology (Vol. 247, Issue 7, pp. 1701–1710). Springer Science and Business Media LLC. https://doi.org/10.1007/s00217-021-03740-y
39. Dovi, K. A. P., Solah, V. A., Taylor, J. R. N., & Johnson, S. K. (2024). Dough-making performance of composite whole-grain sorghum and whole-grain wheat flours as assessed by a micro-doughLAB assay. In Cereal Chemistry (Vol. 101, Issue 2, pp. 334–343). Wiley. https://doi.org/10.1002/cche.10761
40. Bruno, M. C., Posik, D. M., Zappa, M. E., Baroni, V., Wunderlin, D., Giovambattista, G., & García, P. P. (2020). Genetic identification of flax, chia and sesame seeds in processed foods. In Food Control (Vol. 118, p. 107374). Elsevier BV. https://doi.org/10.1016/j.foodcont.2020.107374
41. Calvo-Lerma, J., Asensio-Grau, A., García-Hernández, J., Heredia, A., & Andrés, A. (2022). Exploring the Impact of Solid-State Fermentation on Macronutrient Profile and Digestibility in Chia (Salvia hispanica) and Sesame (Sesamum Indicum) Seeds. In Foods (Vol. 11, Issue 3, p. 410). MDPI AG. https://doi.org/10.3390/foods11030410
42. Abdullah, M., Tufail, T., Hussain, M., Nadeem, M., Owais, M., Zulkiffal, M., Hammad Tanveer, M., & Al Jbawi, E. (2022). Effect of sprouted barley flour on the quality wheat of bread, biscuits and cakes. In Cogent Food & Agriculture (Vol. 8, Issue 1). Informa UK Limited. https://doi.org/10.1080/23311932.2022.2122272
43. Sun, W., Zhang, W., Xu, Y., Wang, P., & Shen, W. (2023). Dough rheology, pasting property, and steamed bread quality of wheat flour as affected by the addition of sprouted wheat flour. In International Journal of Food Properties (Vol. 26, Issue 1, pp. 2160–2170). Informa UK Limited. https://doi.org/10.1080/10942912.2023.2244699
44. C eacute sar, M. B., Natalia, B. B., Jane, M. L. N. G., & Sydnei, M. S. (2016). Influence of enzymes and ascorbic acid on dough rheology and wheat bread quality. In African Journal of Biotechnology (Vol. 15, Issue 3, pp. 55–61). Academic Journals. https://doi.org/10.5897/ajb2015.14931
45. Verheyen, C., Albrecht, A., Elgeti, D., Jekle, M., & Becker, T. (2015). Impact of gas formation kinetics on dough development and bread quality. In Food Research International (Vol. 76, pp. 860–866). Elsevier BV. https://doi.org/10.1016/j.foodres.2015.08.013
46. Samokhvalova, O., Oliinyk, S., Stepankova, G., & Shydakova-Kameniuka, O. (2022). Research of the influence of xanthan gum on rheological properties of dough and quality of bread made from sprouted wheat grain. In Acta Innovations (Issue 45, pp. 62–71). Research and Innovation Centre Pro-Akademia. https://doi.org/10.32933/actainnovations.45.6
47. Marklinder, I., Johansson, L., Haglund, Å., Nagel-Held, B., & Seibel, W. (1996). Effects of flour from different barley varieties on barley sour dough bread. In Food Quality and Preference (Vol. 7, Issues 3–4, pp. 275–284). Elsevier BV. https://doi.org/10.1016/s0950-3293(96)00033-x
48. Zafar, T. A., Allafi, A. R., Alkandari, D., & Al-Othman, A. (2020). Rheological characteristics of wheat–chickpea composite flour doughs and effect of Amla powder (Phyllanthus emblica L.) addition on the functional properties of bread. In Food Science and Technology International (Vol. 27, Issue 3, pp. 264–275). SAGE Publications. https://doi.org/10.1177/1082013220950068

49. Sayaslan, A., & Şahin, N. (2018). Effects of fermented-chickpea liquor (chickpea yeast) on whole-grain wheat flour bread properties. In Quality Assurance and Safety of Crops & Foods (Vol. 10, Issue 2, pp. 183–192). Codon Publications. https://doi.org/10.3920/qas2017.1225

50. Salgado, V. dos S. C. N., Zago, L., Fonseca, E. N. da, Calderari, M. R. da C. M., Citelli, M., & Miyahira, R. F. (2023). Chemical Composition, Fatty Acid Profile, Phenolic Compounds, and Antioxidant Activity of Raw and Germinated Chia (Salvia hispanica L.) Seeds. In Plant Foods for Human Nutrition (Vol. 78, Issue 4, pp. 735–741). Springer Science and Business Media LLC. https://doi.org/10.1007/s11130-023-01115-0

51. Lee, S. B. (2013). Quality Characteristics of Bread Added on Chia seed Powder. In Korean Journal of Human Ecology (Vol. 22, Issue 6, pp. 723–736). Korean Association of Human Ecology. https://doi.org/10.5934/kjhe.2013.22.6.723

52. Ariya, M., Esmaeilinezhad, Z., Naghizadeh, M. M., Dindarloo, M. M., Karimi, F., Kaveh, F., Marzban, S., Hormozi, K., & Barati-Boldaji, R. (2022). The barriers to whole-grain consumption among Iranian students. In Scientific Reports (Vol. 12, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-022-19606-6

53. Medveckienė, B., Kulaitienė, J., Jarienė, E., Vaitkevičienė, N., & Hallman, E. (2020). Carotenoids, Polyphenols, and Ascorbic Acid in Organic Rosehips (Rosa spp.) Cultivated in Lithuania. In Applied Sciences (Vol. 10, Issue 15, p. 5337). MDPI AG. https://doi.org/10.3390/app10155337

54. Comettant-Rabanal, R., Carvalho, C. W. P., Ascheri, J. L. R., Chávez, D. W. H., & Germani, R. (2021). Extruded whole grain flours and sprout millet as functional ingredients for gluten-free bread. In LWT (Vol. 150, p. 112042). Elsevier BV. https://doi.org/10.1016/j.lwt.2021.112042

55. Navarro, J. L., Losano Richard, P., Moiraghi, M., Bustos, M., León, A. E., & Steffolani, M. E. (2024). Effect of Different Wheat Sprouting Conditions on the Characteristics of Whole-Wheat Flour. In Food Technology and Biotechnology (Vol. 62, Issue 2, pp. 264–274). Faculty of Food Technology and Biotechnology - University of Zagreb. https://doi.org/10.17113/ftb.62.02.24.8435

56. Ariya, M., Esmaeilinezhad, Z., Naghizadeh, M. M., Dindarloo, M. M., Karimi, F., Kaveh, F., Marzban, S., Hormozi, K., & Barati-Boldaji, R. (2022). The barriers to whole-grain consumption among Iranian students. Research Square Platform LLC. https://doi.org/10.21203/rs.3.rs-1710700/v1

Funds:

This research received no external funding.

Acknowledgments:

The authors are very grateful to the leaderships of the Almaty Technological University and Taraz State University named after M. Kh. Dulaty for their help and support.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Liliya Alashbayeva, Taraz Regional University named after M.Kh. Dulaty, Suleimenov Street, 7, 080000, Taraz, Republic of Kazakhstan,
Tel.: +77072743085
E-mail: orken_liliya@mail.ru
ORCID: https://orcid.org/0000-0003-3099-5988

Makhamedkali Kenzhekhojayev, Taraz Regional University named after M.Kh. Dulaty, Suleimenov Street, 7, 080000, Taraz, Republic of Kazakhstan,
Tel.: + 77477251172
E-mail: k.mahamed@mail.ru
ORCID: https://orcid.org/0000-0001-6924-4589

Assel Borankulova, Taraz Regional University named after M.Kh. Dulaty, Suleimenov Street, 7, 080000, Taraz, Republic of Kazakhstan,
Tel.:+ 77017884551
E-mail: asel.boor@mail.ru
ORCID: https://orcid.org/0000-0003-3099-5988

Bayan Muldabekova, Almaty Technological University, Department of Technology of Bakery Products and Processing Industries, Tole bi Street, 100, 050012, Almaty, Republic of Kazakhstan,
Tel.: +77777044565
E-mail: bayan_10.04@mail.ru
ORCID: https://orcid.org/0000-0003-1848-4288

***Madina Yakiyayeva**, Almaty Technological University, Research Institute of Food Technologies, Tole bi 100, 050012, Almaty, Republic of Kazakhstan,
Tel.: +77011626749
E-mail: yamadina88@mail.ru
ORCID: https://orcid.org/0000-0002-8564-2912

Sholpan Tursunbayeva, Almaty Technological University, Department of Technology of Bakery Products and Processing Industries, Tole bi Street, 100, 050012, Almaty, Republic of Kazakhstan,
Tel.: +77077540777
E-mail: sh-tursunbayeva@mail.ru
ORCID: https://orcid.org/0000-0001-9645-3634

Zhanerke Dyusembaeva, Almaty Technological University, 1st year master's student of the Department of Technology of Bakery Products and Processing Industries, Tole bi Street, 100, 050012, Almaty, Republic of Kazakhstan,
Tel.: +77781890871
E-mail: zhanerkedusembaeva@gmail.com
ORCID: https://orcid.org/0009-0004-4526-6459

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 29.9.2024
Revised: 30.10.2024
Accepted: 12.11.2024
Published: 22.11.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 1006-1027
https://doi.org/10.5219/2028
ISSN: 1337-0960 online
www.potravinarstvo.com

Determination of the optimal storage zone of functional beverages based on sprouted grain extracts using mathematical models

Almaz Moldakarimov, Auelbek Iztayev, Nurzhan Muslimov, Madina Yakiyayeva, Bayan Muldabekova, Sholpan Tursunbayeva, Fatima Dikhanbayeva, Saida Shintassova, Zhanerke Dyusembaeva

ABSTRACT

Beverages based on sprouted cereals are an excellent basis for creating new types of functional foods, as they are rich in nutrients. Beverages made from sprouted grains aim to improve daily nutrition, prioritising food safety. The proper storage of these drinks depends on the processing techniques used, including chemical preservatives and the conditions under which they are stored. Thus, using a mathematical model, this study aimed to determine the optimal storage zone of functional beverages from sprouted raw materials with preservatives. The results of our study showed that the optimum storage temperature and citric acid content of wheat extract were 2.9% and +11°C; barley 2.4% and 18°C; triticale 2.2% and +11°C; sunflower 2.8% and +14°C; rapeseed 2.7% and +16°C; safflower 2.3% and +17°C; flax 2.6% and +17°C; soya 2.4% and +18°C; pea 2.3% and +18°C; chickpea 2.3% and +18°C, respectively. Overall, these outcomes theoretically support the processing of beverages from sprouted grains. Thus, for practical application, it is recommended to implement controlled storage environments with the recommended temperatures and ensure that citric acid is correctly dosed at the identified optimal levels to enhance the shelf life of beverages.

Keywords: beverage, mathematical model, storage, shelf life

INTRODUCTION

The need to enrich products with biologically active substances and dietary fibre is a major prerequisite for developing food products that meet the needs of consumers. Sprouting, also known as germination, is a natural process often used for various grains, legumes, and seeds, and it offers several benefits for nutrition, flavour, and digestion **[1]**. Sprouts of wheat and other cereals contain fibres, vitamins of the B group, antioxidants, and macro- and microelements, which have a positive effect on human health **[2]**. The germination process initiates various transformations as it revitalises the seed's metabolism. This results in the degradation of macronutrients and antinutritional substances while stimulating the synthesis of secondary metabolites that could provide health benefits **[3]**. Beverages are an excellent basis for creating new types of functional products. Currently, sprouted wheat, barley, rye, corn, buckwheat, triticale, rice, and sorghum are used to manufacture functional beverages **[4]**, **[5]**, **[6]**. In Kazakhstan, while cereal crops are primarily used for bread production, the population's modern shift towards a healthy lifestyle and demand for nutritious products has prompted manufacturers to diversify their grain-based offerings.

The developed beverages are intended to enrich the daily diet, so food safety issues are the main prerequisite for their use. Processing methods influence the effective storage of beverages, the presence of chemical preservatives, storage conditions, temperatures and the packaging materials. It is well known that the thermal method is widely used to increase the shelf life of food products. However, the existing heat treatment methods

reduce the nutritional value of food products **[7]**. Food spoilage and high preservation of food products are serious economic problems for producers, indicating the possibility of preserving food safety indicators through natural preservative application **[8]**.

Previous studies analysed changes in amylase activity, carbohydrate and during germination of domestic cereal grain varieties such as triticale, wheat and barley **[9]**; and protein-protease complex of leguminous crops in Kazakhstan **[10]**; as well as the technology of beverages based on extracts from sprouted grains and seeds **[11]**. However, no studies have been conducted on the optimisation of storage conditions of functional beverages based on these raw materials. Thus, using a mathematical model, this study aimed to determine the optimal storage zone of functional beverages from sprouted raw materials with preservatives.

Scientific Hypothesis

Mathematical modelling has identified the optimal storage zone for functional beverages made from sprouted raw materials with preservatives. The model predicts that specific temperature and citric acid concentration will maximise the beverage's shelf life.

MATERIAL AND METHODOLOGY

Samples

The research objectives are turbid liquids, close to emulsions, with suspensions products of hydrolysis of starchy part of cereals (wheat, triticale, barley), legumes (soya, pea, chickpea) and oilseeds (sunflower, rapeseed, flax, safflower) (Figure 1). Further details about the conditions for sprouting grains and oilseeds and the microbiological assessment can be found in our earlier research **[12]**. Muslimov et al. (2023) **[13]** provide information on extraction technology.

Chemicals

Sodium hydroxide (purity ≥ 99.9%) (Topan, Kazakhstan), phenolphthalein (purity ≥ 98.0%), citric acid (purity ≥ 99%) (Abris+, Russia), distilled water (purity ≥ 99%) (Terranova, Kazakhstan). All chemicals were of analytical grade quality.

Instruments

Burettes, flasks, laboratory glass pipettes, glasses, glass funnels (Kazlabpribor, Kazakhstan), electric heating plate (Tomanalit, Kazakhstan), and stopwatch (LabTime, Kazakhstan).

Laboratory Methods

The shelf life of functional beverages was determined using extracts from sprouted grains and oilseeds according to the requirements of Methodical Instructions (MUK) 4.2.1847-04 **[14]**. Next, the acidity was determined following GOST 6687.4-86 **[15]**.

According to GOST 28188-2014, the suggested shelf life for soft drinks is as follows: when stored at temperatures between 0 and +18 °C, drinks made without preservatives should be consumed within 30 days of production. For those that contain preservatives or are pasteurised, the shelf life can extend to a maximum of 6 months. This research examined two beverage storage methods utilising extracts from sprouted cereal grains, legumes, and oilseeds: one method involved no preservatives, while the other included citric acid as a preservative. Experimental studies investigated the preservation of functional drinks for 6 months from 14.03.2023 to 10.09.2023 (180 days).

According to GOST 28188-2014 “Soft drinks. General technical conditions,” acidity is one of the main quality indicators of beverages based on plant raw materials. The core of the acid determination method involved titrating the research samples with an alkaline solution and storing them for varying durations. A minimum of two parallel measurements is required.

Description of the Experiment

Sample preparation: During 30 days, the functional beverages were stored at 18±2°C, with a humidity of not more than 75%.

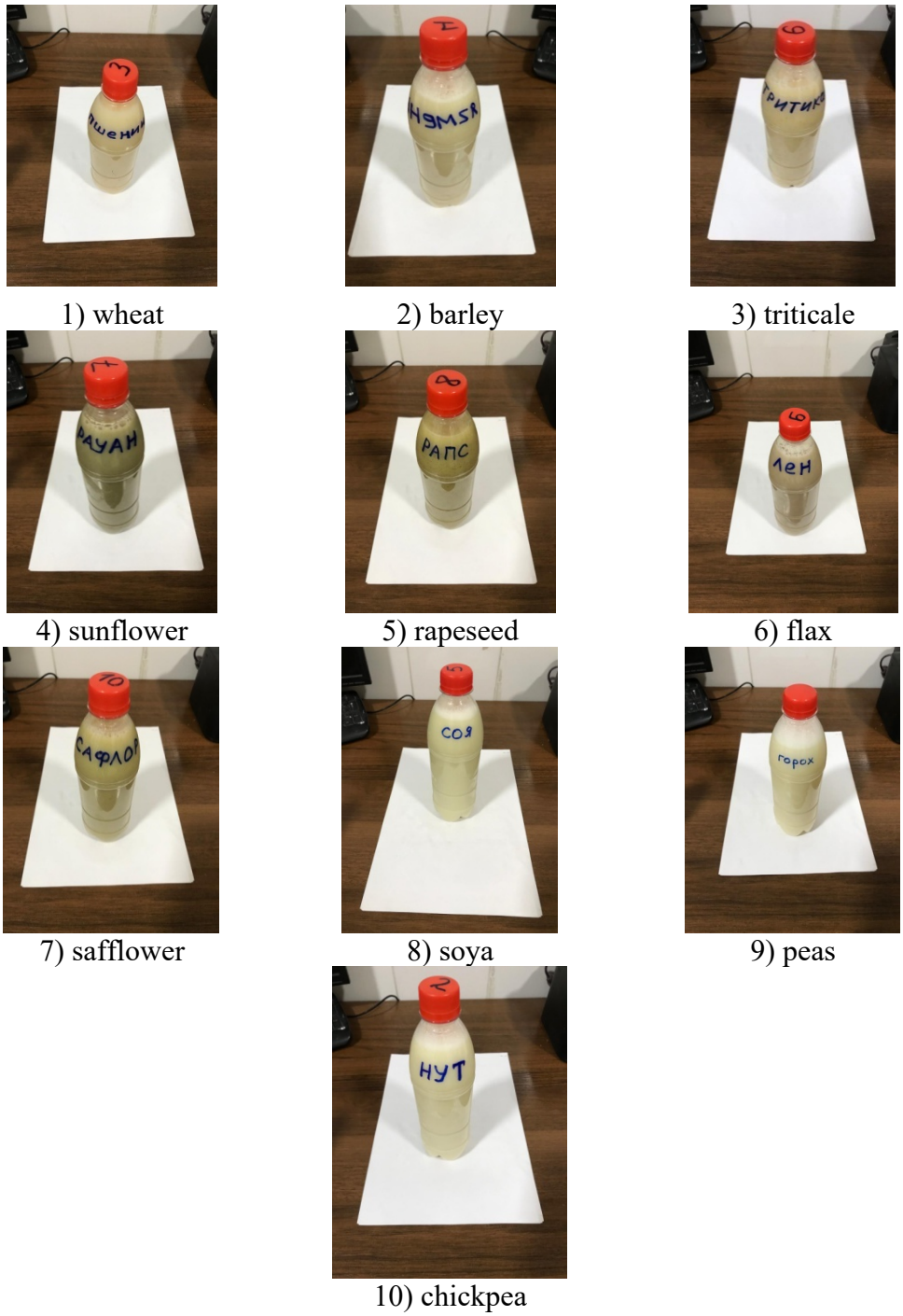
Number of samples analyzed: 10

Number of repeated analyses: 30

Number of experiment replication: 3

Design of the experiment:

In the first stage, we investigated the acidity change during the storage of functional beverages based on the obtained extracts from sprouted grains. Then, we studied the process of storing objects using citric acid as a preservative. Methods of mathematical processing of statistical data were used to process the results of laboratory studies, after which a model in three-dimensional space was constructed. To determine the optimal zones of the storage process of functional beverages with the addition of preservatives, graphs with contours of the calculated response surface were plotted.



1) wheat 2) barley 3) triticale

4) sunflower 5) rapeseed 6) flax

7) safflower 8) soya 9) peas

10) chickpea

Figure 1 Extracts from different sprouted grains and seeds.

Statistical Analysis

Statistical processing of laboratory study results was carried out using mathematical methods. Calculations were performed using the Statgraphics Centurion software package (v19). Data were analysed by an analysis of variance (ANOVA) procedure. *P*-values are less than 0.05, indicating that they significantly differ from zero at the 95% confidence level.

When processing the experimental results and investigating the response functions, we use the second-order equation of the following form:

$$y = b_0 + \sum^{n} b_i\, x_i + \sum_{i<j}^{n} b_{ij}\, x_i x_j + \sum^{n} b_{ii} {x_i}^2 + \ldots,$$

Where: y is the estimated value of the optimisation criterion; x_1, x_2, ..., x_n - independent variables (factors).

The experiment utilised a central composite design (CCD 2^2+star) of the second order. This method was employed to develop a mathematical model for the storage process of functional beverages that incorporate preservatives. This experimental setup examined two factors (K=2), resulting in 13 experimental trials. Among these trials, 5 were conducted at the central point to provide a baseline, while 6 coefficients were used to formulate the regression equation. The analysis also revealed that there were 7 degrees of freedom available for estimating the residual variance, which is essential for assessing the accuracy and reliability of the model.

RESULTS AND DISCUSSION

Determination of the acidity with and without preservatives

A significant portion of food loss can be attributed to food degradation during storage, transportation, or processing **[16]**. Employing precise methodologies to forecast and compute the shelf life of products within particular environmental parameters can yield invaluable insights for optimising storage and distribution strategies pertinent to those products **[17]**, **[18]**.

Acidity significantly influences functional beverages' quality, flavour, and preservation. These beverages are often designed to provide health benefits beyond basic nutrition **[19]**. Table 1 presents the dynamics of acidity changes in beverages based on germinated grain extracts during storage. The data analysis indicates that the acidity decreased on average by 0.8-1.1 cm3 1 M NaOH solution/100 cm3 of the drink throughout the storage period.

Table 1 Changes in acidity during storage of functional beverages based on obtained extracts from sprouted grains.

Beverages based on extracts	Storage time, days					
	5	10	15	20	25	30
Wheat	2.4	2.3	2.1	2.0	1.7	1.5
Barley	2.5	2.3	2.1	1.9	1.8	1.6
Triticale	2.8	2.5	2.2	2.0	1.9	1.8
Sunflower	3.3	3.0	2.8	2.5	2.3	2.2
Rapeseed	3.2	3.1	2.9	2.6	2.4	2.3
Safflower	3.2	3.0	2.8	2.5	2.4	2.2
Falx	3.3	3.1	2.7	2.5	2.4	2.3
Soya	3.5	3.3	3.0	2.8	2.6	2.4
Peas	3.1	3.0	2.8	2.5	2.4	2.3
Chickpea	3.0	2.8	2.5	2.4	2.2	2.1

Meanwhile, citric acid is often added to beverages for its natural preservative properties. It helps prevent microbial growth and prolongs shelf life **[20]**. Table 2 exhibits the acidity changes by adding 1%, 2%, and 3% citric acid. Analysis of the presented data shows that with increasing storage time, the acidity values of the extracts decrease, which can be attributed to microbial metabolism and chemical reactions, such as the breakdown of certain compounds, which can lead to the production of alkaline byproducts, which can neutralise the acidity **[21]**. Generally, plant-based beverages tend to have a shorter shelf life than dairy products due to their high moisture content, processing conditions, and lower acidity levels **[22]**.

Table 2 Change of acidity with addition of preservatives.

Extracts	Duration of storage, months					
	1	2	3	4	5	6
Wheat	2.9	2.8	2.5	2.4	2.0	1.8
Barley	3.0	2.8	2.5	2.3	2.2	1.9
Triticale	3.4	3.0	2.6	2.4	2.3	2.2
Sunflower	4.0	3.6	3.4	3.0	2.8	2.6
Rapeseed	3.8	3.7	3.5	3.1	2.9	2.8
Safflower	3.8	3.6	3.4	3.0	2.9	2.6
Flax	4.0	3.7	3.2	3.0	2.9	2.8
Soybeans	4.2	4.0	3.6	3.4	3.1	2.9
Peas	3.7	3.6	3.4	3.0	2.9	2.8
Chickpea	3.6	3.4	3.0	2.9	2.6	2.5
with 2% citric acid						
Wheat	3.5	3.3	3.0	2.9	2.4	2.2
Barley	3.6	3.3	3.0	2.7	2.6	2.3
Triticale	4.0	3.6	3.2	2.9	2.7	2.6
Sunflower	4.8	4.3	4.0	3.6	3.3	3.2
Rapeseed	4.6	4.5	4.2	3.7	3.5	3.3
Safflower	4.6	4.3	4.0	3.6	3.5	3.2
Flax	4.8	4.5	3.9	3.6	3.5	3.3
Soybeans	5.0	4.8	4.3	4.0	3.7	3.5
Peas	4.5	4.3	4.0	3.6	3.5	3.3
Chickpea	4.3	4.0	3.6	3.5	3.2	3.0
with 3% citric acid						
Wheat	4.1	4.0	3.6	3.5	2.9	2.6
Barley	4.3	4.0	3.6	3.3	3.1	2.8
Triticale	4.8	4.3	3.8	3.5	3.3	3.1
Sunflower	5.7	5.2	4.8	4.3	4.0	3.8
Rapeseed	5.5	5.4	5.0	4.5	4.1	4.0
Safflower	5.5	5.2	4.8	4.3	4.1	3.8
Flax	5.7	5.4	4.7	4.3	4.1	4.0
Soybeans	6.0	5.7	5.2	4.8	4.5	4.1
Peas	5.4	5.2	4.8	4.3	4.1	4.0
Chickpea	5.2	4.8	2.5	4.1	3.8	3.6

Development of the mathematical model and evaluation of adequacy

Using optimization techniques, mathematical models can help identify the best combination of temperature and citric acid concentration that maximises the shelf-life or sensory qualities of the beverages. Previously, mathematical models were used to quantify functional beverage components, enabling a better understanding of how they interact and influence the final beverage product **[23]**, **[24]**. The interplay between acidity, storage duration, and temperature is vital in formulating and preserving functional beverages. Understanding these parameters is essential for manufacturers seeking to optimise flavour, enhance health benefits, and ensure product safety **[25]**.

Based on experimental studies of the storage process of functional drinks with the addition of preservatives, the following factors have been established: citric acid content x_1 (C, %) and temperatures x_2 (T, °C) influencing the optimisation criteria, including wheat extract acidity y_1, barley extract acidity y_2, triticale extract acidity y_3, sunflower extract acidity y_4, rapeseed extract acidity y_5, safflower extract acidity y_6, flax extract acidity y_7, soya extract acidity y_8, pea extract acidity y_9, chickpea extract acidity y_{10}. Next, we coded the intervals and levels of variation of input parameters, which are presented in Table 3. The planning matrix is presented in Table 4.

Table 3 Coding of intervals and levels of variation of input factors.

Factors		Variation levels					Variation intervals
Natural	**Encoded**	**-1.414**	**-1**	**0**	**+1**	**+1.414**	
Citric acid content, %	x_1	0.58	1	2	3	3.41	1
Storage temperature, °C	x_2	1.17	4	14	24	6.83	10

Table 4 Rotatable planning matrix of experimental studies of the storage process of functional beverages with the addition of preservatives.

Encoded values		Natural values		Experimental values									
x_1	x_2	C. %	T. °C	y_1	y_2	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}
2	3	4	5	6	7	9	10	11	12	13	14	15	16
0	-1.414	2.0	0	3.43	3.05	3.83	4.56	5.15	3.72	5.42	4.17	3.62	3.46
-1.414	0	0.58	14	2.97	3.01	3.40	4.34	4.81	3.80	5.13	4.20	3.70	3.60
0	0	2.0	14	3.97	4.00	4.48	5.15	5.29	5.11	5.54	5.58	5.01	4.83
-1	-1	1.0	4	3.17	3.20	3.62	4.98	4.25	4.06	4.66	4.48	3.96	3.85
0	0	2.0	14	3.94	3.90	4.37	5.09	5.15	4.98	5.42	5.45	4.88	4.71
0	0	2.0	14	3.96	4.00	4.48	5.07	5.22	5.11	5.48	5.58	5.01	4.83
+1	-1	3.0	4	3.87	3.80	4.26	5.21	4.81	4.53	5.13	5.31	4.75	4.58
+1	+1	3.0	24	3.57	3.60	3.62	4.95	5.15	4.74	5.42	5.03	4.48	4.34
0	+1.414	2.0	28	2.95	4.30	3.40	4.75	5.00	4.90	5.42	6.00	5.40	5.20
0	0	2.0	14	3.94	4.10	4.58	5.21	5.22	5.04	5.48	5.72	5.14	4.95
-1	+1	1.0	24	3.22	3.10	3.51	4.46	4.74	3.93	5.07	4.34	3.83	3.72
+1.414	0	3.41	14	4.11	3.70	3.83	5.12	5.36	4.32	5.59	5.17	4.62	4.46
0	0	2.0	14	3.95	3.90	4.37	5.09	5.22	4.88	5.48	5.45	4.88	4.71

Note: x_1: citric acid content (C, %); x_2: temperatures (T, °C); y_1: wheat extract acidity; y_2: barley extract acidity; y_3: triticale extract acidity; y_4: sunflower extract acidity; y_5: rapeseed extract acidity; y_6: safflower extract acidity; y_7: flax extract acidity; y_8: soya extract acidity; y_9: pea extract acidity; y_{10}: chickpea extract acidity.

Tables 5-14 summarise the results of the analysis of variance for acidity.

Table 5 Analysis of variance (ANOVA) for wheat extract acidity.

Mean	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
x_1	0.885914	1	0.885914	77.24	0.0000
x_2	0.10784	1	0.10784	9.40	0.0182
x_1^2	0.232648	1	0.232648	20.28	0.0028
$x_1 x_2$	0.030625	1	0.030625	2.67	0.1463
x_2^2	0.890953	1	0.890953	77.68	0.0000
Lack of fit	0.0802885	7	0.0114698	-	-
Pure error	2.12689	12	-	-	-
Total (corr.)	0.885914	1	0.885914	77.24	0.0000

The ANOVA analyses the variability in the acidity of wheat extract by dividing it into distinct components associated with each factor (see Table 5). It subsequently assesses the statistical significance of these factors by comparing the root mean square (RMS) value to the estimated experimental error. The R^2 statistic indicates that the fitted model accounts for 96.2251% of the variability in the acidity of wheat extract, indicating that most of the differences in acidity can be attributed to the factors included in the model. This suggests that the model provides an excellent fit. The adjusted R^2 of 93.53% is ideal for comparing models with varying numbers of independent variables. The standard error was 0.1071, indicating the standard deviation of the residuals. The mean absolute error (MAE) of 0.0571 represents the average of the residuals. The Durbin-Watson (DW) statistic assesses whether there is a significant correlation among the residuals based on their order in the dataset, with a *P*-value exceeding 5%, suggesting no serial autocorrelation at the 5% significance level. The ANOVA results show that all factors are statistically significant ($P<0.05$) except for the interaction term x_1x_2 ($P>0.05$).

Table 6 Analysis of variance (ANOVA) for barley extract acidity.

Mean	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
x_1	0.538619	1	0.538619	76.95	0.0009
x_2	0.269291	1	0.269291	38.47	0.0034
x_1^2	0.780695	1	0.780695	111.53	0.0005
$x_1 x_2$	0.0025	1	0.0025	0.36	0.5823
x_2^2	0.213044	1	0.213044	30.43	0.0053
Lack of fit	0.552585	3	0.184195	-	-
Pure error	0.028	4	0.007	-	-
Total (corr.)	2.29371	12	-	-	-

In Table 6, the R-squared value reveals that the model accounts for 94.69% of the variability in acidity, indicating a strong fit. The adjusted R^2 is 56.61%. The standard error of the estimate is 0.083666. The MAE is 0.167304, providing another measure of model accuracy. The ANOVA results demonstrate that all factors are statistically significant except for the interaction term x_1x_2, which has an insignificant *P*-value, implying that this particular interaction does not explain the variability in acidity.

Table 7 Analysis of variance (ANOVA) for triticale extract acidity.

Mean	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
x_1	0.230556	1	0.230556	21.76	0.0023
x_2	0.23056	1	0.23056	21.76	0.0023
x_1^2	1.03716	1	1.03716	97.89	0.0000
$x_1 x_2$	0.070225	1	0.070225	6.63	0.0368
x_2^2	1.03716	1	1.03716	97.89	0.0000
Lack of fit	0.0741665	7	0.0105952	-	-
Pure error	2.44049	12	-	-	-
Total (corr.)	0.230556	1	0.230556	21.76	0.0023

The R^2 statistic indicates that the above model explains 96.96% of the variability in acidity of triticale extract (Table 7). The adjusted R^2 is 94.7903%. The standard deviation of the residuals is 0.102933. The MAE is 0.0687698.

Table 8 Analysis of variance (ANOVA) for the acidity of sunflower extract.

Mean	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
x_1	0.415455	1	0.415455	125.14	0.0004
x_2	0.0326788	1	0.0326788	9.84	0.0349
x_1^2	0.1445	1	0.1445	43.52	0.0027
$x_1 x_2$	0.0169	1	0.0169	5.09	0.0870
x_2^2	0.22948	1	0.22948	69.12	0.0011
Lack of fit	0.241928	3	0.0806427	-	-
Pure error	0.01328	4	0.00332	-	-
Total (corr.)	1.05237	12	-	-	-

In Table 8, the R^2 statistic reveals that the fitted model accounts for 95.7492% of the variability in the acidity of sunflower extract. The adjusted R^2 statistic stands at 58.4272%. The standard error of estimation indicates that the standard deviation of the residuals is 0.0576194. Meanwhile, the MAE is 0.105824. The ANOVA results show that all factors are statistically significant except for x_1x_2 ($P > 0.05$).

Table 9 Analysis of variance (ANOVA) for the acidity of rapeseed extract.

Mean	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
x_1	0.381857	1	0.381857	155.86	0.0002
x_2	0.0477198	1	0.0477198	19.48	0.0116
x_1^2	0.163111	1	0.163111	66.58	0.0012
$x_1 x_2$	0.005625	1	0.005625	2.30	0.2043
x_2^2	0.173937	1	0.173937	70.99	0.0011
Lack of fit	0.374984	3	0.124995	-	-
Pure error	0.0098	4	0.00245	-	-
Total (corr.)	1.11817	12	-	-	-

In Table 9, the R^2 indicates that the above model explains 95.588% of the variability in acidity. The adjusted R^2 is 41.008%. The estimation's standard error shows that the residuals' standard deviation is 0.04975. The MAE is 0.11815. The results of the ANOVA indicate statistical significance of all factors except for x_1x_2, since the *P* value was insignificant ($P>0.05$).

Table 10 Analysis of variance (ANOVA) for acidity of safflower extract

Mean	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
x_1	0.507722	1	0.507722	53.84	0.0018
x_2	0.382273	1	0.382273	40.54	0.0031
x_1^2	1.40556	1	1.40556	149.05	0.0003
$x_1 x_2$	0.0289	1	0.0289	3.06	0.1549
x_2^2	0.732523	1	0.732523	77.68	0.0009
Lack of fit	0.3864	3	0.1288	-	-
Pure error	0.03772	4	0.00943	-	-
Total (corr.)	3.24883	12	-	-	-

Furthermore, the R^2 statistic shows that the fitted model explains 95.7492% of the variability in the acidity of safflower extract (Table 10). The adjusted R^2 statistic is 58.4272%. The standard error of estimation was 0.0576194. Additionally, the MAE is 0.105824, while the interaction term x_1x_2 was insignificant ($P>0.05$).

Table 11 Analysis of variance (ANOVA) for acidity of flax extract

Mean	Sum of squares	Degree of freedom e	Mean square	F-value	*P*-value
x_1	0.270309	1	0.270309	150.17	0.0003
x_2	0.0612497	1	0.0612497	34.03	0.0043
x_1^2	0.136348	1	0.136348	75.75	0.0010
$x_1 x_2$	0.0036	1	0.0036	2.00	0.2302
x_2^2	0.0841739	1	0.0841739	46.76	0.0024
Lack of fit	0.26964	3	0.0898798	-	-
Pure error	0.0072	4	0.0018	-	-
Total (corr.)	0.807908	12	-	-	-

The R^2 statistic indicates that the fitted model explains 95.7338% of the variability in acidity of flax extract (Table 11). The adjusted R^2 statistic is 41.2579%. The standard error of the estimation shows that the standard deviation of the residuals is 0.04264. The MAE is 0.107692. The results of the ANOVA indicate statistical significance of all factors except for x_1x_2, since the *P* value was insignificant ($P>0.05$).

Table 12 Analysis of variance (ANOVA) for acidity of soya extract.

Mean	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
x_1	1.0453	1	1.0453	82.76	0.0008
x_2	0.587531	1	0.587531	46.52	0.0024
x_1^2	1.4672	1	1.4672	116.17	0.0004
$x_1 x_2$	0.0049	1	0.0049	0.39	0.5671
x_2^2	0.467554	1	0.467554	37.02	0.0037
Lack of fit	1.15181	3	0.383937	-	-
Pure error	0.05052	4	0.01263	-	-
Total (corr.)	4.58851	12	-	-	-

The R^2 statistic shows that the model accounts for 93.7969% of the variation in acidity of soya extract (Table 12). The adjusted R^2 statistic is 55.0804%. The estimation's standard error shows that the residuals' standard deviation is 0.112383. The MAE is 0.237423. The results of the ANOVA indicate statistical significance of all factors except for x_1x_2, since the *P* value was insignificant ($P>0.05$).

Table 13 Analysis of variance (ANOVA) for acidity of pea extract.

Mean	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
x_1	0.939183	1	0.939183	79.39	0.0009
x_2	0.560367	1	0.560367	47.37	0.0023
x_1^2	1.29825	1	1.29825	109.74	0.0005
$x_1 x_2$	0.0049	1	0.0049	0.41	0.5549
x_2^2	0.459473	1	0.459473	38.84	0.0034
Lack of fit	1.07904	3	0.35968	-	-
Pure error	0.04732	4	0.01183	-	-
Total (corr.)	4.214	12	-	-	-

The R^2 statistic reveals that the fitted model accounts for 93.271% of the variability in acidity of pea extract (Table 13). The adjusted R-squared statistic is 54.1788%. The estimation's standard error shows that the residuals' standard deviation is 0.108766. The MAE is 0.229697. The results of the ANOVA indicate statistical significance of all factors, except for x_1x_2, since the *P* value was insignificant ($P>0.05$).

Table 14 Analysis of variance (ANOVA) for acidity of chickpea extract

Mean	Sum of squares	Degree of freedom	Mean square	F-value	*P*-value
x_1	0.823184	1	0.823184	81.67	0.0008
x_2	0.546392	1	0.546392	54.21	0.0018
x_1^2	1.1263	1	1.1263	111.74	0.0005
$x_1 x_2$	0.003025	1	0.003025	0.30	0.6130
x_2^2	0.443084	1	0.443084	43.96	0.0027
Lack of conformity	1.01048	3	0.336826	-	-
Pure error	0.04032	4	0.01008	-	-
Total (corr.)	3.83248	12	-	-	-

The R-squared statistic indicates that the fitted model explains 92.5818% of the variability in the acidity of chickpea extract (Table 14). The adjusted R-squared statistic is 52.9973%. The estimation's standard error shows that the residuals' standard deviation is 0.100399. The MAE is 0.219821. The results of the ANOVA indicate statistical significance of all factors except for x_1x_2 ($P>0.05$).

The interaction effects of the input factors are significant when the P-values from Tables 5-14 are compared with the corresponding regression coefficients in Table 15.

Table 15 Coefficients of regression equations of output parameters.

Coefficients	Optimisation criteria										
	y_1	y_2	y_3	y_4	y_5	y_6	y_7	y_8	y_9	y_{10}	y_{11}
b_0	1.771	1.451	2.725	1.682	4.005	3.647	2.018	4.13	2.011	1.598	1.617
b_1	1.188	1.634	1.562	1.899	0.713	0.884	1.931	0.786	2.247	2.12	1.969
b_2	0.106	-0.072	0.014	0.118	0.031	0.059	0.096	0.046	0.107	0.105	0.102
b_{11}	-0.183	-0.335	-0.377	-0.386	-0.144	-0.153	-0.45	-0.14	-0.459	-0.432	-0.402
b_{12}	-	-	0.015	-0.013	-	-	-	-	-	-	-
b_{22}	-0.004	-0.002	-	-0.004	-0.002	-0.002	-0.003	-0.001	-0.003	-0.003	-0.003

It follows from the analysis of variance that the influence of all remaining coefficients of the equation on the output parameter y is statistically significant (their corresponding values of significance levels P are less than 0.05). Consequently, the regression equation (mathematical model of the process) can be written in the following form:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{12}x_1x_2 + b_{11}x_1^2 + b_{22}x_2^2$$

Thus, the regression equations for the process of storage of functional drinks with the addition of citric acid will be as follows:

$$y_1 = 1.771 + 1.188x_1 + 0.106x_2 - 0.183x_1^2 - 0.004x_2^2$$

$$y_2 = 1.451 + 1.634x_1 - 0.072x_2 - 0.335x_1^2 - 0.002x_2^2$$

$$y_3 = 1.682 + 1.899x_1 + 0.118x_2 - 0.013x_1x_2 - 0.386x_1^2 - 0.004x_2^2$$

$$y_4 = 4.005 + 0.713x_1 + 0.031x_2 - 0.144x_1^2 - 0.002x_2^2$$

$$y_5 = 3.647 + 0.884x_1 + 0.059x_2 - 0.153x_1^2 + 0.002x_2^2$$

$$y_6 = 2.018 - 0.1574x_1 + 1.2616x_2 - 0.001x_1^2 + 0.308x_2^2$$

$$y_7 = 4.13 + 0.786x_1 + 0.046x_2 - 0.14x_1^2 - 0.001x_2^2$$

$$y_8 = 2.011 + 2.247x_1 + 0.107x_2 - 0.459x_1^2 - 0.003x_2^2$$

$$y_9 = 1.598 + 2.12x_1 + 0.105x_2 - 0.432x_1^2 - 0.003x_2^2$$

$$y_{10} = 1.617 + 1.969x_1 + 0.102x_2 - 0.402x_1^2 - 0.003x_2$$

The analysis of three-dimensional spatial models shows that the necessary values of the optimisation criterion y are achieved in the considered search area. This indicates that the variations of the initial factors in the planning of experiments are sufficiently taken into account. Figures 2-11 show graphical representations of dependence graphs.

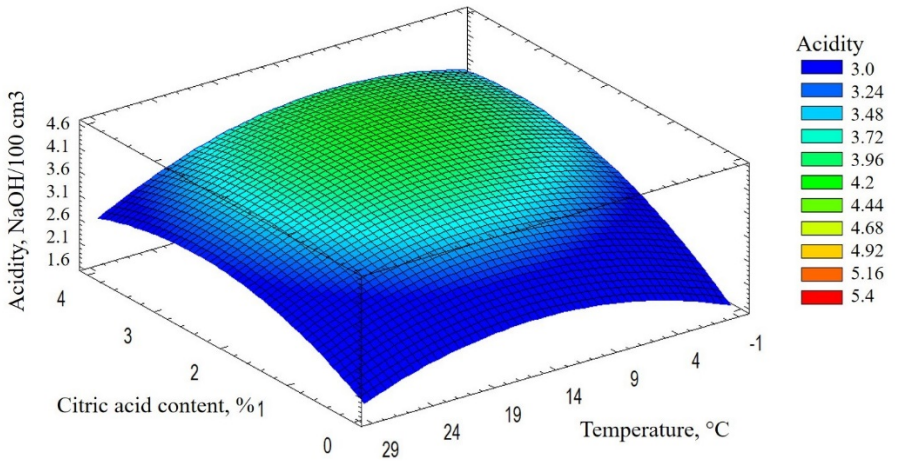


Figure 2 Three-dimensional space model characterising the dependence of $y_1=f(C, T)$ of citric acid content and storage temperature on wheat extract acidity.

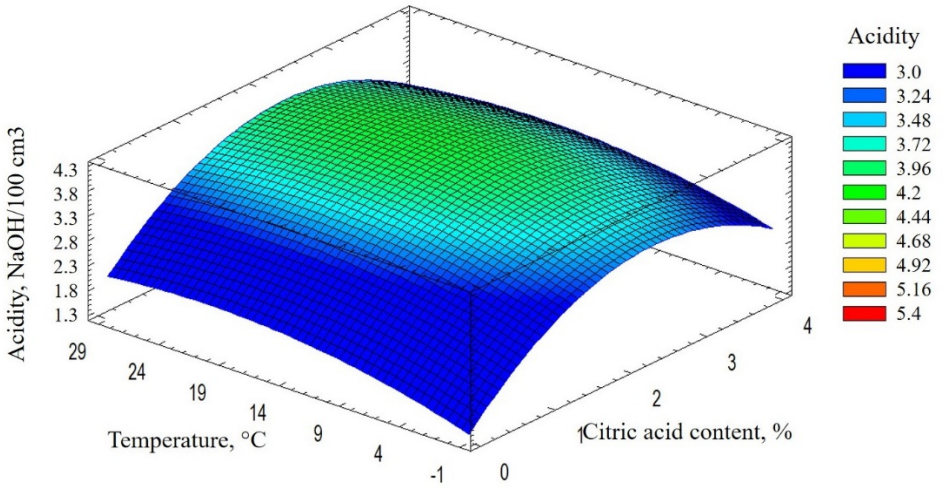


Figure 3 Three-dimensional space model characterising the dependence of $y_1=f(C, T)$ of citric acid content and storage temperature on barley extract acidity.

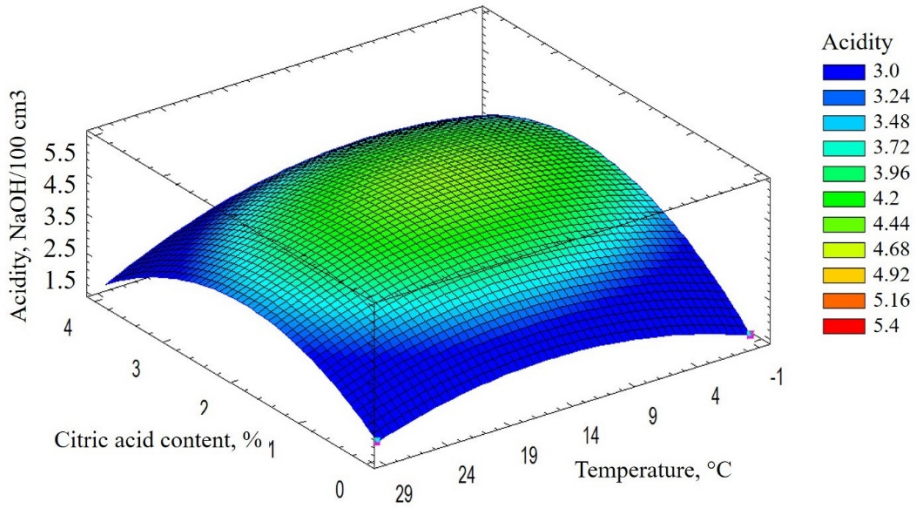


Figure 4 Three-dimensional space model characterising the dependence of $y_1=f(C, T)$ of citric acid content and storage temperature on triticale extract acidity.

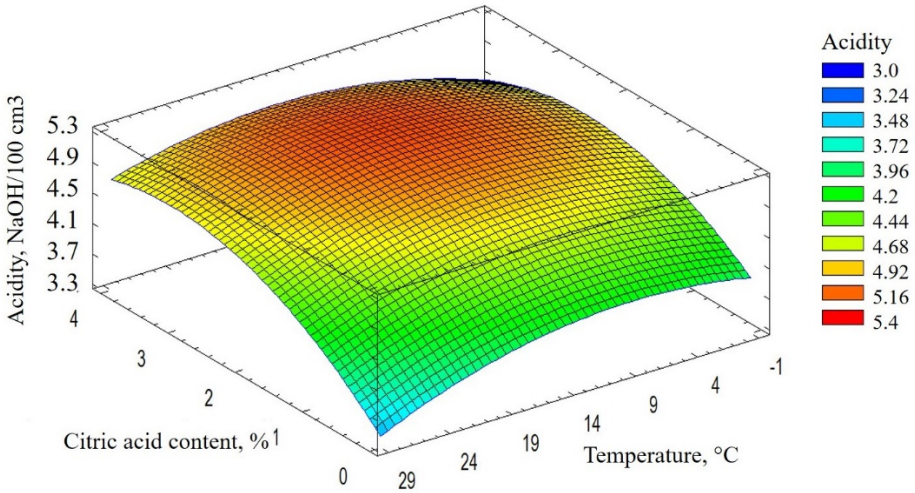


Figure 5 Three-dimensional space model characterising the dependence of $y_1=f(C, T)$ of citric acid content and storage temperature on sunflower extract acidity.

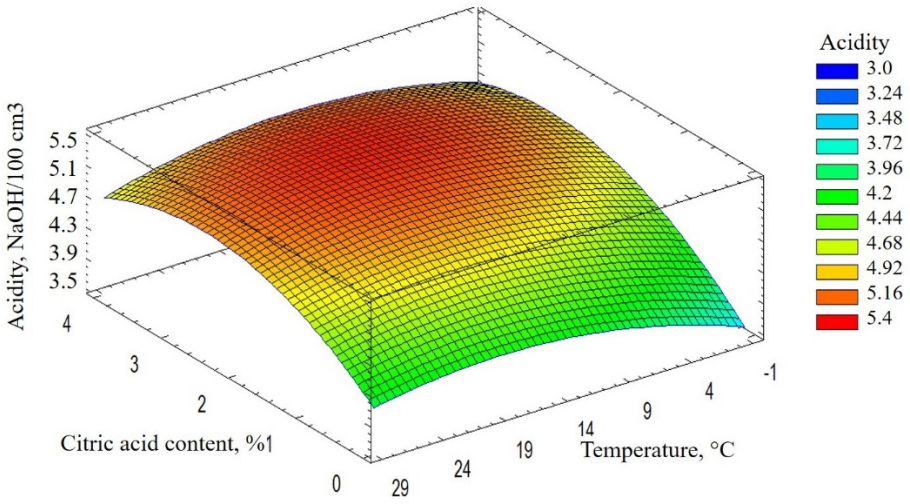


Figure 6 Three-dimensional space model characterising the dependence of $y_1=f(C, T)$ of citric acid content and storage temperature on rape extract acidity.

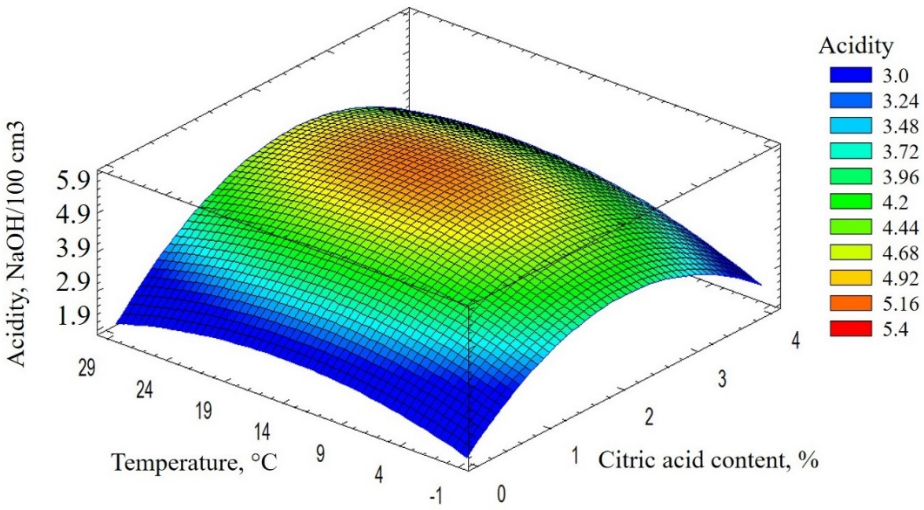


Figure 7 Three-dimensional space model characterising the dependence of $y_1=f(C, T)$ of citric acid content and storage temperature on safflower extract acidity.

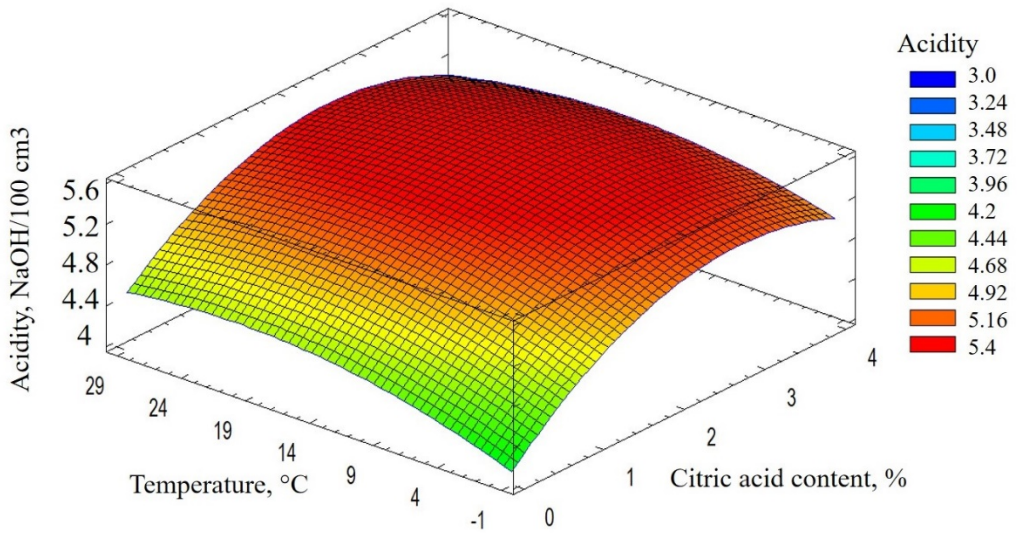


Figure 8 Three-dimensional space model characterising the dependence of $y_1=f(C, T)$ of citric acid content and storage temperature on flax extract acidity.

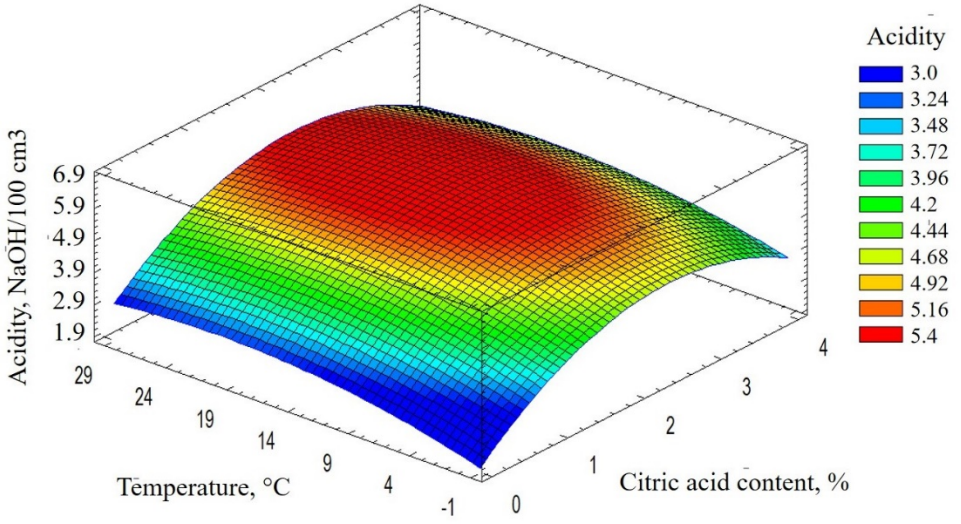


Figure 9 Three-dimensional model in space characterising the dependence of $y_1=f(C, T)$ citric acid content and storage temperature on soybean extract acidity.

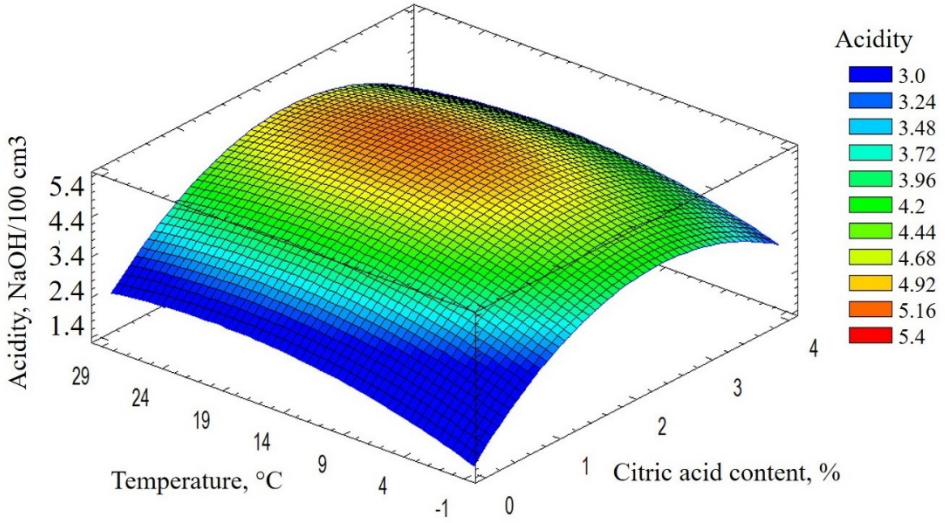


Figure 10 Three-dimensional space model characterising the dependence of $y_1=f(C, T)$ of citric acid content and storage temperature on pea extract acidity.

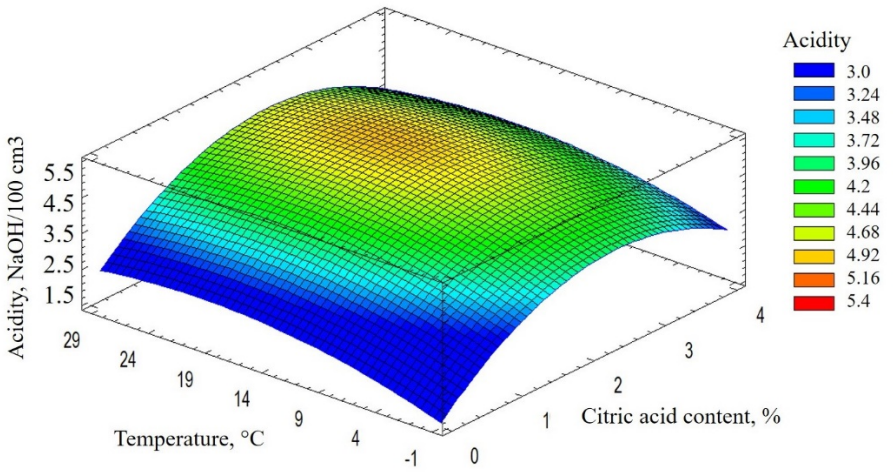


Figure 11 Three-dimensional space model characterising the dependence of $y_1=f(C, T)$ of citric acid content and storage temperature on chickpea extract acidity.

To determine the optimum zones for storing functional beverages with the addition of a preservative, graphs with contours of the calculated response surface were plotted, which are presented in Figures 12-21.

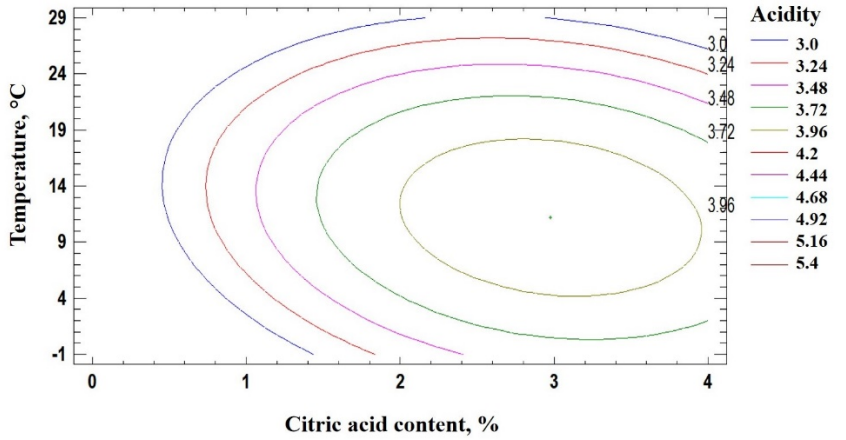


Figure 12 Contours of the calculated response surface characterising the dependence of citric acid content and storage temperature on wheat extract acidity.

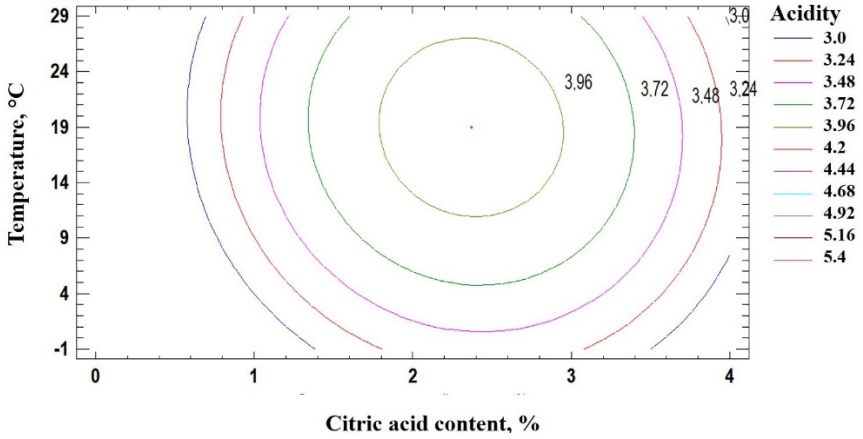


Figure 13 Contours of the calculated response surface characterising the dependence of citric acid content and storage temperature on barley extract acidity.

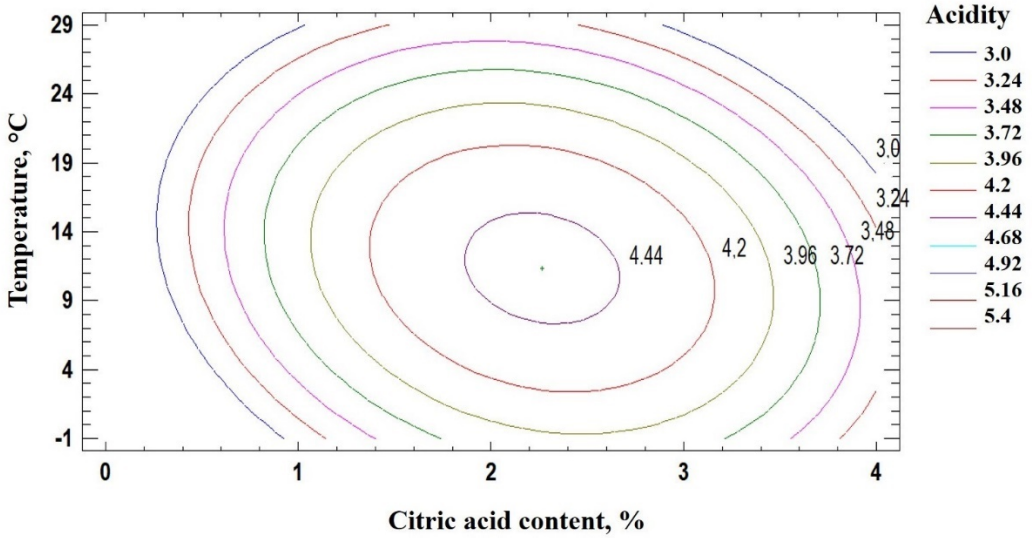


Figure 14 Contours of the calculated response surface characterising the dependence of citric acid content and storage temperature on triticale extract acidity.

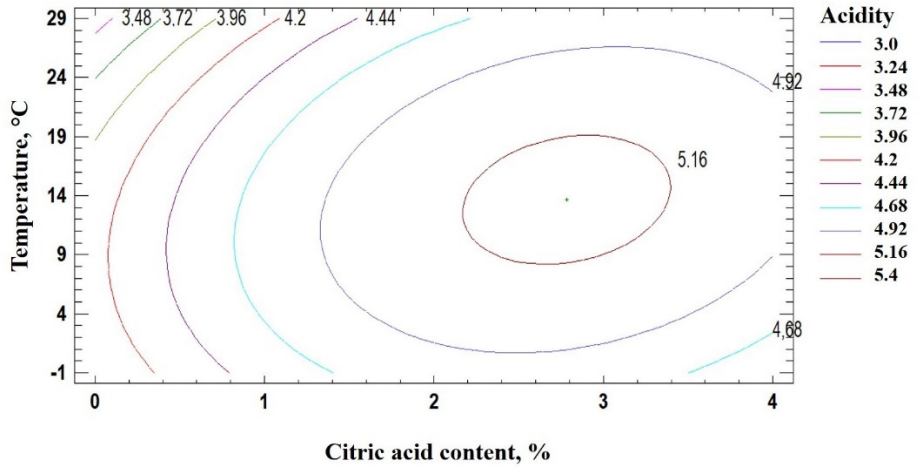


Figure 15 Contours of the calculated response surface characterising the dependence of citric acid content and storage temperature on sunflower extract acidity.

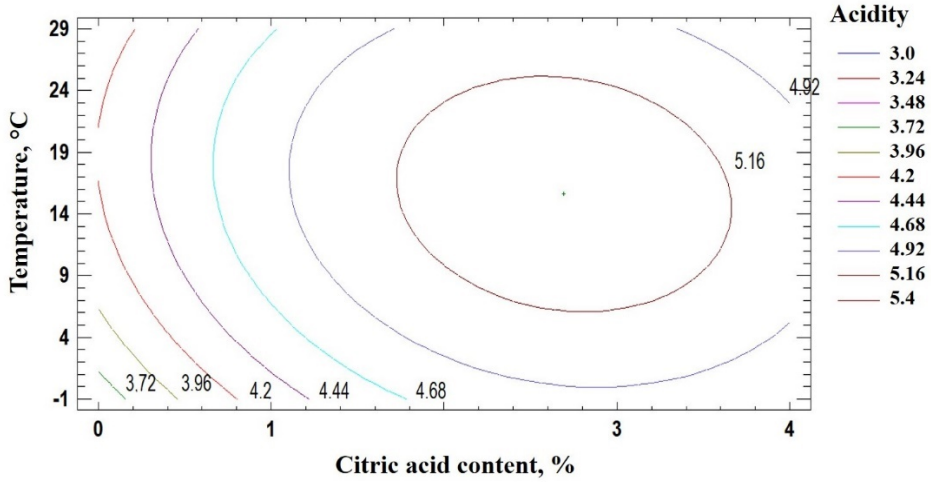


Figure 16 Contours of the calculated response surface characterising the dependence of citric acid content and storage temperature on rapeseed extract acidity.

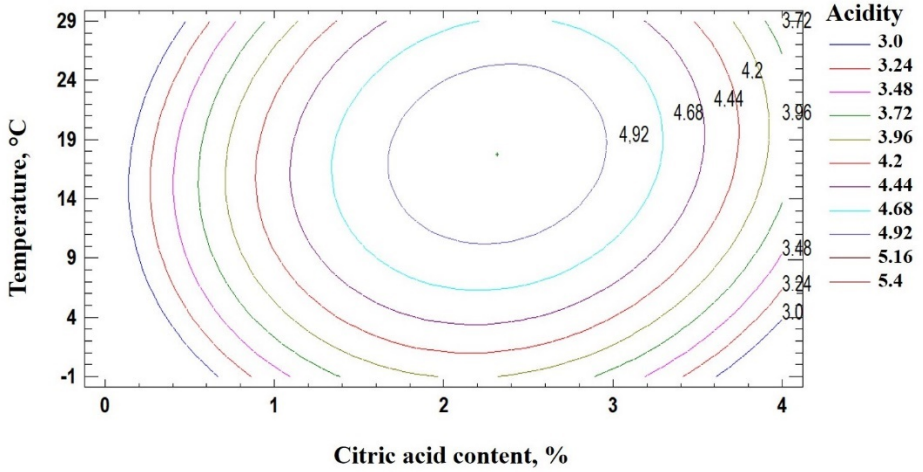


Figure 17 Contours of the calculated response surface characterising the dependence of citric acid content and storage temperature on the acidity of the safflower extract acidity.

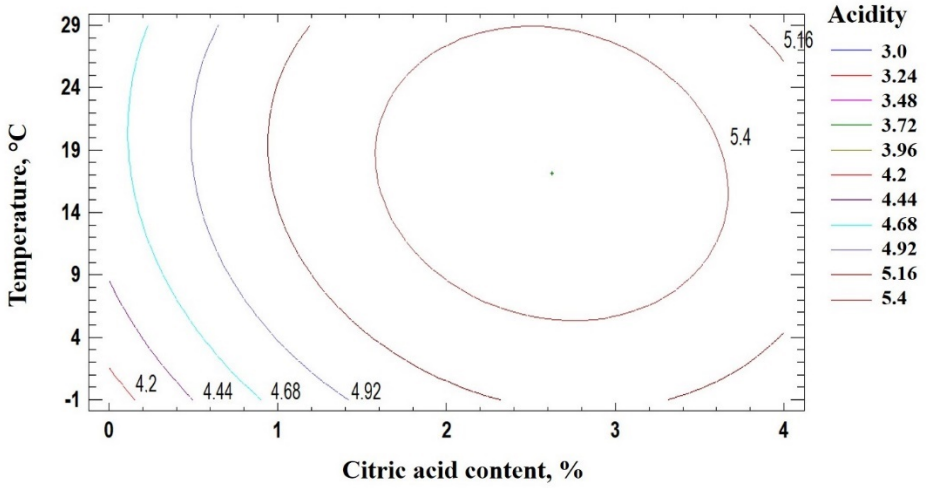


Figure 18 Contours of the calculated response surface characterising the dependence of citric acid content and storage temperature on flax extract acidity.

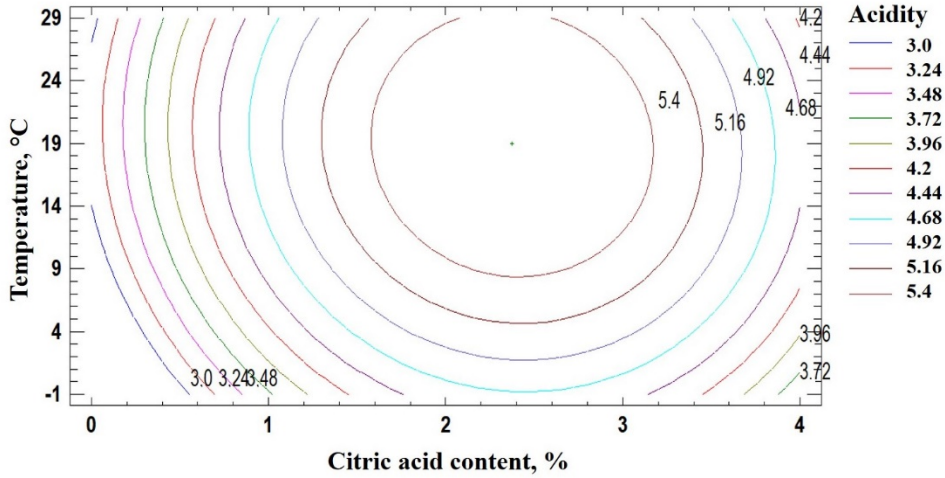


Figure 19 Contours of the calculated response surface characterising the dependence of citric acid content and storage temperature on soybean extract acidity.

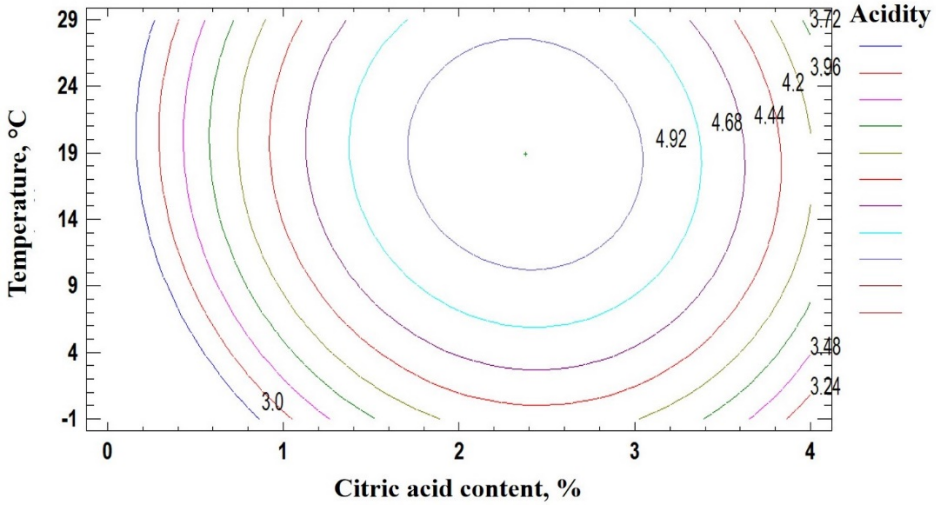


Figure 20 Contours of the calculated response surface characterising the dependence of citric acid content and storage temperature on the acidity of the pea extract.

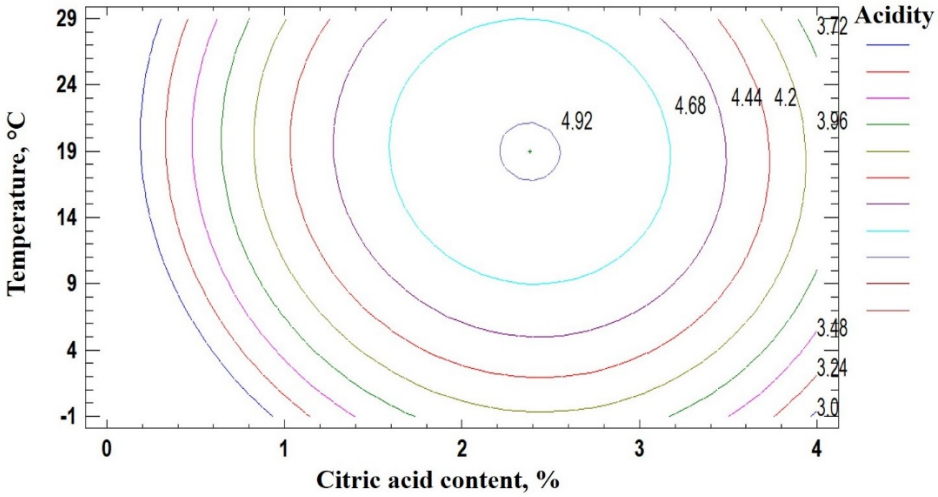


Figure 21 Contains the calculated response surface characterising the dependence of citric acid content and storage temperature on chickpea extract acidity.

It was established that the optimum zone of storage of research objects with the use of preservatives for wheat extract, which is achieved when the content of citric acid and storage temperature was 2.9% and +11°C; for barley extract was 2.4% and 18°C; for triticale, the extract was 2.2% and +11°C; for sunflower, the extract was 2.8% and +14°C; for rapeseed, the extract was 2.7% and +16°C; for safflower, the extract was 2.3% and +17°C; for flax, extract was 2.6% and +17°C; for soya, extract was 2.4% and +18°C; for pea, extract was 2.3% and +18°C; for chickpea extract was 2.3% and +18°C, respectively.

Response surface methodology (RSM) is an essential statistical and mathematical technique for optimising processes and understanding the relationships between several explanatory variables and one or more response variables **[26]**. In studying the shelf life of beverages, RSM offers advantages. Although many studies are using mathematical models analysing different beverages **[27]**, **[28]**, **[29]**; grains **[30]**, **[31]**, **[32]**, **[33]** and food products **[34]**, **[35]** from different aspects, there remains a notable deficiency in scholarly inquiry focused specifically on the optimal storage conditions for non-alcoholic functional beverages based on germinated grain extracts. For example, it was noted that soybeans harvested with a moisture content of 23%, dried at 80 °C, and stored at temperatures below 23 °C preserved their oil content (25.89%), crude protein (35.69%), and lipid acidity (5.54 mL), as demonstrated through mathematical modelling and multivariate analysis **[36]**.

Furthermore, the regression equation developed has been chosen as the mathematical model for predicting the shelf life of the functionally enriched sugarcane juice based on the independent variables **[37]**. A binary logistic regression model has been created to predict the growth of spoilage microorganisms in craft beer, demonstrating strong goodness of fit and accurate predictions in prior research **[38]**. Additionally, there has been a concise overview of the application of mathematical models for estimating the shelf life of coffee when stored under accelerated conditions **[28]**. Storage studies showed that the germinated brown rice beverage has beneficial nutritional properties, including high levels of total phenolic content, γ-oryzanol, niacin and γ-Aminobutyric acid **[39]**. The beverage with three parts jujube concentrate, two parts water, and 0.1% citric acid demonstrated better sensory and microbial quality than the other samples **[40]**. It was observed that the functional multigrain probiotic drink can be kept in refrigeration (4°C) for a maximum of 2 weeks without any decline in quality **[41]**. These findings indicate that the optimal formulation can significantly enhance sensory and microbial quality attributes, extending the drink's usability.

In addition, the results of the ANOVA analysis indicate that the interaction terms between citric acid content and temperature (x_1x_2) are not statistically significant across several scenarios. While the non-significant interaction terms between citric acid content and temperature suggest a consistent independent effect of each variable, it is crucial to acknowledge the current model's limitations. One possible limitation is that the model may oversimplify the complex biological or chemical interactions occurring in the beverage matrix, for example, additional factors such as pH, sugar content, or the presence of other acids may influence the relationship between temperature, citric acid content, and sensory characteristics of beverages. In addition, the ranges of citric acid and temperature investigated may not reflect potential nonlinear effects or other interactions that may be relevant at different levels or combinations of these variables. Given these considerations, including more independent variables (e.g. pH or presence of other flavour compounds) in the analysis could provide a more complete model that takes into account interactions not assessed in this study. Also, more advanced statistical techniques, such as

mixed-effects models, could account for individual variations and allow for a more thorough examination of interactions **[42]**. Additionally, we plan to enhance our model's reliability by conducting further experiments to assess the statistical significance of citric acid content and temperature interactions in different beverage types. By expanding our research to include external data, we hope to validate our existing model and provide more robust conclusions in our upcoming studies.

Current storage technologies may not adequately maintain the optimal conditions required for preserving products, particularly in less industrialized markets where traditional storage facilities often struggle to control humidity and temperature precisely **[43]**. This challenge is compounded by the significant financial investments that innovative storage techniques may require, creating barriers for smaller companies in the functional beverage industry. However, the findings from the present study offer promising advancements specifically tailored to the functional beverage sector, particularly concerning the storage of beverages derived from sprouted grains and oilseeds. Manufacturers can create products with longer shelf lives without compromising flavour or nutritional value by identifying optimal storage parameters such as the concentration of citric acid as a preservative and appropriate temperature ranges. This research supports the development of new functional beverages that emphasise the nutritious properties and stability of sprouted grains and highlights the economic benefits of effective storage parameters. Besides, implementing these optimised storage practices can reduce waste due to spoilage, lower logistics costs, and ultimately increase inventory turnover rates. Therefore, the insights gathered from this study could pave the way for smaller brands to enhance their competitive edge while fostering sustainable growth within the functional beverage market.

CONCLUSION

Collectively, a mathematical model has been developed in this study, which makes it possible to determine the optimal parameters of the process of storage of research objects. The study outcomes revealed the optimum zone of storage of functional beverages with the use of citric acid as a preservative for wheat (2.9% and +11°C), barley (2.4% and 18°C), triticale (2.2% and +11°C), sunflower (2.8% and +14°C), rapeseed (2.7% and +16°C); safflower (2.3% and +17°C), flax (2.6% and +17°C), soya (2.4% and +18°C), pea (2.3% and +18°C), chickpea (2.3% and +18°C) extracts. These results provide theoretical support for the storage of sprouted grain beverages. Thus, for practical application, it is recommended to implement controlled storage environments with the recommended temperatures and ensure that citric acid is correctly dosed at the identified optimal levels to enhance the shelf life of beverages. Additionally, monitoring microbial activity and sensory properties during storage can help ensure product quality and safety over time.

REFERENCES

1. Majzoobi, M., Wang, Z., Teimouri, S., Pematilleke, N., Brennan, C. S., & Farahnaky, A. (2023). Unlocking the Potential of Sprouted Cereals, Pseudocereals, and Pulses in Combating Malnutrition. In Foods (Vol. 12, Issue 21, p. 3901). MDPI AG. https://doi.org/10.3390/foods12213901
2. Maqbool, Z., Khalid, W., Mahum, Khan, A., Azmat, M., Sehrish, A., Zia, S., Koraqi, H., AL-Farga, A., Aqlan, F., & Khan, K. A. (2023). Cereal sprout-based food products: Industrial application, novel extraction, consumer acceptance, antioxidant potential, sensory evaluation, and health perspective. In Food Science & Nutrition (Vol. 12, Issue 2, pp. 707–721). Wiley. https://doi.org/10.1002/fsn3.3830
3. Peñaranda, J. D., Bueno, M., Álvarez, F., Pérez, P. D., & Perezábad, L. (2021). Sprouted grains in product development. Case studies of sprouted wheat for baking flours and fermented beverages. In International Journal of Gastronomy and Food Science (Vol. 25, p. 100375). Elsevier BV. https://doi.org/10.1016/j.ijgfs.2021.100375
4. Janiak, M. A., Karamać, M., Sulewska, K., Amarowicz, R., Denev, P., & Slavova-Kazakova, A. (2023). Phenolic Profile and Antioxidant Potential of Beverages from Buckwheat and Side Streams after Beverages Production. In Processes (Vol. 11, Issue 11, p. 3205). MDPI AG. https://doi.org/10.3390/pr11113205
5. Zenkova, M., Melnikova, L., & Timofeeva, V. (2023). Non-Alcoholic Beverages from Sprouted Buckwheat: Technology and Nutritional Value. In Food Processing: Techniques and Technology (pp. 316–325). Kemerovo State University. https://doi.org/10.21603/2074-9414-2023-2-2435
6. Patra, M., Bashir, O., Amin, T., Wani, A. W., Shams, R., Chaudhary, K. S., Mirza, A. A., & Manzoor, S. (2023). A comprehensive review on functional beverages from cereal grains-characterization of nutraceutical potential, processing technologies and product types. In Heliyon (Vol. 9, Issue 6, p. e16804). Elsevier BV. https://doi.org/10.1016/j.heliyon.2023.e16804
7. Mishra, S., Singh, R., Upadhyay, A., Mishra, S., & Shukla, S. (2023). Emerging trends in processing for cereal and legume-based beverages: A review. In Future Foods (Vol. 8, p. 100257). Elsevier BV. https://doi.org/10.1016/j.fufo.2023.100257

8. Neelam, M., & Mishra, S. (2018). Effects of Food Additives and Preservatives on Processed Food. In Asian Journal of Science and Applied Technology (Vol. 7, Issue 2, pp. 30–32). Centre for Research and Innovation. https://doi.org/10.51983/ajsat-2018.7.2.1031
9. Muslimov, N., Dalabaev, A., Ospanov, A., Sadibaev, A., Moldakarimov, A. (2022a) Changes in the carbohydrate-amylase complex germination of cereal crops. Journal of Hygienic Engineering and Design. (Volume 40, pp. 114-118).
10. Muslimov, N., Dalabaev, A., Timurbekova, A., Sadibaev, A,. Moldakarimov, A., Spandiyarov, Y. (2022b). Changes in the protein-protease complex of germinated grains of leguminous crops. Journal of Hygienic Engineering and Design. (Volume 40, pp. 132-138).
11. Muslimov, N., Ospanov, A., Timurbekova, A., Dalabaev, A. (2023). Technology of drinks based on extracts from sprouted grains and seeds: monograph. Astana, p. 175. (In Russian)
12. Muslimov, N., Kabylda, A., Dalabaev, A. (2022c). Study of the microflora of cereals, legumes and oilseeds during their germination for food purposes. Food Processing Industry (Vol. 8, pp. 42-45). (in Russian).
13. Muslimov, N., Ospanov, A., Alzhaxina, N., Dalabayev, A., Tuyakova, A., & Sadibaev, A. (2023). The economic essence of electro-pulse extraction technology in the production of extracts from sprouted grains of cereal crops. In Economic Annals-XXI (Vol. 201, Issues 1–2, pp. 33–43). Institute of Society Transformation sp. z o.o. https://doi.org/10.21003/ea.v201-04
14. Methodical Instructions (MUK) 4.2.1847-04 "Sanitary-epidemiological evaluation of justification of shelf life and storage conditions of food products". (in Russian).
15. GOST 6687.4-86 "Non-alcoholic beverages. kvass and syrups. Method of acidity determination". (in Russian).
16. Marimuthu, S., Saikumar, A., & Badwaik, L. S. (2024). Food losses and wastage within food supply chain: a critical review of its generation, impact, and conversion techniques. In Waste Disposal & Sustainable Energy. Springer Science and Business Media LLC. https://doi.org/10.1007/s42768-024-00200-7
17. Cui, F., Zheng, S., Wang, D., Tan, X., Li, Q., Li, J., & Li, T. (2023). Recent advances in shelf life prediction models for monitoring food quality. In Comprehensive Reviews in Food Science and Food Safety (Vol. 22, Issue 2, pp. 1257–1284). Wiley. https://doi.org/10.1111/1541-4337.13110
18. García, M. R., Ferez-Rubio, J. A., & Vilas, C. (2022). Assessment and Prediction of Fish Freshness Using Mathematical Modelling: A Review. In Foods (Vol. 11, Issue 15, p. 2312). MDPI AG. https://doi.org/10.3390/foods11152312
19. BehnamNik, A., & Vazifedoost, M. (2020). Optimizing the functional beverage formulation from the co-crystalized powder of *Securigera securidaca* seed extract. In Journal of Food Science and Technology (Vol. 57, Issue 7, pp. 2443–2451). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-020-04279-8
20. Vara, S., Karnena, M. K., & Dwarapureddi, B. K. (2019). Natural Preservatives for Nonalcoholic Beverages. In Preservatives and Preservation Approaches in Beverages (pp. 179–201). Elsevier. https://doi.org/10.1016/b978-0-12-816685-7.00006-9
21. Purewal, S. S., Kamboj, R., Sandhu, K. S., Kaur, P., Sharma, K., Kaur, M., Salar, R. K., Punia, S., & Siroha, A. K. (2022). Unraveling the effect of storage duration on antioxidant properties, physicochemical and sensory parameters of ready-to-serve Kinnow-Amla beverages. In Applied Food Research (Vol. 2, Issue 1, p. 100057). Elsevier BV. https://doi.org/10.1016/j.afres.2022.100057
22. Patra, T., Rinnan, Å., & Olsen, K. (2021). The physical stability of plant-based drinks and the analysis methods thereof. In Food Hydrocolloids (Vol. 118, p. 106770). Elsevier BV. https://doi.org/10.1016/j.foodhyd.2021.106770
23. Usta-Gorgun, B., Yilmaz-Ersan, L., & Sahin, S. (2022). Optimization of formulation and process conditions of chestnut-based functional beverage using response surface methodology. In Journal of Food Science and Technology (Vol. 59, Issue 8, pp. 3210–3219). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-022-05475-4
24. Habib, H., Singh, J., Kumar, A., Amin, T., Bhat, T.A., Aziz, N., & Ercişli, S. (2023). Optimization of Functional Beverage Using Germinated Pseudocereals. In Journal of Food Chemistry & Nanotechnology (Vol. 9). United Scientific Group. https://doi.org/10.17756/jfcn.2023-s1-015
25. Sharma, S., Singh, A., Sharma, S., Kant, A., Sevda, S., Taherzadeh, M. J., & Garlapati, V. K. (2021). Functional foods as a formulation ingredients in beverages: technological advancements and constraints. In Bioengineered (Vol. 12, Issue 2, pp. 11055–11075). Informa UK Limited. https://doi.org/10.1080/21655979.2021.2005992

26. Worku Kidane, S. (2021). Application of Response Surface Methodology in Food Process Modeling and Optimization. In Response Surface Methodology in Engineering Science. IntechOpen. https://doi.org/10.5772/intechopen.100113

27. Schreurs, M., Piampongsant, S., Roncoroni, M., Cool, L., Herrera-Malaver, B., Vanderaa, C., Theßeling, F. A., Kreft, Ł., Botzki, A., Malcorps, P., Daenen, L., Wenseleers, T., & Verstrepen, K. J. (2024). Predicting and improving complex beer flavor through machine learning. In Nature Communications (Vol. 15, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41467-024-46346-0

28. Cueva Ríos, M. A., Fernández Rosillo, F., Quiñones Huatangari, L., & Milagros Cabrejos Barrios, E. (2023). Estimation of coffee shelf life under accelerated storage conditions using mathematical models - Systematic review. In Czech Journal of Food Sciences (Vol. 41, Issue 2, pp. 92–102). Czech Academy of Agricultural Sciences. https://doi.org/10.17221/163/2022-cjfs

29. Maselesele, T. L., Molelekoa, T. B. J., Gbashi, S., & Adebo, O. A. (2023). The Optimisation of Bitter Gourd-Grape Beverage Fermentation Using a Consolidated Response Surface Methodology (RSM) and Artificial Neural Network (ANN) Approach. In Plants (Vol. 12, Issue 19, p. 3473). MDPI AG. https://doi.org/10.3390/plants12193473

30. Fazeli Burestan, N., Afkari Sayyah, A. H., & Taghinezhad, E. (2020). Mathematical modeling for the prediction of some quality parameters of white rice based on the strength properties of samples using response surface methodology (RSM). In Food Science & Nutrition (Vol. 8, Issue 8, pp. 4134–4144). Wiley. https://doi.org/10.1002/fsn3.1703

31. de Oliveira, L. C., Balbinoti, T. C. V., Alvarez, D. C., de Matos Jorge, L. M., & Jorge, R. M. M. (2023). Modeling and thermodynamic analysis of the hydration and germination of triticale seeds. In Journal of Cereal Science (Vol. 113, p. 103756). Elsevier BV. https://doi.org/10.1016/j.jcs.2023.103756

32. Anukiruthika, T., & Jayas, D. S. (2024). Mathematical modeling for management of stored-grain ecosystems: Approaches, opportunities, and research needs. In Journal of Stored Products Research (Vol. 106, p. 102304). Elsevier BV. https://doi.org/10.1016/j.jspr.2024.102304

33. Iztayev, A., Yakiyayeva, M., Kulazhanov, T., Kizatova, M., Maemerov, M., Stankevych, G., Toxanbayeva, B., & Chakanova, Z. (2018). Efficient mathematical models of ion-ozon cavitation treatment for long-term storage of grain legume crops. Acta Technica CSAV (Ceskoslovensk Akademie Ved) (Vol. 63, Issue 1, pp. 1-8).

34. Sheikhi, A., Mirdehghan, S. H., Arab, M. M., Eftekhari, M., Ahmadi, H., Jamshidi, S., & Gheysarbigi, S. (2020). Novel organic-based postharvest sanitizer formulation using Box Behnken design and mathematical modeling approach: A case study of fresh pistachio storage under modified atmosphere packaging. In Postharvest Biology and Technology (Vol. 160, p. 111047). Elsevier BV. https://doi.org/10.1016/j.postharvbio.2019.111047

35. Kumar, V., Ahire, J. J., & Taneja, N. K. (2024). Advancing microbial food safety and hazard analysis through predictive mathematical modeling. In The Microbe (Vol. 2, p. 100049). Elsevier BV. https://doi.org/10.1016/j.microb.2024.100049

36. Lima, R. E., Coradi, P. C., Nunes, M. T., Bellochio, S. D. C., da Silva Timm, N., Nunes, C. F., de Oliveira Carneiro, L., Teodoro, P. E., & Campabadal, C. (2021). Mathematical modeling and multivariate analysis applied earliest soybean harvest associated drying and storage conditions and influences on physicochemical grain quality. In Scientific Reports (Vol. 11, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-021-02724-y

37. Rajalakshmi, R., Sujatha, G., Serma Saravana Pandian, A., Rita, N., Perasiriyan, V., Karpoora Sundara Pandian, N. (2022) Application of predictive modeling to assess the shelf life of functional enriched sugarcane juice. In International Journal of Agriculture Sciences (Volume 14, Issue 1, pp. 11049-11051).

38. Rodríguez-Saavedra, M., Pérez-Revelo, K., Valero, A., Moreno-Arribas, M. V., & González de Llano, D. (2021). A Binary Logistic Regression Model as a Tool to Predict Craft Beer Susceptibility to Microbial Spoilage. In Foods (Vol. 10, Issue 8, p. 1926). MDPI AG. https://doi.org/10.3390/foods10081926

39. Jabeen, R., Jan, N., Naseer, B., Sarangi, P. K., Sridhar, K., Dikkala, P. K., Bhaswant, M., Hussain, S. Z., & Inbaraj, B. S. (2024). Development of Germinated-Brown-Rice-Based Novel Functional Beverage Enriched with γ-Aminobutyric Acid: Nutritional and Bio-Functional Characterization. In Foods (Vol. 13, Issue 8, p. 1282). MDPI AG. https://doi.org/10.3390/foods13081282

40. Shams Najafabadi, N., Sahari, M. A., Barzegar, M., & Hamidi Esfahani, Z. (2021). Quality characteristics, nutraceutical profile, and storage stability of functional beverage prepared from jujube (Ziziphus jujuba var vulgaris) fruit. In Journal of Food Processing and Preservation (Vol. 45, Issue 4). Hindawi Limited. https://doi.org/10.1111/jfpp.15201

41. Kokwar, M. A., Arya, S. S., & Bhat, M. S. (2021). A cereal-based nondairy probiotic functional beverage: An insight into the improvement in quality characteristics, sensory profile, and shelf-life. In Journal of Food Processing and Preservation (Vol. 46, Issue 1). Hindawi Limited. https://doi.org/10.1111/jfpp.16147
42. Yu, Z., Guindani, M., Grieco, S. F., Chen, L., Holmes, T. C., & Xu, X. (2022). Beyond t test and ANOVA: applications of mixed-effects models for more rigorous statistical analysis in neuroscience research. In Neuron (Vol. 110, Issue 1, pp. 21–35). Elsevier BV. https://doi.org/10.1016/j.neuron.2021.10.030
43. Sridhar, A., Ponnuchamy, M., Kumar, P. S., & Kapoor, A. (2020). Food preservation techniques and nanotechnology for increased shelf life of fruits, vegetables, beverages and spices: a review. In Environmental Chemistry Letters (Vol. 19, Issue 2, pp. 1715–1735). Springer Science and Business Media LLC. https://doi.org/10.1007/s10311-020-01126-2

Funds:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments:

We thank “Kazakh Research Institute of Processing and Food Industry” LLP for co-operation.

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

Almaz Moldakarimov, Almaty Technological University, Research Institute of Food Technologies, Tole bi 100, 050012, Almaty, Republic of Kazakhstan,
Tel.: +77476868689
E-mail: mister_almaz89@mail.ru
ORCID: https://orcid.org/0000-0001-8309-9285

Auelbek Iztayev, Almaty Technological University, Research Institute of Food Technologies, Tole bi 100, 050012, Almaty, Republic of Kazakhstan
Tel.: +77002162256
E-mail: iztayev.a@mail.ru
ORCID: https://orcid.org/0000-0002-7385-482X

Nurzhan Muslimov, Sh. Murtaza International Taraz Innovation Institute, Sh. Murtaza St. 101A, 080000, Taraz, Republic of Kazakhstan
Tel.: +7474868866
E-mail: n.muslimov@inbox.ru
ORCID: https://orcid.org/0000-0002-0302-2817

Madina Yakiyayeva, Almaty Technological University, Research Institute of Food Technologies, Tole bi 100, 050012, Almaty, Republic of Kazakhstan
Tel.: +77011626749
E-mail: yamadina88@mail.ru
ORCID: https://orcid.org/0000-0002-8564-2912

Bayan Muldabekova, Almaty Technological University, Department of Technology of Bakery Products and Processing Industries, Tole bi Street, 100, 050012, Almaty, Republic of Kazakhstan
Tel.: +77777044565
E-mail: bayan_10.04@mail.ru
ORCID: https://orcid.org/0000-0003-1848-4288

*Sholpan Tursunbayeva, Almaty Technological University, Department of Technology of Bakery Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstan
Tel.: +77011626749
E-mail: sh-tursunbayeva@mail.ru
ORCID: https://orcid.org/0000-0001-9645-3634

Fatima Dikhanbayeva, Almaty Technological University, Department of Food Technology, Tole bi 100, 050012, Almaty, Republic of Kazakhstan
Tel.: +77477555985
E-mail: fatima6363@mail.ru
ORCID: https://orcid.org/0000-0003-4257-3774

Saida Shintassova, Almaty Technological University, Faculty of Food Technology, Department of Technology of Bakery Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstam
Tel.: +77772160603
E-mail: saida_atu@mail.ru
ORCID: https://orcid.org/0000-0001-6269-4675

Zhanerke Dyusembaeva, Almaty Technological University, Department of Technology of Bakery Products and Processing Industries, Tole bi 100, 050012, Almaty, Republic of Kazakhstan
Tel.: +77781890871
E-mail: zhanerkedusembaeva@gmail.com
ORCID: https://orcid.org/0009-0004-4526-6459

Corresponding author: *

potravinárstvo®
OPEN ACCESS
Received: 12.10.2024
Revised: 14.11.2024
Accepted: 30.11.2024
Published: 4.12.2024

Slovak Journal of Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 1028-1049
https://doi.org/10.5219/2030
ISSN: 1337-0960 online
www.potravinarstvo.com

Development and study of the nutritional value and storage stability of a soft cottage cheese product enriched with collagen and antioxidant-rich plant extracts

Yerlan Zharykbasov, Aitbek Kakimov, Klara Zharykbasova, Zhainagul Kakimova, Guldana Raimkhanova, Zhanna Kozykenova

ABSTRACT

This study investigates the development of a novel soft cottage cheese product enriched with collagen concentrate from poultry processing by-products and antioxidant-rich plant extracts for the adaptive nutrition of athletes. Collagen concentrate was obtained from chicken skin, bone tissue, and feet through enzymatic hydrolysis and freeze-drying. Antioxidant-rich extracts were prepared from sea buckthorn and cinnamon rosehip using ethanol extraction. The plant extract demonstrated high antioxidant potential, containing 1.98% phenolic compounds, 29.8 mg/100g vitamin A, 48.9 mg/100g vitamin E, and 756.4 mg/100g vitamin C. The antioxidant extract demonstrated significant immune-boosting effects in experimental rats by enhancing lymphocyte and T-cell counts. Various ratios of collagen concentrate and plant extract were tested in the cottage cheese product. Optimal water-holding capacity and effective viscosity were achieved with a 6:4 or 8:4 collagen-to-extract ratio, balancing collagen's gelation properties with the antioxidant benefits. The addition of 8% dry collagen concentrate and 4% sea buckthorn and rosehip extract resulted in an enhanced nutritional profile, particularly through increased polyunsaturated fatty acids (Omega-3 and Omega-6), vitamins A, C, E, and essential minerals like calcium, phosphorus, and magnesium. Storage stability studies indicated optimal preservation of product structure at 0-2°C for up to 96 hours, maintaining a viscosity loss coefficient between 15.0-15.8%. This enhanced soft cottage cheese product demonstrates improved nutritional profiles and antioxidant properties while maintaining structural stability, making it a promising functional food for athletes and health-conscious consumers.

Keywords: collagen concentrate, plant extracts, functional dairy product, polyunsaturated fatty acids, antioxidant, texture stability

INTRODUCTION

In recent years, globally and in Kazakhstan, significant attention has been directed toward developing innovative, nutritionally balanced products, particularly for athletes. Manufacturing functional foods enriched with complex additives from animal and plant-based sources is gaining momentum as a promising approach to meet the growing demand for optimal nutrition. Dairy products, a staple in many diets, are an ideal platform for incorporating plant-based bioactive compounds, which enhance their health-promoting properties. These fortified products not only contribute to overall health by supporting physiological functions and preventing diseases but also play a crucial role in restoring the body's functional indicators after intense physical activity and aiding the recovery of the musculoskeletal system during rehabilitation **[1]**, **[2]**, **[3]**, **[4]**.

One key functional ingredient that plays a significant role in this recovery process is collagen. This animal-derived protein has been shown to enhance connective tissue repair and alleviate pain by boosting collagen production. Athletes who consume collagen-containing foods can maximise their physical performance **[5]**, **[6]**.
Collagen extracted from secondary products of the meat, fish and poultry processing industries is widely used in producing food additives. Collagen from secondary protein raw materials of the poultry processing industry, including chicken feet, feathers, scallops, bones and skin, is in particular demand. It was found that collagen from the skin contains the largest amount of proline and oxyproline (about 20%) and nearly none of the aromatic and sulfur-containing amino acids, which makes it especially valuable for further research **[7]**, **[8]**, **[9]**, **[10]**.

Integrating plant-derived bioactive compounds and extracts into dairy products offers multiple benefits. These include extending shelf life through antioxidant and antimicrobial activities, imparting positive health effects, and improving physicochemical, textural, and organoleptic properties **[11]**, **[12]**. Various plant sources, such as fruit peels, herbs, and byproducts, have been successfully utilised to fortify yoghurt, cheese, and kefir, enhancing their nutritional profiles and potential health benefits **[13]**, **[14]**, **[15]**.

As consumer interest in functional foods continues to rise, incorporating plant-based additives into dairy products represents a valuable strategy for producing nutritious, health-promoting food products that align with modern dietary trends.

While incorporating plant-based additives into dairy products offers numerous benefits, it is crucial to understand the specific functional ingredients that make these fortified products particularly valuable for athletes and their unique nutritional needs. Plant-based antioxidants-rich supplements offer targeted advantages, particularly for athletes **[16]**, **[17]**. These antioxidants, including phenolic compounds, carotenoids, and vitamins, are critical in combating oxidative stress and muscle damage caused by intense physical activity. Antioxidants are necessary to reduce the level of free radicals, so it is recommended that athletes include antioxidant-rich plant foods in their diet **[18]**. Using foods containing antioxidants in athletes' diets helps reduce tissue damage and accelerate their recovery after high physical loads during training and competition **[19]**, **[20]**.

This work plans to investigate fruit and berry plants to obtain biologically active additives (BAA) with pronounced antioxidant properties since they are characterised by an increased content of antioxidants **[21]**, **[22]**.

Taking into account the relevance of the production of specialised milk products for the adaptive nutrition of athletes, in this paper, we aimed to develop a formulation of a soft cottage cheese product with the application of collagen concentrate from secondary raw materials of the poultry processing industry and dietary supplements from plant raw materials with a high content of antioxidants. This combination of functional ingredients in cottage cheese products is unique. It simultaneously strengthens the musculoskeletal system due to collagen and protects the body from oxidative stress due to antioxidants, which is especially important for athletes.

Scientific hypothesis

Incorporating specific proportions of dry collagen concentrate derived from secondary poultry processing by-products and antioxidant-rich extracts from sea buckthorn and cinnamon rosehip into soft cottage cheese will significantly enhance the product's nutritional and biological value—particularly benefiting athletes by supporting musculoskeletal health and providing antioxidant protection—without adversely affecting its organoleptic properties or shelf life.

MATERIAL AND METHODOLOGY

Samples

Fresh cow's milk was purchased from farmers' markets in Semey. Fruit and berry plants (sea buckthorn and cinnamon rosehip) were collected during route expeditions in the Abay region of the Republic of Kazakhstan. Chicken skin, bone tissue, and chicken feet were obtained after deboning of bird carcasses purchased in a specialized store of the poultry processing enterprise “Ardager” (Semey, Kazakhstan).

Chemicals and biological material

Hydrochloric acid (mass fraction of hydrochloric acid (HCl), 35-38%, pure for analysis, Snabservice Astana LLP, Astana, Kazakhstan).
Sodium hydroxide, NaOH, (mass fraction of sodium hydroxide (NaOH), 99.3%, Snabservice Astana LLP, Astana, Kazakhstan).
Ethyl alcohol (70%, Snabservice Astana LLP, Astana, Kazakhstan)
Sodium chloride (40%, Kelun-Kazfarm LLP, Almaty, Kazakhstan)
Papain enzyme (Sigma-Aldrich, Burlington, United States).

Instruments

Scanning electron microscope "JSM-639 LV" (JEOL Co., Ltd., Tokyo, Japan).
Vacuum freezing dryer TOPT-10C (TOPTION Co., Ltd., Xi'an, China).
LVDV-2T rotary viscometer (Dongguan Lonroy Equipment Co., Ltd, China).
Spectrophotometer Shimadzu UV-1800 (Shimadzu Corporation, Kyoto, Japan).

Laboratory Methods

1) Physico-chemical methods of analysis to study the composition of the extract:

- determination of flavonoids by spectrophotometry method according to GOST R 55312-2012 **[23]**;
- determination of vitamin A by spectrophotometry according to GOST 12823-1-2014 **[24]**;
- determination of vitamin C by titrimetric method according to GOST 34151-2017 **[25]**;
- determination of vitamin E by colorimetric method according to GOST 12822-2014 **[26]**.

2) Immunological methods of research

Forty white mongrel rats weighing 180-200 grams at three ages were used to study the effect of dietary supplements from plant raw materials with pronounced antioxidant properties on the state and metabolic processes in the immune system under in vivo conditions of laboratory animals. The use of animals in the experiment is carried out in compliance with the norms and rules regulated by the legislation of the Republic of Kazakhstan, and international recommendations of the European Convention for the Protection of Vertebrate Animals used for experiments for scientific or other purposes. During the experiment, all animals are in the same standard vivarium conditions.

Total leukocyte and lymphocyte counts were determined using a haematology analyser. The content of T-helper (CD4+) and T-suppressor (CD8+) cells was determined by flow cytometry using monoclonal antibodies labelled with fluorescent dyes. IgA, IgM and IgG immunoglobulin concentrations were determined by enzyme-linked immunosorbent assay (ELISA). The latex particle uptake test by macrophages was used to evaluate phagocytic activity. CIC levels were determined using the immune complex precipitation test. The mitogen-assisted lymphocyte proliferation test was used to determine PTML. All data are presented as median (Q1-Q3). For statistical analysis, the Mann-Whitney test was used to compare control and experimental groups. The level of significance was set at $p < 0.05$.

3) Determination of the mass fraction of collagen

To conduct the study in a conical flask with a capacity of 250 cm^3, 1 g of sample was put into it, and 100 ml of hydrochloric acid was added, with further hydrolysis for 8 hours. The resulting hydrolysate is subjected to filtration and placed in a measuring flask with a capacity of 250 cm^3. The flask's contents are cooled, and distilled water is poured to the mark on the flask with further stirring. In a 100 cm^3 measuring flask, 1 ml of hydrolysate and 60 ml of distilled water were neutralised with NaOH solution to pH 6.0 using indicator paper **[27]**.

The flask's contents are topped up to the mark with distilled water and stirred. 4 ml of the hydrolysate solution is poured into a test tube, and 2 ml of the prepared oxidation reagent is stirred and held for 20 minutes at room temperature. Then, 2 ml of the prepared colour reagent is added to the test tube with the hydrolysate solution; the test tube is closed with aluminium foil and then kept in a water bath at 60 ºC for 15 minutes. At the same time, two control solutions are prepared using distilled water instead of hydrolysate. Test tubes with experimental and control samples are cooled, and after 30 minutes, the optical density is measured on a spectrophotometer.

From the plotted calibrated graph, the concentration of oxyproline is determined.

A) The mass fraction of oxyproline in % is calculated according to formula (1):

$$X = \frac{C \times 250 \times 100 \times 100}{m \times V \times 10^6}, \quad (1)$$

Where:

C – concentration of oxyproline in the sample solution found from the calibration graph, µg/cm^3;
250 – volume of hydrolysate, cm^3;
100 – the volume of the solution obtained after dilution of the hydrolysate, cm^3;
100 – percentage conversion factor;
m – sample mass, g;
V – the volume of hydrolysate sampled for neutralization, cm^3;
10^6 – µg to g conversion factor.

B) Mass fraction of collagen (X_1) in % is calculated according to formula (2):

$$X_1 = K - X, \quad (2)$$

Where:

K – conversion factor of oxyproline to collagen (8.07);

X – a mass fraction of oxyproline calculated by formula 1.

4) Methodology for determining the pore size of dry collagen on a scanning electron microscope (SEM) - "JSM-639 LV JEOL" (Japan).

Dry collagen samples were ground into powder and then applied to copper plates for electron microscope. The surface of the collagen concentrate is coated with a thin layer of platinum with a layer thickness of 5-10 nm. The surface of the sample is scanned by electron beam, then the obtained images are fixed with different magnifications for detailed analysis of collagen structure. Pore sizes (distances between collagen fibrils) are measured on the obtained SEM-images using image analysis software.

Calculate the mean value of pore size, standard deviation, coefficient of variation.

Mean value (χ_m):

The mean value of pores is defined as the sum of all values divided by the number of these pores (3):

$$\chi_m = \frac{1}{N}\sum_{i=1}^{N}\chi_i , \quad (3)$$

Where:

χ_i – pore diameter, μm;

N – number of measurements.

Standard deviation (σ)

Standard deviation measures the average deviation of each value of a data set from the mean and is calculated using the formula (4):

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(\chi_i - \chi_{cp})^2} , \quad (4)$$

Where:

χ_m – mean pore size, μm;

χ_i – pore size, μm;

N – number of measurements.

Coefficient of variation (CV):

CV shows the magnitude of the standard deviation of the relative mean and is calculated using the formula (5):

$$CV = \frac{\sigma}{\chi_{cp}} \times 100\% , \quad (5)$$

Where:

σ – standard deviation, μm;

χ_m – mean pore size, μm.

5) Methodology for determining water-holding capacity (WHC) according to **[28]**.

To determine the water-holding capacity 100 mg sample is taken, put it on filter paper, pressed with a device planimeter to release moisture. Then the area of the total spot and the area of the spot left by the product are measured.

The WHC (%) is calculated according to the formula (6):

$$WHC = \frac{m_w - 8.4\,(S_1 - S_2)}{m} \times 100 , \quad (6)$$

Where:

m_w – moisture content in the sample, mg;

S_1 – area of the total spot, cm^2;

S_2 – sample spot area, cm^2;

m – sample weight, mg.

6) The effective viscosity was determined on an LVDV-2T rotary viscometer (Dongguan Lonroy Equipment Co., Ltd, China).

7) The composition of non-fat soft curd product is determined based on interstate standards:
- mass fraction of fat by acid method according to GOST 5867-2023 **[29]**;
- mass fraction of protein by the Kjeldahl method according to GOST 31957-2012 **[30]**;
- mass fraction of carbohydrates by potentiometric method according to GOST 34304-2017 **[31]**;
- determination of fatty acid composition using gas chromatography according to GOST 32915-2014 **[32]**;
- determination of amino acid composition according to MVI MN 1363-2000 "Method for determination of amino acids in food products by high-performance liquid chromatography" **[33]**;
- Determination of vitamin A content according to GOST EN 12823-1-2014 by high-performance liquid chromatography **[24]**;
- Determination of vitamin E content according to GOST EN 12822-2014 by high-performance liquid chromatography **[26]**;
- determination of vitamin C content according to GOST 34151-2017 by high-performance liquid chromatography **[25]**;
- determination of vitamin D content according to GOST 12821-2014 by high-performance liquid chromatography **[34]**;
- determination of calcium, magnesium and potassium content by the spectrometric method according to GOST ISO8070/IDF 119-2014 **[35]**;
- determination of phosphorus content according to GOST 30615-99 using a spectrophotometer **[36]**.

Description of the Experiment

The method of obtaining an extract: From cruciferous sea buckthorn and cinnamon rosehip consists of the following stages: purification of plant raw materials, washing at a temperature not exceeding 20 ºC, drying and weighing of the plant raw materials, grinding, extraction in a batch extractor with a stirrer, purification of extract from ballast substances by sedimentation for 12-15 hours with further filtration of alcoholic extract and evaporation of extractant in the extraction apparatus at a temperature of 76 °C for 2 hours, evaporation of water at a temperature of 80 °C and obtaining a viscous plastic-like mass of yellow-brown colour. For the extraction of berry plants, 75 % ethyl alcohol in the ratio 1:5 (berry : alcohol) was used.

Method of obtaining dry collagen concentrate: The method of processing chicken skin consists of the following stages: cutting chicken skin 2-3 cm in size; cooking chicken skin at a temperature of 65 ºC for 3 hours; cooling the mixture with chicken skin to a temperature of 36 ºC; adding papain enzyme to the mixture with chicken skin in a ratio of 1: 10, respectively; thermostatic the mixture at 36 ºC for 24 hours; cooling the mixture to 20-25 ºC; settling the mixture at 20-25 ºC for 60 minutes; separating the solid phase from the gel; centrifugation to separate the fat from the gel at 1000 rpm; and cooling the chicken skin gel to 5-6 ºC;

The method of processing chicken bone tissue and feet consists of the following stages: selection and cleaning of chicken bone tissue and feet, washing of raw materials, chopping into pieces of 2-3 cm; and making a mixture of chicken bone tissue and feet (the ratio of 40: 60, respectively); heat treatment of raw materials for 3-4 hours at a temperature of 65 ºC; cooling of the mixture of chicken bone tissue and feet to a temperature of 25 ºC; fine grinding of the mixture; introduction of papain enzyme into the mixture of chicken bone tissue and feet at a ratio of 1:10, respectively; thermostating of the mixture at a temperature of 36 ºC for 24 hours; cooling of the obtained collagen concentrate to 5-6 ºC.

Figure 1 Dry collagen concentrate.

Gel and paste collagen concentrate are blended in a 1:1 ratio and thoroughly mixed. The obtained composition was dried in a vacuum-freezer TOPT-10C (TOPTION Co., Ltd., Xi'an, China) at minus 44 °C until the humidity reached 8 - 10%. After drying, the powder contained 46.5% of collagen. Figure 1 shows dry collagen concentrate in powder form. The powder has a light, slightly beige colour and uniform texture. The consistency of the concentrate is finely dispersed, indicating that it is finely milled, which may contribute to better dissolution and assimilation when used in the production of cottage cheese products. A flowchart of collagen concentrate preparation is present in Figure 2.

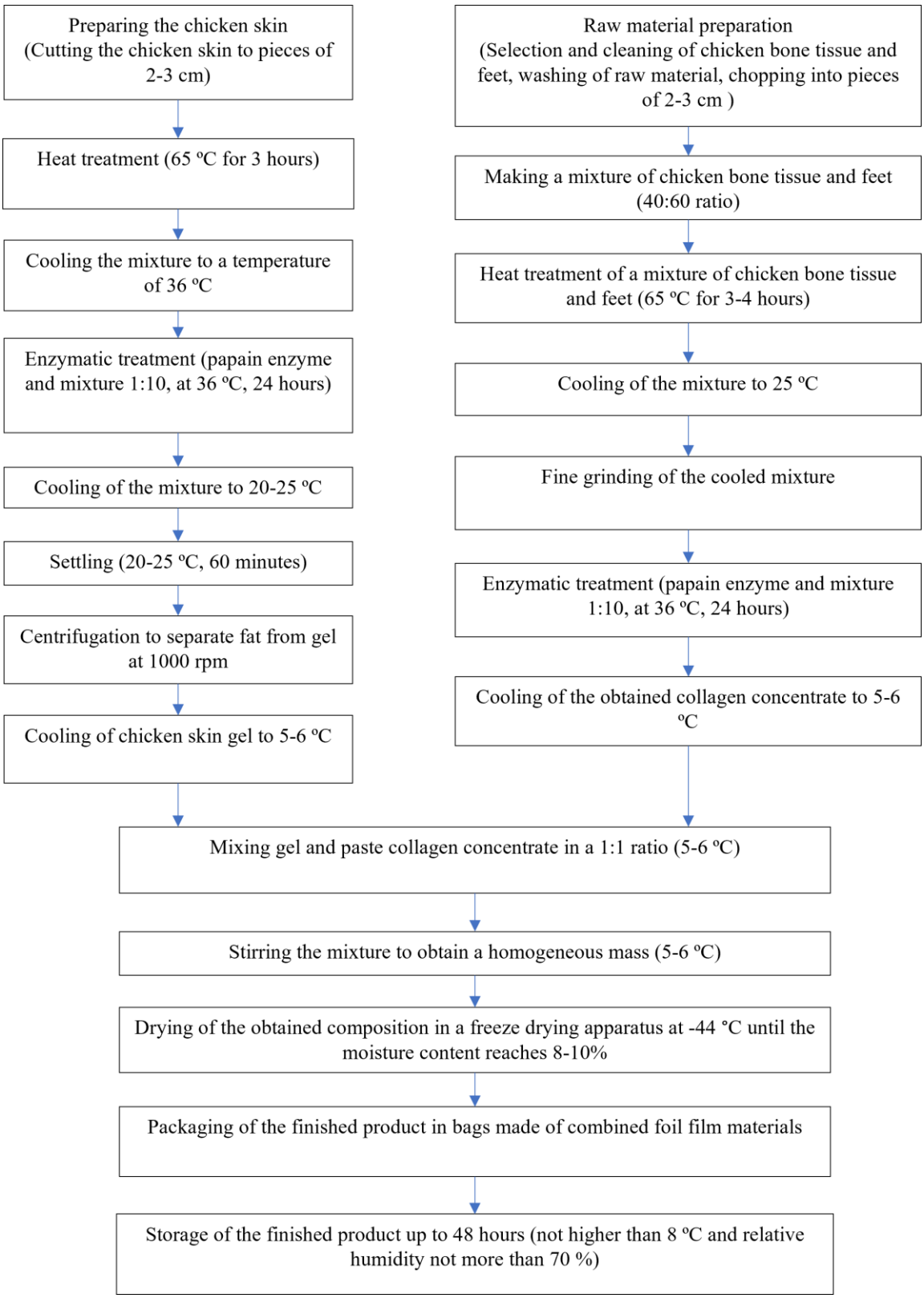


Figure 2. Flowchart of collagen concentrate preparation.

Technology of soft non-fat cottage cheese product preparation.

The main raw material for the production of cottage cheese products is cow's milk, which has an acidity not exceeding 18 °T. The technological production of non-fat cottage cheese products includes cooling to 8-10 ° C and, cleaning milk from mechanical impurities on the separator milk purifier, heating milk to 30-35 ° C with further separation to obtain skim milk. Skim milk is pasteurised at a temperature of 76-78 ° C with a holding time of 30-40 seconds, cooling of milk to the leavening temperature to 30-32 ° C.

During fermentation of milk 5% starter, prepared on pure cultures of mesophilic and thermophilic lactic acid streptococcus, 40% sodium chloride solution (at the rate of 400 g of anhydrous salt per 1 ton of milk) and 1% rennet enzyme are added to the milk. Fermentation of skim milk is carried out at a temperature of 30-32 ° C for 4-6 hours to reach a titratable acidity of 71-73 °T. After the formation of the clot, it is cut into 1-2 cm cubes, and slowly heated to 35-40 ° C with exposure for 20-30 minutes to improve the separation of whey.

Whey is removed by self-pressing at 25-28°C for 30-40 minutes. Pressing of the clot continues under the same conditions for 1-2 hours until the moisture content of the product reaches 65-70%. The curd is ground on a colloid mill to obtain a homogeneous mass, heated to 35 °C, and added with constant mixing dry collagen concentrate (8%) and extract composition of sea buckthorn and rosehip cinnamon (4%). The finished product is cooled to 4-6 ° C, packed in airtight containers, and stored at 0-2 °C for no more than 96 hours.

Number of samples analysed: 30 samples of cottage cheese were analysed.
Number of repeated analyses: Each study was carried out 3 times.
Number of experiment replications: The study was repeated three times, with the experimental data processed using mathematical statistics methods.

Statistical Analysis

Each experiment was conducted in triplicate to ensure data reliability and statistical power. Standard deviation was calculated to assess the variability of the data, providing insight into the consistency of measurements. One-way Analysis of Variance (ANOVA) was used to determine significant differences among the experimental groups for parameters such as water-holding capacity, viscosity, and mechanical stability. Tukey's Honest Significant Difference (HSD) test was applied post-hoc to identify specific groups that showed statistically significant differences ($p < 0.05$). The Mann-Whitney test was used to compare immune response parameters between control and experimental groups due to the non-parametric distribution of the data. The results were graphically represented using Microsoft Excel for clarity and interpretation.

RESULTS AND DISCUSSION

Study of the microstructure of collagen concentrate

The highest collagen content characterises selected secondary products of the poultry processing industry. The method of obtaining dry collagen concentrate is based on the enzymatic process using the enzyme papain for hydrolysis of raw materials. This method allows for preserving the biological value of collagen due to the soft processing of raw materials. A comparative study by Munasinghe et al. shows that combining acetic acid and pepsin increases collagen production. However, the results of these studies showed that using papain enzyme for processing is preferable as it minimises the possible changes in protein structure associated with acid exposure and provides a more stable result **[37]**.

Studies were carried out to determine the pore size of dry collagen concentrate. When developing curd products enriched with dry collagen concentrate, it is important to consider their structure and pore size. Collagen pores, which are the spaces between collagen fibrils, can significantly affect the curd product's texture uniformity and water-holding capacity **[38]**. Figure 3 shows a micrograph of dry collagen concentrate at 100, 200 and 300x magnification.

As can be seen from Figure 3, the dry collagen concentrate is characterised by non-uniformity in pore size (6.32 μm; 11.33 μm; 14.00 μm; 14.8 μm; 16.12 μm). The average pore size of the obtained dry collagen concentrate is 12.514 μm, with a standard deviation of about 4.08 μm. The coefficient of variation of 32.6% indicates heterogeneity in pore size, which may affect the consistency and water-holding capacity of the curd product.

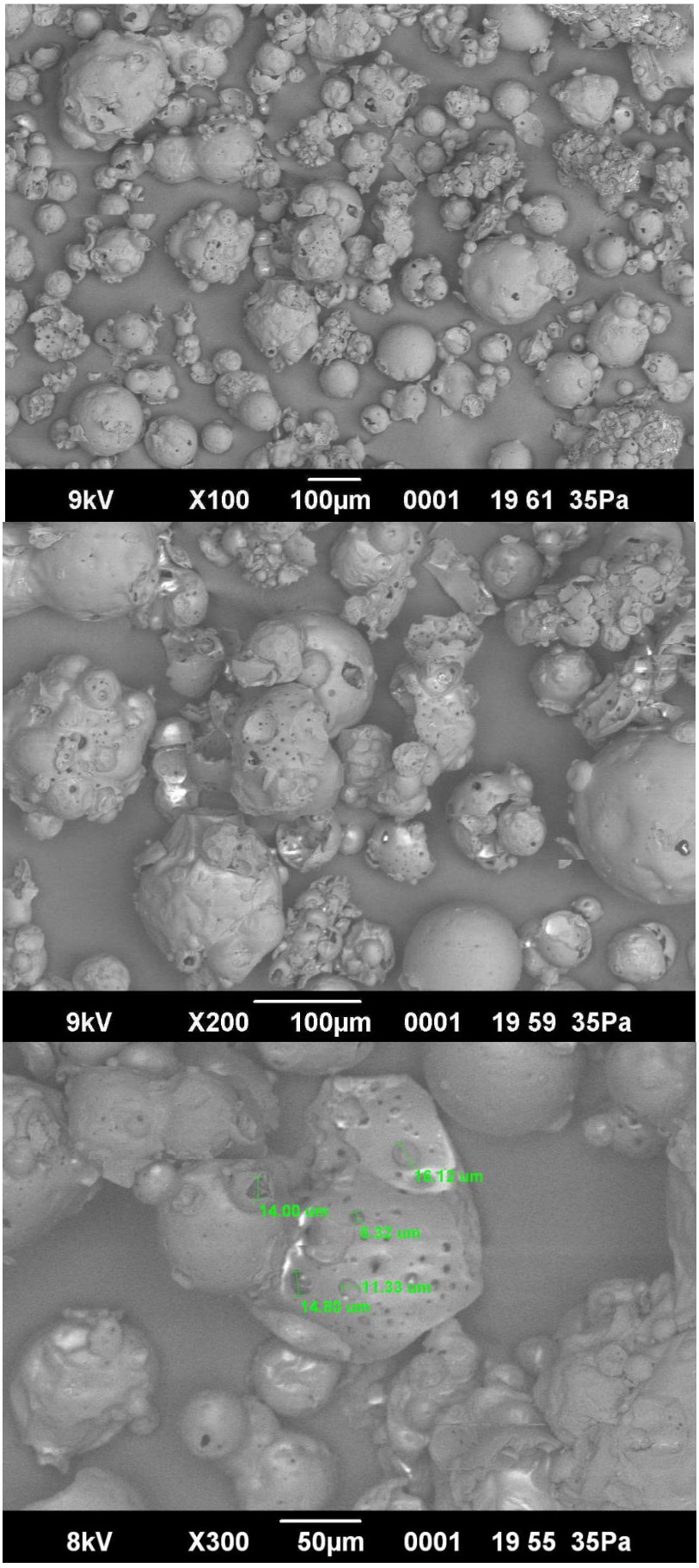


Figure 3. Microstructure of dry collagen concentrate.

Study of antioxidant activity of extract from fruit-berry plants

To conduct experimental studies, 5 species of fruit and berry plants with pronounced antioxidant properties were selected based on the analysis of literature sources: sea buckthorn, red haw hawthorn, black chokeberry, cinnamon rosehip, and Russian olive. Route expeditions were carried out to select fruit and berry plants, taking into account their distribution area and frequency of occurrence in the Abai region of the Republic of Kazakhstan. During the route expeditions, the territories of different zones were covered: mountainous, steppe, forest-steppe, and forest. Based on the route expeditions, the frequency of occurrence and area of distribution of fruit and berry plants with pronounced antioxidant properties in the Abai region were determined (Table 1, Figure 4).

Table 1 Frequency of occurrence and area of distribution of fruit and berry plants in the Abay region of the Republic of Kazakhstan.

#	Family	Plant genus and species	Frequency of occurrence	Sampling place	Geographic coordinates of the sampling location
1	*Elaeagnaceae*	Sea buckthorn (Hippophae rhamnoides)	Soc	Pine forest of Semey city	Suburbs of Semey city, Abay region Kazakhstan 50°55'07.9"N 79°34'22.5"E
2	*Rosaceae*	Red haw hawthorn (*Crataegus sanguinea*)	Cop	Borodulikha, Beskaragai, Abai districts	1) Borodulikha district, Novopokrovka village, Abay region Kazakhstan 50°40'10.2" N 80°27'49.7" E 2) Beskaragai district, Sosnovka village, Abay region Kazakhstan 51°27'07.5" N 79°30'03.7" E 3) Abai district, foothills of the Chingiztau mountain range, Abai region, Kazakhstan 48°38'00" N 79°10'00" E
3	*Rosacae*	Black chokeberry (*Aronia melanocarp*)	Cop	Summer houses plots of Semey city	Summer houses in eastern settlement of semey city, Abay Region Kazakhstan 50°23'30.1"N 80°21'14.7"E
4	*Rosacae*	Cinnamon rosehip (*Roza cinnamomea*)	Cop	Beskaragai district, Pine forest of Semey city	Pine forest of Semey city, Beskaragai district, Glukhovka village, Abay region Kazakhstan 50°29'43.8" N 79°52'09.8" E Beskaragai district, Kanonerka village, Abay region, Kazakhstan 50°43'24.6" N 79°41'26.2" E
5	*Elaeagnaceae*	Russian olive *Elaeagnus oxycarpa*	Sol	Beskaragai district	Beskaragai district, Glukhovka village, Abay region Kazakhstan 50°29'43.8" N 79°52'09.8" E

Note: Symbols in the table: Socialis (Soc) - «very much»; Copiosa (Cop) - «average»; Solitaries (Sol)- «very rarely». (latitude): 50.4955 N, (longitude): 79.8694 E.

Figure 4 Photo of fruit and berry plants grown in the Abay region of the Republic of Kazakhstan.
Note: a) Sea buckthorn (*Hippophae rhamnoides*); b) Red haw hawthorn (*Crataegus sanguinea*); c) Black chokeberry (*Aronia melanocarp*); d) Cinnamon rosehip (*Roza cinnamomea*); e) Russian olive (*Elaeagnus oxycarpa*)

As can be seen from Table 1, taking into account the density of growth and constancy of species composition of the studied plants, 4 species of plants necessary for the experimental work were collected: sea buckthorn, red haw hawthorn, black chokeberry, cinnamon rosehip. The population density of Russian olives was very rare.
As a result of a comparative analysis of the chemical composition of 4 species of fruit and berry plants, a composition of two berries was used to obtain the extract: sea buckthorn and cinnamon rosehip. To extract berry plants, it is necessary to use 75% ethyl alcohol in a ratio of 1:5 (berry : alcohol). The results of the study of the composition of the obtained extract are presented in Table 2.

Table 2 Chemical composition of fruit and berry plant extract.

Name	Content			
	phenolic compounds, %	**vitamin A, mg/100 g**	**vitamin E, mg/100 g**	**vitamin C, mg/100 g**
Sea buckthorn and cinnamon rosehip composition extract	1.98	29.8	48.9	756.4

Based on these data, it can be concluded that the extract of sea buckthorn and rosehip composition has high antioxidant potential due to its significant content of phenolic compounds (1.98%) and vitamins A (29.8 mg/100g), E (48.9 mg/100g) and C (756.4 mg/100g). This extract can effectively neutralise free radicals and protect cells from oxidative stress, making it useful for various food products to improve their antioxidant properties **[39]**.

To determine the antioxidant potential of the obtained extract, studies of its effect on the condition and metabolic processes in the organs of the immune system of experimental animals in laboratory conditions were carried out. The studies were carried out on white mongrel rats. The animals were randomly divided into control and experimental groups. The results of the study are presented in Table 3.

Table 3 Comparative analysis of cellular and humoral immunity indicators.

Indicator	First control group of animals Me (Q1-Q3)	Second experimental group of animals Me (Q1-Q3)	U	Z	P
Leukocytes	8.500 (7.700-9.100)	8.650 (7.900-9.125)	69.5	-0.306	0.760
Lymphocytes	3.600 (3.200-3.800)	4.400 (3.725-4.450)	28.5	-2.587	0.01*
T-helpers	0.790 (0.700-0.850)	1.015 (0.885-1.152)	23.0	-2.886	0.004*
T-suppressors	0.47 (0.43-0.61)	0.67 (0.49-0.78)	37.0	-2.111	0.03*
T-lymphocytes	1.40 (1.33-1.52)	1.66 (1.51-1.83)	31.0	-2.444	0.015*
B-lymphocytes	0.6 (0.5-0.7)	0.7 (0.6-0.9)	34.5	-2.310	0.021*
Phagocytic count	2.6 (2.0-3.0)	2.2 (1.9-2.5)	52.0	-1.280	0.201
Phagocytic index	52 (49-56)	47 (43-52)	42.5	-1.810	0.07
Circulating immune complex	21 (19-24)	22.5 (18.75-22.50)	62.5	-0.694	0.488
Reaction of leukocyte migration inhibition	21 (19-24)	22.5 (17.75-26.75)	60.0	-0.836	0.403
HCT (Phagocyte activity)	3 (2-5)	4 (2-7)	97.0	-0.451	0.652
IgA	7.129 (5.624-8.610)	6.122 (4.853-6.804)	43.0	-1.775	0.07
IgM	7.406 (6.992-8.308)	6.485 (5.902-7.734)	46.0	-1.610	0.107
IgG	5.896 (5.337-6.179)	6.698 (5.018-7.926)	65.0	-0.555	0.579

Note: Mann-Whitney U Criterion was used for two independent samples. * level of statistical significance $p < 0.05$.

The study results showed that the obtained extract significantly affects experimental rats' immune system by increasing the number of lymphocytes, T-helper cells, T-suppressors, T-lymphocytes and B-lymphocytes. The extract significantly increases the number of lymphocytes, indicating its stimulating effect on cellular immunity and may increase the body's overall ability to mount an immune response. An increase in T-helper cells indicates an enhanced coordination of the immune response, as T-helper cells play a key role in activating other immune cells **[40]**. Increased T suppressors may indicate better control of the immune response and prevention of over-activation of the immune system. Increased levels of T-lymphocytes indicate an overall activation of cellular immunity. An increase in the number of B-lymphocytes indicates a stimulating effect of the extract on humoral immunity **[41]**, **[42]**.

Study of the influence of different doses of dry collagen concentrate and extract from fruit and berry plants on the quality indicators of soft curd product

In the next stage, the effect of the obtained functional ingredients on the quality indicators of soft curd products was investigated. The research was carried out to prepare a formulation of cottage cheese products for sports nutrition. The influence of different doses of a mixture of dry collagen concentrate and extract of sea buckthorn and rosehip composition on the properties of soft curd product was investigated. For the study, 9 experimental samples of soft curd products were selected, in which the above components were added in different ratios (Table 4).

Table 4 Ratio of components of the experimental sample.

Sample	Ratio, %	
	collagen concentrate	sea buckthorn and rosehip composite extract
Sample 1	4	2
Sample 2	4	4
Sample 3	4	6
Sample 4	6	2
Sample 5	6	4
Sample 6	6	6
Sample 7	8	2
Sample 8	8	4
Sample 9	8	6

These ratios (Table 4) are selected for a comprehensive study of the influence of the doses of the ingredients on the quality of soft curd products. This approach will allow identifying the optimal proportions that will provide the product's physicochemical, organoleptic and functional properties. The mixture of dry collagen-containing concentrate and extract of sea buckthorn and rosehip composition was added to the ready curd mass, which is made according to traditional technology. It is known that the stability and structural characteristics of collagen are affected by temperature **[43]**. At temperatures above 40°C, collagen loses its structural stability, which may destroy its gel-forming properties, which are important for creating structure in food products **[44]**. Therefore, the studies were conducted at temperatures of 20 °C; 25 °C; 30 °C; 35 °C; and 40 °C.

The study results of the effect of different doses of the mixture of dry collagen concentrate and extract of sea buckthorn and rosehip composition on the water-holding capacity of cottage cheese products at different temperatures of their incorporation are presented in Figure 5.

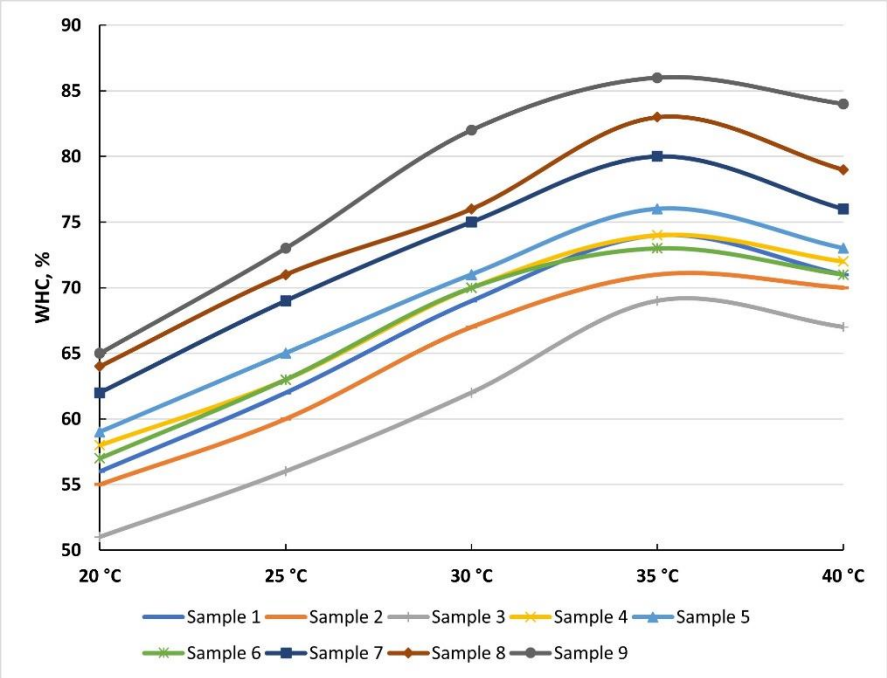


Figure 5 Dynamics of change in water-holding capacity of cottage cheese product.

As seen in Figure 5, all the experimental samples show an increase in the water-holding capacity of the curd product with an increase in temperature up to 35°C. This is probably due to the improvement of the collagen gelation process at this temperature, which increases the water-holding capacity of the product **[45]**. At 40°C, a decrease in water-holding capacity was observed. This may be due to partial denaturation of collagen and deterioration of its gelation properties.

The samples (1st, 2nd, 3rd, 6th) show the lowest values of water-holding capacity at all investigated temperatures. Reduced content of collagen and extract characterises the first experimental sample. The second and sixth experimental samples contain dry collagen concentrate and extract of sea buckthorn and rosehip composition in equal ratios. In the third experimental sample, the extract of sea buckthorn and rosehip composition

is contained more. The higher content of the extract reduces the hydrophilic properties of the product, which leads to a decrease in the ability to retain moisture despite the presence of collagen.
With the increase in the content of dry collagen concentrate in the experimental samples, an increase in the water-holding capacity of the soft curd product is observed. Balanced results with good water-holding capacity are observed in the fourth and fifth experimental samples, in which collagen concentrate and extract content is 6:2 and 6:4, respectively.

The samples with a high collagen-containing concentration (7th, 8th and 9th) show the highest water-holding capacity.

For a comprehensive understanding of the rheological properties of the curd product after determining the dynamics of changes in water-holding capacity, studies of the effect of different doses of a mixture of dry collagen concentrate and extract of sea buckthorn and rosehip composition on the change in the effective viscosity of the curd product at a temperature of 35°C were carried out. This temperature was chosen as the optimum temperature based on the study of the dynamics of change in the water-holding capacity of the curd product. The results of the study are presented in Figure 6. Some studies have reported that the gelation properties of collagen are impaired at temperatures above 50°C to 70°C **[46]**, **[47]**. Still, the results of these studies show a decrease in water-holding capacity at temperatures above 35°C, which is probably due to the deterioration of its gelation properties.

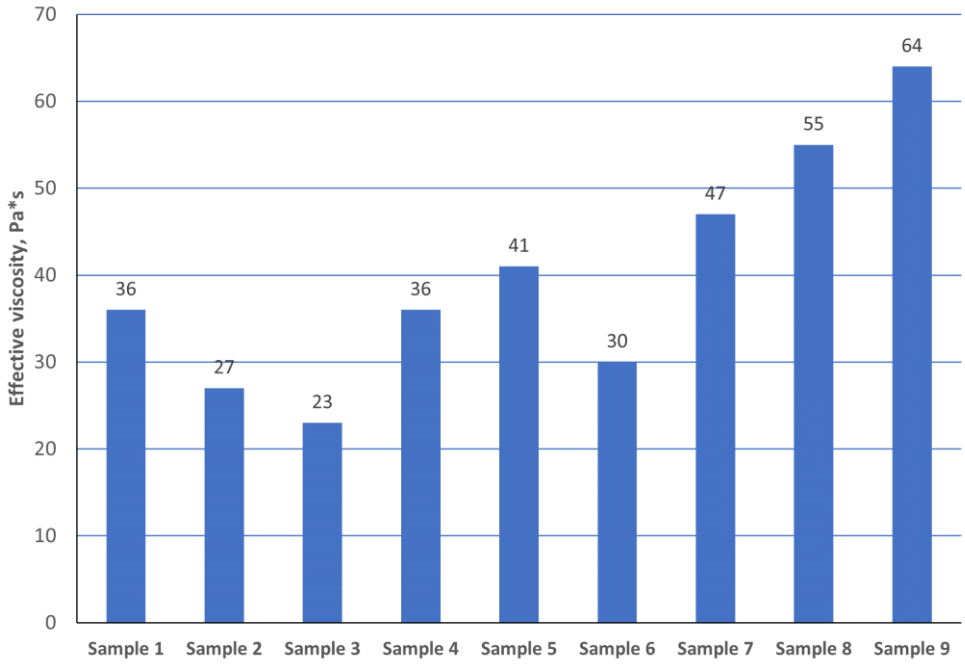


Figure 6 Effect of different doses of applied ingredients on effective viscosity.

As can be seen from Figure 5, the effective viscosity of the curd product depends on the ratio of dry collagen concentrate and sea buckthorn and rosehip extract: an increase in the proportion of collagen (especially at ratios of 8:4 and 8:6) leads to a significant increase in viscosity, indicating the formation of a denser and more stable product structure. At lower extract contents (4:2, 6:2), viscosity also remains high, indicating the ability of collagen to retain moisture and form a stable texture.

On the contrary, when the extracted content is increased to 4:6 and 6:6, viscosity decreases, which is associated with excess hydration and a decrease in product density. It should be noted that the results of the effective viscosity study correlate well with the water-holding capacity of the curd product: samples with high collagen content and optimum extract level (8:4 and 8:6), which show the highest viscosity also have the best water-holding capacity, indicating a dense and stable product structure. At the same time, samples (2nd, 3rd and 6th) with lower viscosity (4:4; 4:6 and 6:6) show decreased moisture retention capacity due to excess hydration and reduced density of the structure.

Thus, the best textural characteristics and product stability are achieved at ratios that balance collagen and extract content, as evidenced by viscosity and moisture retention properties.
In the next stage, the study of indicators that characterise the stability of the curd product structure to destruction (viscosity loss coefficient and mechanical stability coefficient) at the addition of different doses of a mixture of

dry collagen concentrate and extract of sea buckthorn and rosehip composition was carried out. The results of the study are presented in Figure 7 and Figure 8.

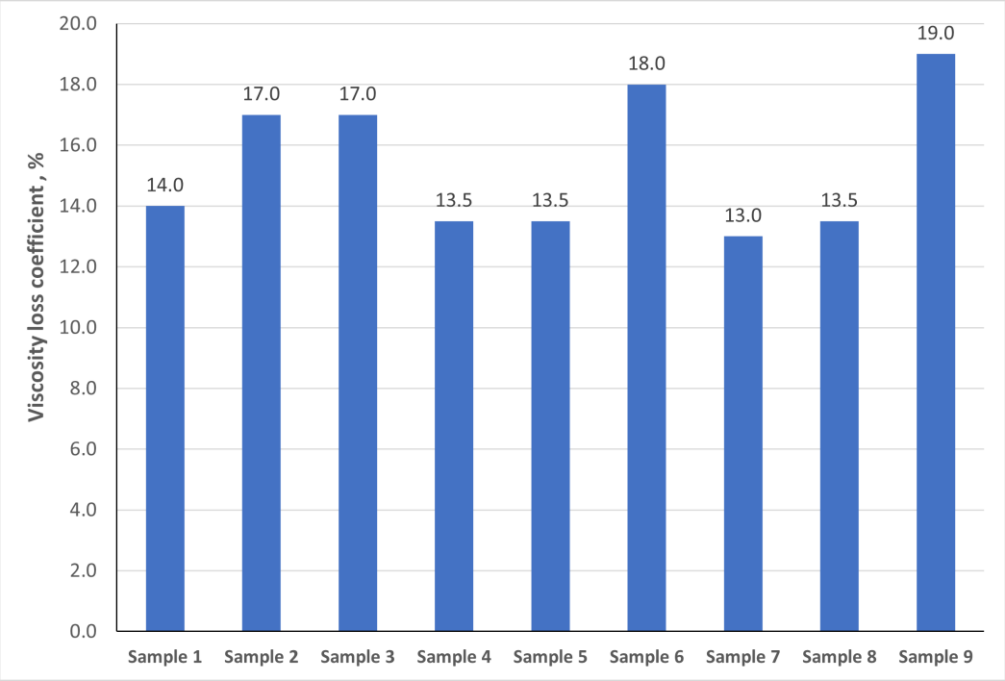


Figure 7 Viscosity loss coefficient of curd product when adding different amounts of functional ingredients.

As can be seen from Figure 7, the highest viscosity loss ratio (17-19%) was observed in the second, third, sixth and ninth samples. This result may indicate increased extract content in these ratios increases viscosity loss. This is probably because a higher concentration of extract changes the physicochemical properties of the compositions, making them less stable and increasing the fluidity of the systems.

The lowest coefficient of viscosity loss (13.5-14.0 %) is found in the first, fourth, fifth, seventh, and eighth samples. In these samples, the collagen concentrate content exceeds the extract amount by 2-4 times. This may indicate that a higher concentration of collagen component leads to an increase in the viscosity of the samples, probably due to the formation of a denser network of collagen fibers.

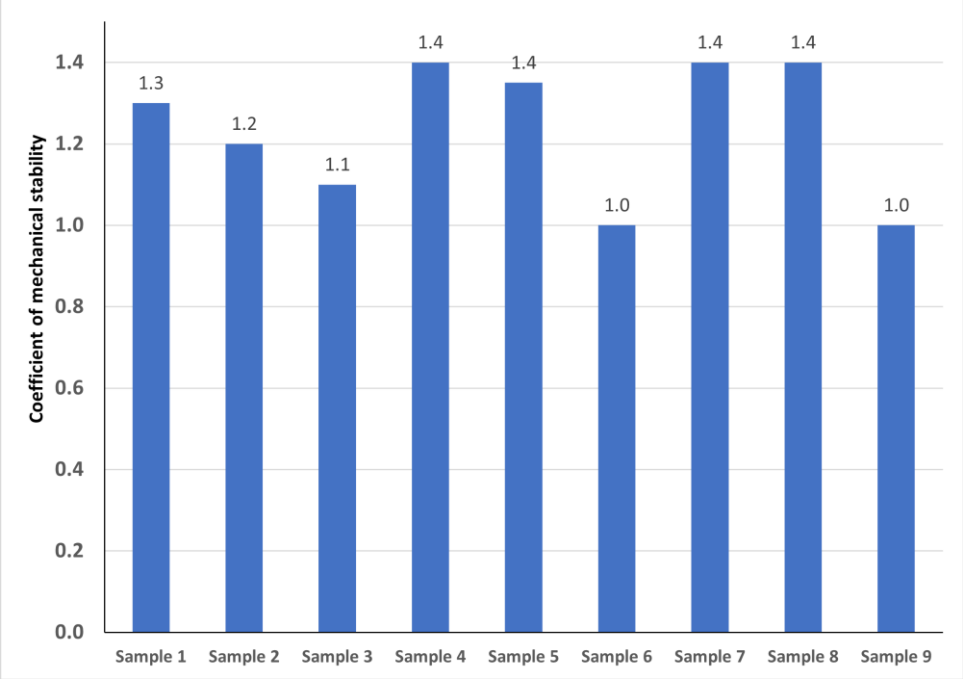


Figure 8 Coefficient of mechanical stability of cottage cheese product with the addition of different amounts of functional ingredients.

As can be seen from Figure 8, the highest coefficient of mechanical stability of the curd product is observed in the experimental samples in which the lowest viscosity loss coefficient indices (first, fourth, fifth, seventh and eighth) were established. The results show that increasing the proportion of dry collagen concentrate in the experimental samples also affects the increase in the stability of the curd product.

Study of nutritional and biological value of non-fat soft curd product

Based on the research conducted, a non-fat soft curd product was obtained. Recipe of cottage cheese product is presented in Table 5. By the recipe, after separation of whey and cooling to a temperature of 8 °C a dry collagen concentrate and extract composition of sea buckthorn and cinnamon rosehip are added to the curd mass. Nonfat soft curd product with a creamy texture and soft pink color, has a rich sweet-sour taste with a slight flavor of berry fillers (Figure 9).

Table 5. Recipe for cottage cheese product

#	Ingredient	Ingredient usage (kg per 1 t, excluding losses)
1	Low-fat soft cottage cheese product	88.0
2	Dry collagen concentrate	8.0
3	Sea buckthorn and rosehip composite extract	4.0
	Total	100

Figure 9 Appearance of low-fat soft curd product.

As can be seen in Figure 8 the curd product has fine flecks that are evenly distributed throughout the mass. These flecks result from sea buckthorn and cinnamon rosehip extract, which give the product flavour, aromatic characteristics, and visual texture. Such flecks add textural variation to the product, creating a pleasant contrast against its delicate pink hue. The nutritional and biological value of the finished product was investigated. The results of the study are presented in Table 6.

Table 6 Nutritional and biological value of experimental and control samples of soft curd product.

#	Nutrients	Control sample	Experimental sample
1.	Protein, g	16.00±0.30	16.40±0.33
2.	Fats, g	0.50±0.01	0.80±0.01*
3.	Carbohydrates, g	2.70±0.06	2.90±0.04
4.	Fatty acid composition, %:		
5.	Sum of polyunsaturated fatty acids, including	0.25±0.00	1.30±0.02*
	Sum of omega-3 polyunsaturated fatty acids	0.05±0.00	0.30±0.00*
	Sum of omega-6 polyunsaturated fatty acids	0.20±0.00	1.00±0.01*
6.	Vitamins in 100 g:		
	Vitamin A, mcg	20.00±0.30	75.00±0.79*
	Vitamin D, mcg	0.05±0.00	0.08±0.00
	Vitamin E, mg	0.10±0.00	0.21±0.01*
	Vitamin C, mg	1.00±0.01	6.00±0.10*
7.	Mineral substances mg/100 g		
	Calcium	100.00±1.26	119.00±1.32*
	Phosphorus	141.00±2.40	172.00±2.53
	Magnesium	10.00±0.16	19.00±0.28*
	Potassium	80.00±1.19	96.00±1.36*
8.	Essential amino acids, mg/100g		
	Tryptophan	0.10±0.00	0.15±0.00*
	Leucine	1.10±0.02	1.40±0.02*
	Isoleucine	0.60±0.01	0.80±0.01*
	Valine	0.70±0.01	0.80±0.01
	Threonine	0.50±0.00	0.53±0.00
	Lysine	0.90±0.01	1.20±0.02*
	Methionine + cystine	0.20±0.00	0.25±0.00*
	Phenylalanine + tyrosine	0.40±0.01	0.60±0.01*
	Energy value, kcal	78.76	83.8

Note: *indicate a significant difference from the control sample ($p<0.05$).

The data presented in Table 6 show that the experimental sample shows a slight increase in the mass fraction of proteins, fats, and carbohydrates in the soft curd product due to the addition of functional fillers. A study of a soft curd product identified omega-3 and omega-6 fatty acids. These fatty acids are crucial for maintaining cardiovascular health, reducing inflammation, and improving cognitive function and body repair **[48]**. Consequently, they are particularly important in athletes' nutrition, aiming for peak performance and rapid recovery.

The determination of vitamins A, D, E, and C is justified by their key role in maintaining immune function, antioxidant defense, skin and bone health, and ensuring normal metabolism, which is especially important for maintaining high physical activity and recovery of athletes. The determination of calcium, phosphorus, magnesium and potassium is substantiated by their critical importance in maintaining the musculoskeletal system, energy metabolism and optimal cardiovascular function, making this product particularly useful in athletes' nutrition **[49]**.

Introducing 8% collagen concentrate and 4% sea buckthorn and rosehip extract significantly increases the biological value of non-fat cottage cheese products. In particular, there is a significant increase in the content of polyunsaturated fatty acids (Omega-3 and Omega-6). The vitamin and mineral composition of the experimental sample was also significantly enriched, which is especially evident in vitamins A, C and E, as well as calcium, phosphorus and magnesium.

As a result of these changes, the prototype demonstrates a more balanced composition in terms of antioxidant protection and immune system support, which is achieved due to the increased content of polyunsaturated fatty acids Omega-3 and Omega-6, as well as vitamins A, C and E, compared to the control sample. The increased content of essential amino acids in the experimental sample, such as leucine, lysine and tryptophan, confirms the high biological value of the product. These changes can be attributed to the addition of collagen-containing concentrate, sea buckthorn, and rosehip extract, making the product more functional and healthy for consumers.

Several studies have explored using plant extracts to enhance the functional properties of cheese products. Caleja et al. (2016) investigated the incorporation of microencapsulated fennel and chamomile extracts into cottage cheese. Their findings suggest that microencapsulation offers a promising strategy to maintain higher

antioxidant activity after storage (7 days) compared to free extracts while preserving the nutritional profile of the cheese **[50]**.

Similarly, Ribeiro et al. (2016) examined the effects of rosemary extracts (both free and microencapsulated) on the antioxidant activity of cottage cheese. While both forms enhanced antioxidant activity, the free rosemary extract displayed a more pronounced effect at the initial time point (0 days) and after 7 days of storage **[51]**. These findings highlight the potential of rosemary for functional cheese development but also suggest that delivery methods may influence the extent and duration of its benefits.

Beyond rosemary and chamomile, Carocho et al. (2016) explored the use of basil leaves in "Serra da Estrela" cheese. Their research demonstrated that basil leaves, particularly in the form of decoctions, significantly increased the cheese's antioxidant activity, reduced moisture content, and preserved unsaturated fatty acids and proteins **[52]**. This study suggests that basil offers a multi-functional approach, improving the cheese's shelf life and nutritional value.

Investigation of shelf life of nonfat soft curd product by the indicators characterising the stability of its structure

A key factor affecting curd products' quality and consumer properties is its structure. The structural stability of the curd product plays an important role in forming texture and consistency and retaining these characteristics throughout shelf life. The structure's instability can lead to the separation of whey, change in consistency, and deterioration of the organoleptic parameters of the finished product **[53]**.

To determine the optimal conditions for storage of soft curd product, ensuring the maintenance of its texture and consistency throughout the shelf life, studies of changes in the coefficient of viscosity loss and mechanical stability of the product at different temperatures (0 °C, 2 ° C, 4 ° C, 6 ° C and 8 ° C) and storage time (24 hours, 48 hours, 72 hours and 96 hours are carried out.) The selected temperature regimes (0 °C, 2 °C, 4 °C, 6 °C and 8 °C) and time intervals (24 hours, 48 hours, 72 hours and 96 hours) allow a comprehensive evaluation of how the addition of collagen concentrate and extract of sea buckthorn and rosehip composition affects the preservation of the product structure during the shelf life. This will make it possible to develop recommendations on optimal storage conditions to ensure maximum conservation of the textural characteristics of the product and its consumer properties. The result of the study of change in the viscosity coefficient of soft curd product during storage is shown in Figure 10 at the initial value of viscosity loss coefficient after the addition of fillers 13.5%.

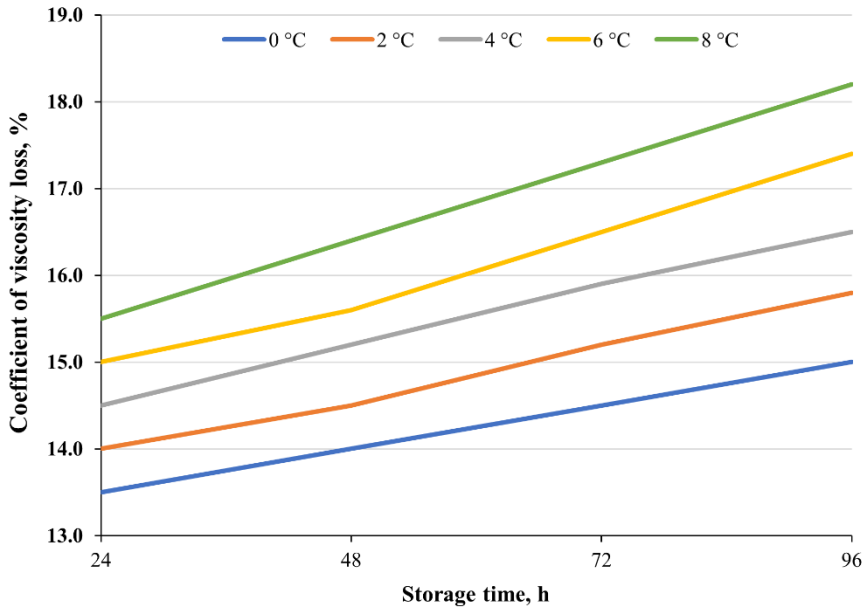


Figure 10 Change in coefficient of soft curd product viscosity loss during storage.

As can be seen from Figure 10, when the curd product was stored at 0-2 °C for 96 hours, the coefficient of viscosity loss was in the range of 15.0-15.8%. Under these conditions, the product retained its typical soft and creamy consistency, which is common for soft curd. This effect may be due to the effect of collagen concentrate on the microstructure of the curd product, which helps to increase its stability and maintain textural characteristics under the specified storage conditions.

At a temperature of 4 °C for 72 hours, the viscosity loss coefficient was 15.9%; at a temperature of 6 °C for 48 hours - 15.6%; at a temperature of 8 °C for 24 hours - 15.5%. When the viscosity loss coefficient exceeded 16%, there was whey release and a decrease in the curd product's creaminess during storage, probably due to the destruction of the protein-collagen network.

At the next stage, the change in the mechanical stability coefficient of the soft curd product during storage at its initial value of 1.4 was investigated. The results of the study are presented in Figure 11.

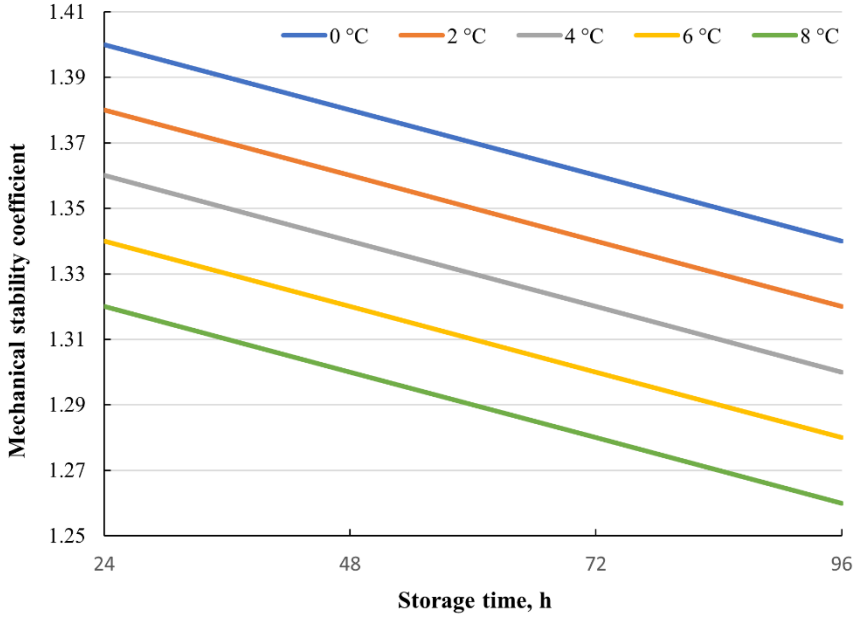


Figure 11 Change of mechanical stability coefficient of soft curd product during storage.

As shown from Figure 11, the mechanical stability coefficient gradually decreases with increasing temperature and storage time, indicating a gradual degradation of the product's structural integrity. At lower temperatures (0-2 °C), the decrease in stability is slower than at higher temperatures (6-8 °C), at which the product loses mechanical stability much faster. When the mechanical stability coefficient falls below 1.28 (after 96 hours of storage at 4-6 °C or 72 hours at 6-8 °C), the curd product has a loose consistency with a pronounced whey release. These data emphasise the importance of maintaining a low temperature to preserve the structural properties of the curd product during long storage times.

CONCLUSION

This study developed a functional soft cottage cheese product enriched with dry collagen concentrate and antioxidant-rich extracts from sea buckthorn and cinnamon rosehip. The formulation, which includes 8% collagen concentrate and 4% antioxidant extract, significantly enhances the nutritional value of the product, providing an optimal balance of polyunsaturated fatty acids, essential vitamins (A, C, and E), and minerals (such as calcium, phosphorus, magnesium, and potassium). Compared to the control cheese product, the enriched version notably increased omega-3 and omega-6 fatty acids. Additionally, the enriched product contained higher levels of vitamins A, C, and E. The study demonstrated that the enriched cottage cheese product maintained structural stability and textural integrity during storage at 0-2°C for 96 hours. The balance between collagen and plant extracts allowed the product to achieve desirable water-holding capacity, viscosity, and mechanical stability. The inclusion of collagen improved the product’s gelation properties, while the antioxidants from plant extracts provided additional health benefits without compromising the sensory attributes of the cottage cheese. Overall, the developed soft cottage cheese product delivers enhanced nutritional and biological value and retains its sensory qualities, making it a promising option for athletes and health-conscious consumers seeking functional dairy products to support their active lifestyle.

REFERENCES

1. Arenas-Jal, M., Suñé-Negre, J. M., Pérez-Lozano, P., & García-Montoya, E. (2019). Trends in the food and sports nutrition industry: A review. In Critical Reviews in Food Science and Nutrition (Vol. 60, Issue 14, pp. 2405–2421). Informa UK Limited. https://doi.org/10.1080/10408398.2019.1643287
2. Malsagova, K. A., Kopylov, A. T., Sinitsyna, A. A., Stepanov, A. A., Izotov, A. A., Butkova, T. V., Chingin, K., Klyuchnikov, M. S., & Kaysheva, A. L. (2021). Sports Nutrition: Diets, Selection Factors, Recommendations. In Nutrients (Vol. 13, Issue 11, p. 3771). MDPI AG. https://doi.org/10.3390/nu13113771
3. Hernández, H., Nunes, M. C., Prista, C., & Raymundo, A. (2022). Innovative and Healthier Dairy Products through the Addition of Microalgae: A Review. In Foods (Vol. 11, Issue 5, p. 755). MDPI AG. https://doi.org/10.3390/foods11050755
4. Zia, F.; Sumbal, S. Physical performance and functional food. J. Hum. Nutr. Food Sci. 2019, 7, 1128.
5. Kviatkovsky, S. A., Hickner, R. C., & Ormsbee, M. J. (2022). Collagen peptide supplementation for pain and function: is it effective? In Current Opinion in Clinical Nutrition and Metabolic Care (Vol. 25, Issue 6, pp. 401–406). Ovid Technologies (Wolters Kluwer Health). https://doi.org/10.1097/mco.0000000000000870
6. Clifford, T., Ventress, M., Allerton, D. M., Stansfield, S., Tang, J. C. Y., Fraser, W. D., Vanhoecke, B., Prawitt, J., & Stevenson, E. (2019). The effects of collagen peptides on muscle damage, inflammation and bone turnover following exercise: a randomized, controlled trial. In Amino Acids (Vol. 51, Issue 4, pp. 691–704). Springer Science and Business Media LLC. https://doi.org/10.1007/s00726-019-02706-5
7. Hashim, P., Mohd Ridzwan, M. S., Bakar, J., Mat Hashim, D. (2015). Collagen in food and beverage industries. In Inernational Food Research Journal (Vol. 22, Issue 1, pp. 1-8). Universiti Putra Malaysia.
8. Tang, C., Zhou, K., Zhu, Y., Zhang, W., Xie, Y., Wang, Z., Zhou, H., Yang, T., Zhang, Q., & Xu, B. (2022). Collagen and its derivatives: From structure and properties to their applications in food industry. In Food Hydrocolloids (Vol. 131, p. 107748). Elsevier BV. https://doi.org/10.1016/j.foodhyd.2022.107748
9. Santana, J. C. C., Gardim, R. B., Almeida, P. F., Borini, G. B., Quispe, A. P. B., Llanos, S. A. V., Heredia, J. A., Zamuner, S., Gamarra, F. M. C., Farias, T. M. B., Ho, L. L., & Berssaneti, F. T. (2020). Valorization of Chicken Feet By-Product of the Poultry Industry: High Qualities of Gelatin and Biofilm from Extraction of Collagen. In Polymers (Vol. 12, Issue 3, p. 529). MDPI AG. https://doi.org/10.3390/polym12030529
10. Radhakrishnan, R., Ghosh, P., Selvakumar, T.A., Shanmugavel, M., Gnanamani, A. (2020). Poultry spent wastes: an emerging trend in collagen mining. Advances in Tissua Engineering & Regenerative Medicine (Vol.6, Issue 2, p. 26-35). MedCrave. https://doi.org/10.15406/atroa.2020.06.00113
11. Kandyliari, A., Potsaki, P., Bousdouni, P., Kaloteraki, C., Christofilea, M., Almpounioti, K., Moutsou, A., Fasoulis, C. K., Polychronis, L. V., Gkalpinos, V. K., Tzakos, A. G., & Koutelidakis, A. E. (2023). Development of Dairy Products Fortified with Plant Extracts: Antioxidant and Phenolic Content Characterization. In Antioxidants (Vol. 12, Issue 2, p. 500). MDPI AG. https://doi.org/10.3390/antiox12020500
12. Lisak Jakopović, K., Repajić, M., Rumora Samarin, I., Božanić, R., Blažić, M., & Barukčić Jurina, I. (2022). Fortification of Cow Milk with Moringa oleifera Extract: Influence on Physicochemical Characteristics, Antioxidant Capacity and Mineral Content of Yoghurt. In Fermentation (Vol. 8, Issue 10, p. 545). MDPI AG. https://doi.org/10.3390/fermentation8100545
13. Tleuova K, Shingisov A, Vetokhin S. Development of technology for the manufacture of yogurt from combined cow and mare's milk enriched with plant extract in Kazakhstan. Plant Sci. Today [Internet]. 2024 Apr. 1 [cited 2024 Oct. 1];11(2). Available from: https://horizonepublishing.com/journals/index.php/PST/article/view/3193
14. Šlapkauskaitė, J., Žalnieraitė, K., Šlapkauskas, A., & Sekmokienė, D. (2018). The influence of aqueous extracts on curd-type cheese quality. In Žemės ūkio mokslai (Vol. 25, Issue 2). Lithuanian Academy of Sciences. https://doi.org/10.6001/zemesukiomokslai.v25i2.3754
15. O'Sullivan, A. M., O'Grady, M. N., O'Callaghan, Y. C., Smyth, T. J., O'Brien, N. M., & Kerry, J. P. (2016). Seaweed extracts as potential functional ingredients in yogurt. In Innovative Food Science & Emerging Technologies (Vol. 37, pp. 293–299). Elsevier BV. https://doi.org/10.1016/j.ifset.2016.07.031
16. Alenisan, M. A., Alqattan, H. H., Tolbah, L. S., & Shori, A. B. (2017). Antioxidant properties of dairy products fortified with natural additives: A review. In Journal of the Association of Arab Universities for Basic and Applied Sciences (Vol. 24, Issue 1, pp. 101–106). Informa UK Limited. https://doi.org/10.1016/j.jaubas.2017.05.001
17. Salehi, F. (2021). Quality, physicochemical, and textural properties of dairy products containing fruits and vegetables: A review. In Food Science and Nutrition (Vol. 9, Issue 8, pp. 4666–4686). Wiley. https://doi.org/10.1002/fsn3.2430

18. Clemente-Suárez, V. J., Bustamante-Sanchez, Á., Mielgo-Ayuso, J., Martínez-Guardado, I., Martín-Rodríguez, A., & Tornero-Aguilera, J. F. (2023). Antioxidants and Sports Performance. In Nutrients (Vol. 15, Issue 10, p. 2371). MDPI AG. https://doi.org/10.3390/nu15102371
19. Li, S., Fasipe, B., & Laher, I. (2022). Potential harms of supplementation with high doses of antioxidants in athletes. In Journal of Exercise Science & Fitness (Vol. 20, Issue 4, pp. 269–275). Elsevier BV. https://doi.org/10.1016/j.jesf.2022.06.001
20. Lamprecht, M. (2014). Antioxidants in Sport Nutrition. CRC Press. https://doi.org/10.1201/b17442
21. Baby, B., Antony, P., & Vijayan, R. (2017). Antioxidant and anticancer properties of berries. In Critical Reviews in Food Science and Nutrition (Vol. 58, Issue 15, pp. 2491–2507). Informa UK Limited. https://doi.org/10.1080/10408398.2017.1329198
22. Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T., & Sochor, J. (2015). Bioactive Compounds and Antioxidant Activity in Different Types of Berries. In International Journal of Molecular Sciences (Vol. 16, Issue 10, pp. 24673–24706). MDPI AG. https://doi.org/10.3390/ijms161024673
23. GOST P 55312-2012 (2020). Propolis. Method for determination of flavonoid compounds. Moscow: Standardinform.
24. GOST 12823-1-2014 (2016). Foodstuffs - determination of vitamin A by high performance liquid chromatography - part 1: measurement of all-trans-retinol and 13-cis-retinol. Moscow: Standardinform.
25. GOST 34151-2017 (2019). Food products. Determination of vitamin C by high-performance liquid chromatography. Moscow: Standardinform.
26. GOST EN 12822-2014 (2015). Foodstuffs. Determination of vitamin E (alpha-, betta-, gamma- and delta-tocopherols) by high-performance liquid chromatography. Moscow: Standardinform.
27. GOST R 50207-92 (2010). Meat and meat products. Method for determination of oxyproline. Moscow: Standardinform.
28. Dolmatova O.I., Sidelnikov E.M. Production of cottage cheese with improved quality indicators. Proceedings of VSUET. 2023. vol. 85. no. 4. pp. 15–19. (in Russian). doi:10.20914/2310-1202-2023-4-15-19
29. GOST 5867-2023 (2024). Milk and milk products. Methods of determination of fat. Moscow: Russian Institute of Standardization.
30. GOST 25179-2014 (2019). Milk and milk products. Methods for determining the mass fraction of protein. Moscow: Standartinform.
31. GOST 34304-2017 (2018). Milk and milk products. Method for the determination of lactose and galactose. Moscow: Standartinform.
32. GOST 32915-2014 (2015). Milk and dairy products. Determination of fatty acid composition of the fat phase by gas chromatography. Moscow: Standartinform.
33. MVI MN 1363-2000 “Method for determination of amino acids in food products by high-performance liquid chromatography”
34. GOST EN 12821-2014 (2014). Foodstuffs. Determination of cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) by high-performance liquid chromatography. Moscow: Standartinform.
35. GOST ISO 8070/IDF 119-2014 (2021). Milk and milk products. Determination of calcium, sodium, potassium and magnesium. Atomic absorption spectrometric method. Moscow: Russian Institute of Standardization.
36. GOST 30615-99 (2003). Raw materials and food products. Method for determination of phosphorus. Minsk: Interstate Council for Standardization, Metrology and Certification.
37. Munasinghe, K. A., Schwarz, J. G., & Nyame, A. K. (2014). Chicken Collagen from Law Market Value By-Products as an Alternate Source. In Journal of Food Processing (Vol. 2014, pp. 1–6). Hindawi Limited. https://doi.org/10.1155/2014/298295
38. Chen, P.-Y., Hsieh, H.-J., & Huang, L. L. H. (2014). Shrinking mechanism of a porous collagen matrix immersed in solution. In Journal of Biomedical Materials Research Part A (p. n/a-n/a). Wiley. https://doi.org/10.1002/jbm.a.35135
39. Nigussie, G., Siyadatpanah, A., Norouzi, R., Debebe, E., Alemayehu, M., & Dekebo, A. (2023). Antioxidant Potential of Ethiopian Medicinal Plants and Their Phytochemicals: A Review of Pharmacological Evaluation. In R. K. Singla (Ed.), Evidence-Based Complementary and Alternative Medicine (Vol. 2023, Issue 1). Wiley. https://doi.org/10.1155/2023/1901529
40. Barakat, H., Alkhurayji, R. I., & Aljutaily, T. (2023). Immune-Boosting Potentiating Properties of Brassica nigra Hydroalcoholic Extract in Cyclophosphamide-Induced Immunosuppression in Rats. In Foods (Vol. 12, Issue 19, p. 3652). MDPI AG. https://doi.org/10.3390/foods12193652
41. Joshua, P. E., Yahaya, J., Ekpo, D. E., Ogidigo, J. O., Odiba, A. S., Asomadu, R. O., Oka, S. A., & Adeniyi, O. S. (2022). Modulation of immunological responses by aqueous extract of Datura stramonium L. seeds on

cyclophosphamide-induced immunosuppression in Wistar rats. In BMC Immunology (Vol. 23, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12865-022-00519-y

42. Birhanu, B. T., Kim, J.-Y., Hossain, Md. A., Choi, J.-W., Lee, S.-P., & Park, S.-C. (2018). An in vivo immunomodulatory and anti-inflammatory study of fermented Dendropanax morbifera Léveille leaf extract. In BMC Complementary and Alternative Medicine (Vol. 18, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s12906-018-2282-x
43. Soutelino, M. E. M., Rocha, R. da S., de Oliveira, B. C. R., Máralém, E. T., & Silva, A. C. de O. (2023). Technological aspects and health effects of hydrolyzed collagen and application in dairy products. In Critical Reviews in Food Science and Nutrition (Vol. 64, Issue 18, pp. 6120–6128). Informa UK Limited. https://doi.org/10.1080/10408398.2022.2163974
44. Zhang, X., Xu, S., Shen, L., & Li, G. (2020). Factors affecting thermal stability of collagen from the aspects of extraction, processing and modification. In Journal of Leather Science and Engineering (Vol. 2, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1186/s42825-020-00033-0
45. Znamirowska, A., Szajnar, K., & Pawlos, M. (2020). Probiotic Fermented Milk with Collagen. In Dairy (Vol. 1, Issue 2, pp. 126–134). MDPI AG. https://doi.org/10.3390/dairy1020008
46. Tang, C., Xu, Y., Zhou, K., Xie, Y., Ma, Y., Li, C., Xu, F., Zhou, H., & Xu, B. (2023). Mechanism behind the deterioration in gel properties of collagen gel induced by high-temperature treatments: A molecular perspective. In Food Research International (Vol. 171, p. 112985). Elsevier BV. https://doi.org/10.1016/j.foodres.2023.112985
47. de Moraes, M. C., & Cunha, R. L. (2013). Gelation property and water holding capacity of heat-treated collagen at different temperature and pH values. In Food Research International (Vol. 50, Issue 1, pp. 213–223). Elsevier BV. https://doi.org/10.1016/j.foodres.2012.10.016
48. Djuricic, I., & Calder, P. C. (2021). Beneficial Outcomes of Omega-6 and Omega-3 Polyunsaturated Fatty Acids on Human Health: An Update for 2021. In Nutrients (Vol. 13, Issue 7, p. 2421). MDPI AG. https://doi.org/10.3390/nu13072421
49. Farag, M. A., Abib, B., Qin, Z., Ze, X., & Ali, S. E. (2023). Dietary macrominerals: Updated review of their role and orchestration in human nutrition throughout the life cycle with sex differences. In Current Research in Food Science (Vol. 6, p. 100450). Elsevier BV. https://doi.org/10.1016/j.crfs.2023.100450
50. Caleja, C., Ribeiro, A., Barros, L., Barreira, J. C. M., Antonio, A. L., Beatriz P.P. Oliveira, M., Barreiro, M. F., & Ferreira, I. C. F. R. (2016). Cottage cheeses functionalized with fennel and chamomile extracts: Comparative performance between free and microencapsulated forms. In Food Chemistry (Vol. 199, pp. 720–726). Elsevier BV. https://doi.org/10.1016/j.foodchem.2015.12.085
51. Ribeiro, A., Caleja, C., Barros, L., Santos-Buelga, C., Barreiro, M. F., & Ferreira, I. C. F. R. (2016). Rosemary extracts in functional foods: extraction, chemical characterization and incorporation of free and microencapsulated forms in cottage cheese. In Food & Function (Vol. 7, Issue 5, pp. 2185–2196). Royal Society of Chemistry (RSC). https://doi.org/10.1039/c6fo00270f
52. Carocho, M., Barros, L., Barreira, J. C. M., Calhelha, R. C., Soković, M., Fernández-Ruiz, V., Buelga, C. S., Morales, P., & Ferreira, I. C. F. R. (2016). Basil as functional and preserving ingredient in “Serra da Estrela” cheese. In Food Chemistry (Vol. 207, pp. 51–59). Elsevier BV. https://doi.org/10.1016/j.foodchem.2016.03.085
53. Kayihura, J. F. (2024). Structural dependence of concentrated skim milk curd on micellar restructuring. In Heliyon (Vol. 10, Issue 1, p. e24046). Elsevier BV. https://doi.org/10.1016/j.heliyon.2024.e24046

Funds:

This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP19679638).

Acknowledgments:

-

Conflict of Interest:

No potential conflict of interest was reported by the author(s).

Ethical Statement:

The study protocols and experimental procedures were approved by the Local Ethical Commission of the Institutional Review Board of Shakarim University of Semey (protocol code P042-2.11 from 20.04.2021).

Contact Address:

Yerlan Zharykbasov
Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan, Tel.: + 7 7222 314 602
E-mail: erlan-0975@mail.ru
ORCID: https://orcid.org/0000-0001-9707-0539

Aitbek Kakimov
Shakarim University, Department of Food technology and biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan, Tel.: + 7 7222 314 602
E-mail: bibi.53@mail.ru
ORCID: https://orcid.org/0000-0002-9607-1684

Klara Zharykbasova*
Alikhan Bokeikhan University, Department of Biotechnology, 11 Mangilik El Street, 070000, Semey, Kazakhstan, Tel.: + 7 702 470 30 39
E-mail: klara_zharykbasova@mail.ru
ORCID: https://orcid.org/0000-0002-2027-3183

Zhainagul Kakimova
Shakarim University, Department of Biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan, Tel.: + 7 7222 314 602
E-mail: zhaynaqul.kakimova@mail.ru
ORCID: https://orcid.org/0000-0002-3501-3042

Guldana Raimkhanova
Shakarim University, Department of Biotechnology, 20A Glinki Street, 071412, Semey, Kazakhstan, Tel.: + 7 7222 314 602
E-mail: nurlankyzy_92@mail.ru
ORCID: https://orcid.org/0000-0003-0899-6781

Zhanna Kozykenova
NCJSC "Semey Medical University", Department of Physiological Disciplines named after T.A. Nazarova, 103 Abay street , 070000, Semey, Kazakhstan, Tel.: + 7 777 583 56 17
E-mail: alicher-02@mail.ru
ORCID: https://orcid.org/0000-0001-7420-2279

Corresponding author: *

We support science and research.

www.haccp.sk