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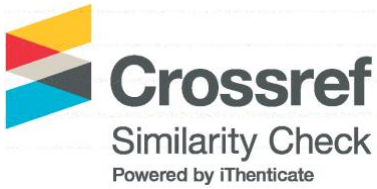

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Verification of the humic substances and PGPB biostimulants beneficial effects on the potato yield and bioactive substances content

Alena Andrejiová, Samuel Adamec, Alžbeta Hegedűsová, Ondrej Hegedűs, Robert Rosa

ABSTRACT

Potatoes are one of the most important sources of nutrients worldwide, but excessive doses of industrial fertilizers are usually used to achieve higher yields. Soil biostimulants are an increasingly used alternative for reducing fertilizer doses and growing healthy agricultural products. In this study, we examined the effects of humic substances (Agriful) and beneficial bacteria (Groundfix) based biostimulants applied by dripping irrigation on the yield and quality of potato tubers in comparison with the conventional N fertilization system. The small trail field experiment was founded in the growing season of 2020 in the Botanical Garden of the Slovak University of Agriculture in Nitra. The highest tubers yield had the combination of biostimulants and N fertilizer – 195.16% above to control. Simultaneously this combination reached an increase in refractometric dry matter content, starch content – 3.6%, and vitamin C content – 20% increase above to control. The Groundfix variant had the highest antioxidant activity with a 16.2% difference compared to the conventional nitrogen fertilization variant. These results show the positive effect of applied biostimulants on the yield and quality of cultivated potatoes.

Keywords: potatoes, yield, biostimulants, humic substances, beneficial bacteria

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) as one of the most important sources of antioxidants and energy (about 14%) in the human diet are together with rice and wheat the most important food crops for human consumption **[1]**, **[2]**, **[4]**. Tubers are a great source of carbohydrates, resistant starch, quality proteins, and vitamin C **[2]**, **[3]**. Plays an important role in the production of antioxidant defense systems by providing essential nutrient antioxidants, such as vitamins, β-carotene, polyphenols, and minerals (especially potassium) **[5]**. *Solanum tuberosum* L. is a typical plant that loves hummus in terms of soil and ecological conditions **[6]**. They prefer heated, deep, light to medium-heavy soils that are not too rocky and have an even water supply, however management of nutrients, especially nitrogen (N), is one of the most important factors in potato production. Within agronomic operations, soil compaction should be avoided as much as possible to achieve quality tuber yields **[7]**, **[8]**. Cropping systems and management practices that improve soil health may greatly enhance crop productivity **[9]**.

Since 1900, soil organic matter has declined drastically in farmlands around the world because of carbon turnover and cropping systems **[10]**. But it is only one of the problems farmers must face nowadays. Feeding the world's rising population is one of the biggest challenges, especially when the agriculture system is facing a multitude of complex problems arising from changing environments due to global climate change **[11]**. Current trends in agriculture are also focusing on enhancing the efficiency of fertilizer use since approximately 65% of applied mineral nitrogen is lost from the plant-soil system through gaseous emissions, runoff, erosion, and

leaching **[12]**. Similarly, plant P uptake is inefficient due to poor soil P solubility, especially for potato (*Solanum tuberosum* L.) plants with relatively poor rooting efficiency **[13]**. To avoid excessive use of external inputs, without compromising potato crop performance, the increase of soil nutrient availability and nutrient use efficiency is fundamental in sustainable potato production **[19]**. Farmers start to understand that focusing on increasing fertilization rates may have a negative impact on food safety and the integrity of the ecosystem **[14]**. This is indicated by the fact that global demand for organic production is increasing by approx. 20% annually **[15]**. Plants are more and more affected by environmental stresses **[16]** while abiotic stresses strongly affect plant growth, development, and quality of production **[17]**. For these reasons plant abiotic stress has been a matter of concern for the maintenance of human life on earth and especially for the world economy **[18]** and the reduction of environmental impact, ensuring both high and stable yield and high quality, is a primary goal in modern agriculture **[20]**. Various potato cultivation techniques have been developed for this purpose **[21]** but the increase of crop stress tolerance through genetic improvements requires long breeding programs and different cultivation environments for crop performance validation **[22]**. The horticultural sector is therefore searching for innovative and sustainable agronomic solutions **[23]** which simultaneously will bring repeated positive results, are easy to handle, and are reasonably priced **[24]**.

The use of plant biostimulants has gained substantial and significant heed worldwide as an environmentally friendly alternative to sustainable agricultural production **[11]**. Humic acid (HA) and plant growth-promoting rhizobacteria (PGPR) are among the most effective methods that utilize natural biologically active substances **[25]**. During evolution, plants have become associated with guilds of plant-growth-promoting rhizobacteria (PGPR), which raises the possibility that individual PGPR populations may have developed mechanisms to counteract one another on plant roots **[24]**. Currently, within industry and agricultural research, there is an increasing curiosity about microbial biostimulants, especially beneficial soil bacteria **[11]**. An increasing number of studies have illustrated the important role of microbiota in crop plant growth **[27]**, **[28]**. Increasing the presence of beneficial soil microorganisms in soil is considered a promising sustainable alternative to support conventional and organic fertilization and may help to improve crop health and productivity **[26]**. Different mechanisms are involved in bacteria-induced plant growth promotion, including biological nitrogen fixation (BNF), mineral solubilization, production of phytohormones, and pathogen biocontrol **[12]**. The claimed benefits of the tested microbial products include improving soil physical and biological health, stimulating beneficial microbial populations, increasing crop yield, root growth, plant establishment, drought tolerance, and reducing fertilizer requirements **[29]**. Each microorganism functions in coordination with the overall soil microbiome to influence plant health and crop productivity **[28]**. While there may not be one simple strategy that can effectively promote the growth of all plants under all conditions **[30]**, every new knowledge is very important.
Humic substances (HS) are the major fraction of the soil organic matter which represent the final stage of a complex interaction between non-living organic matter and microbial communities and are among the most complex and biologically active compounds in the soil **[31]**, **[32]**. Their connection with humus quality plays dynamic roles in soil physical, chemical, and biological functions essential to soil health and plant growth **[33]**, **[10]**. Biostimulant effects of humic substances are characterized by both structural and physiological changes in roots and shoots related to nutrient uptake, assimilation, and distribution (nutrient use efficiency traits) **[34]**. It includes changes in root architecture and growth dynamics but is also extended to the major biochemical pathways since the driving force for most nutrient uptake is the electrochemical gradient across the plasma membrane **[32]**. Stimulatory effects of humic substances (HS) on plant growth have been observed and widely documented **[35]**, however recent research has shown that humic substances provide direct stimulation of plant growth under laboratory conditions **[36]**. They have many other supportive effects on plants **[34]**, **[37]** but the detailed nature of humic substances is still not very understood **[38]**. For example, the mitigating activity of HS can be defined as a phenomenon of lowering the adverse effects of contaminants toxicity and those of abiotic stress factors such as unfavorable temperature, pH, salinity, etc. As a rule, it is related to the detoxifying properties of HS or their beneficial effects on biota **[39]**.

If the application of single bio-effectors has shown satisfactory results, further improvements may arise by combining multiple beneficial soil microorganisms with natural bioactive molecules **[26]**. Enhanced knowledge of the effects on plants' physiology and biochemistry and interaction with rhizosphere and endophytic microbes should lead to achieving increased crop productivity through better use of HS inputs in Agriculture **[32]**. Combinations allow bacteria to interact with each other synergistically, providing nutrients and stimulating each other through physical and biochemical activities that may enhance some beneficial aspects of their physiology **[40]**. The stability and increased consistency of the potato plant response to bacterial inoculation in the presence of humic acid indicated should be a promising biotechnological tool to improve the growth, quality, and adaptation of potatoes to field conditions **[25]**. The challenge of this study was to verify the mutual effectiveness of two selected biostimulants, based on humic substances and PGPB.

Scientific hypothesis

The yield and quality of the potato tubers variety ´Spinela´ cultivated in the Botanical Garden of Slovak University of Agriculture will depend on the biostimulants and nitrogen application, while expected best results after the combined application of humic substances and beneficial bacteria due to their synergic effectivity.

MATERIAL AND METHODOLOGY

Samples

University of Agriculture (SUA) in Nitra, cultivated by the Institute of Horticulture of SUA (48°18'53" N, 18°5'15" E) in 2020. Within our research, we studied the effects of humic substances and beneficial bacteria biostimulant on potato tubers' total and marketable yield, together with their antioxidant activity and refractometric dry matter, starch, vitamin C, and total polyphenols content. Determination of refractometric dry matter, total polyphenols, vitamin C and antioxidant activity (AOA) in potatoes was carried out at the Institute of Horticulture of the Slovak University of Agriculture in Nitra. The analyses of total starch content took place in the laboratories of the Department of Storage and Processing of Plant Products of the FBP SPU in Nitra.

Chemicals

All chemicals were of analytical grade quality. For analyses of refractometric and gravimetric dry matter, we used distilled water. The following reagents were used for the tests of other qualitative parameters: (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%, Acros Organics™, Denmark), 2,2-diphenyl-1-picrylhydrazyl (DPPH, ≤100%, Sigma Aldrich), Folin–Ciocalteu reagent (Merck Germany), gallic acid (GA, Sigma Aldrich), sodium carbonate (solution 20% w/w, Merck Germany), palladium nitrate ($Pd(NO3)_2$, palladium modifier, 0.1 $mol.l^{-1}$), ascorbic acid (AsA, solution 1%, w/w), methanol (pure pro analysis – purity grades of lab reagents, 70%, v/v, Fisher Scientific UK, Loughborough, UK). For starch content analyses HCl, 30% zinc sulfate solution and 15% potassium ferrocyanide solution was used. For Vitamin C analysis 2,6-dichlorophenolindophenol and 5% trichloroacetic acid solution was used.

Animals and Biological Material

Animal materials weren't used in this research. Groundfix is a bacterial biostimulant containing a rich spectrum of soil bacteria influencing plant growth and soil health condition: Bacillus subtilis, Bacillus megatherium, var. phosphaticum, Azotobacter chroococcum, Enterobacter, Paenibacillus polymyxa. The total number of living microorganisms is 0.5-1.5 x 109 CFU/cm^3. This biostimulant also contains other beneficial microorganisms (Lactobacilli, enzyme-producing bacteria), vitamins, phytohormones, amino acids, and other physiologically active substances. Agrigul is humic substances (HS) based biostimulant with a total content of humic substances 50% (humic acids 25% and fulvic acids 25%), nitrogen content (N) 4.5%, phosphorus content (P2O5) 1.0%, potassium content (K2O) 1.0%, remaining organic matter content 45.0%.

Instruments

The refractometric dry matter was measured by using a refractometer type CRUESS DR201-95.

Determination of antioxidant activity was performed with a spectrophotometer Jenway 6301 (Bibby Scientific Ltd., UK).

For determination of total polyphenols was used spectrophotometer Shimadzu UV/VIS-1240. The absorbance of the sample solution was measured at 765 nm of wavelength against blank.

Laboratory Methods

The tubers collected within each replicate and variant were weighed and subsequently sorted according to the applicable UN/ECE quality standards for tubers of late potato varieties for marketing and the proportion of marketable tubers was determined. From the measured data, we calculated the achieved total potato yield as well as the marketable yield of tubers. The refractometric dry matter was measured by measuring the average homogenized potato samples of the individual replications. The total dry matter content was determined by using the gravimetric weighing method. The average homogenized potato samples of the individual replications were dried at 105 °C up until the weight of the samples became stable. Determination of the starch content was carried out polarimetrically by the Ewers method. The polarimetric determination of starch content involves the conversion of starch by hydrolysis into an optically active substance. The analyses took place in the laboratories of the Faculty of Biotechnology and food science of the SUA in Nitra **[42]**. Determination of vitamin C content (ascorbic acid) was conducted by 2,6-Dichloroindophenol Titrimetric Method. Antioxidant activity (AOA) was determined by DPPH method and expressed as % inhibition of the DPPH radicals per g of sample and recalculated to Trolox equivalent (TE) **[43]**. The absorbance of the samples was measured at a wavelength of 517 nm (At_{30}). Total polyphenol content (TPC) was estimated by using Folin–Ciocalteu method using gallic acid as a standard (GAE) spectrophotometrically at 765 nm according to **[44]** and calculated in milligram of GA equivalent (GAE) per kilogram dried weight (d.w.).

Figure 1 Solanum tuberosum L., variety ´Spinela´ (A), Experimental variant at the beginning of vegetation and (B), Sorting of tubers after harvesting.

Description of the Experiment

Sample preparation: Plant raw material (tubers) was weighed on laboratory weights and prepared in the labs at the Institute of Horticulture of SUA. Material for average samples was taken diagonally from the collected tubers and homogenized. 40 ml of methanol (70%, v/v) was added to 1 g of the homogenized mixture into 250 ml extraction flasks and left at room temperature for 20 h and then extracted with a horizontal shaker for 4 h **[80]**. We used 10 g of precisely weighed and homogenized mashed potatoes for individual starch content analyses and Vitamin C analyses.

Number of samples analyzed: We analysed 14 samples.

Number of repeated analyses: All biochemical procedures were duplicated – 2 qualitative analyses for each sample (variant) except for refractometric dry matter, where 3 analyses for each repetition were estimated.

Number of experiment replication: 7 reps. for each observed variant.

Design of the experiment: The small plot field experiment was established by the block method under open field conditions. The tubers were planted on 16 April 2020, with 13 tubers planted in each repetition in a single cultivation distance of 0.75 m x 0.3 m. The area of one experimental plot (repetition) was 3 m^2, and with four repetitions for variant, the area of one experimental variant was 12 m^2. Between the individual experimental variants was a protective line, the width of which was 1 m. The total area of the experiment was 108 m^2.

During the vegetation, additional sprinkler irrigation was applied as needed, based on climatic conditions and soil moisture status. Soil cultivation and weed control were carried out by manual hoeing. During the vegetation, the health status of the plants was monitored, and the plants were treated as necessary with authorized plant protection products. Potato harvesting took place once September 4, 2020.

In the experiment, we observed the differences in the biostimulating effect of selected biostimulants on selected quantitative and qualitative parameters of potato tubers. For these purposes, we have established 6 different variants:

- 1st variant (C) – control variant without applying N fertilizer and biostimulants.
- 2nd variant (N) – conventional variant with a full dose of N fertilizer, but without biostimulants
- 3rd variant (N + G) – conventional variant with a full dose of N fertilizer, with a combination of plant growth promoting bacteria biostimulant – Groudfix (G)
- 4th variant (N + G +A) – conventional variant with a full dose of N fertilizer, with a combination of plant growth promoting bacteria biostimulant – Groudfix (G) and biostimulant based on humic substances – Agriful (A)
- 5th variant (G +A) – variant without applying of N fertilizer, with a combination of plant growth promoting bacteria biostimulant (G) and biostimulant based on humic substances (A)
- 6th variant (G) – variant without applying of N fertilizer, with plant growth promoting bacteria biostimulant (G)

Before the establishment of the experiment, an agrochemical analysis of the soil was performed at the Department of Agrochemistry and Plant Nutrition, Faculty of Agrobiology and Food Resources, SUA in Nitra. The results are presented in Table 1. In the autumn, 2019 cow manure was applied at the dose of 30 $t.ha^{-1}$ and then plowed into the soil. Based on agrochemical analysis of the soil and the recommended standard for growing potatoes according to **[41]**, supplementary fertilization has been done in the form of nitrogen. N fertilizer was added in the form of urea (45.5% N), which is a nitrogen fertilizer with a slow-acting form of nitrogen at the dose of 130 $kg.ha^{-1}$ (100% of the recommended standard) two weeks before the planned planting of potatoes. Based on soil analysis, other macroelements were not applied to the soil.

For experimental purposes, potatoes (*Solanum tuberosum* L.) were selected as a model crop based on their importance to growers in the region. The variety "Spinela" was chosen as an available and recommended variety in an actual growing season. A ´Spinela´ is medium early red-skin variety intended for direct consumption designated based on processing characteristics as cooking type B with starch content in tubers 15-16%. This area is situated in a very warm agroclimatic region and a very dry sub-region. The data in Table 2 shows the weather condition of the experimental site during the growing season of 2020 including average rainfalls and average temperatures.

For starch content analyses was the homogenized sample quantitatively flushed with 100 cm^3 of HCl into a 200 cm^3 volumetric flask. The contents were thoroughly mixed, and the flask was placed in a pre-prepared boiling water bath for 15 minutes. We move the bank in the first three minutes. After 15 minutes, the flask was filled to about 180 cm^3 with distilled water and cooled to 20 °C. The contents of the flask are clarified by adding 2 cm^3 of a 30% zinc sulfate solution and 2 cm^3 of a 15% potassium ferrocyanide solution. After mixing, the clarifier is working for 5 minutes and then the flask is filled with distilled water exactly to the 200 cm^3 mark. After re-mixing, we filter the contents of the flask. The first portion of the filtrate is poured out and a 200 mm long polarimetric tube is filled with the next clear filtrate. AOA analyses of DPPH inhibition and spectrophotometric measurements were performed after a constant time of 30 min. Of note, 0.1 ml of the extract was pipetted into the spectrophotometer cuvette (depending on the nature of the sample) and supplemented with 70% methanol to 2.0 ml, and 4 ml of DPPH solution of about 25 $mg.l^{-1}$ concentration was added. Immediately after the DPPH solution was added, the absorbance of the mixture was measured at 517 nm (At_0). Thirty minutes later, the absorbance of each sample was measured at 517 nm (At_{30}). For the determination of total polyphenols content, the Folin–Ciocalteu phenol reagent was added to a volumetric flask containing 100 µl of extract. The content was mixed, and 5 ml of a sodium carbonate solution (20%, w/w) was added after 3 min. The volume was adjusted to 50 ml by adding distilled water. After 2 hours, the samples were centrifuged for 10 min and the absorbance was measured at the wavelength of 765 nm against blank. Vitamin C: To prepare the titration reagent solution, 0.2 g of 2,6-dichlorophenolindophenol was weighed and diluted with a small amount of distilled water, the reason was to dissolve the reagent. Subsequently, we filtered the titrant through a blue filter and poured it into a measuring flask (500 ml). The reagent was made up to the mark with hot distilled water and cooled in a water bath. The cooled solution was kept in the cold. 10 g of the homogenized potato sample was placed in a volumetric flask, and we poured it with 30 ml of 5% trichloroacetic acid solution and let it macerate for 2 hours in the dark.

Table 1 Agrochemical characteristics of the soil before the experiment establishment in 2020.

pH/KCl	Humus %	Nutrients content in $mg.kg^{-1}$ of the soil			
		N_{an}	P	K	Mg
6.77 Neutral	3.89 Good	19.40 M	325.00 VH	650.00 VH	817 VH

Note: Nutrient content: M – medium content, H – high content, VH – very high content.

Table 2 Evaluation of the mean monthly air temperatures and total precipitations during growing season 2020 according to climatology normal 1961-1990.

Month	t [°C]	Normal (1961-1990)	Characteristic	PRC [mm]	Normal (1961-1990)	Characteristic
V.	15.0	15.1	Normal	91	58	Extra Wet
VI.	20.3	18.0	Extra Warm	14	66	Extremely Dry
VII.	21.4	19.8	Warm	135	52	Extremely Dry
VIII.	19.5	19.3	Normal	35	61	Dry
IX.	17.5	15.6	Warm	37	40	Normal

Statistical Analysis

Differences between variants were tested using a one-way analysis of variance in Statgraphics Centurion XVIII (StatPoint Inc., USA). For the deriving, the relationship between the observed parameters, a Pearson's product-model correlation was determined. We used the LSD test ($p \leq 0.05$) and Microsoft Excel 2010 (USA) to test statistically significant differences in the calculated averages.

RESULTS AND DISCUSSION

Total and marketable yield

Over the past 17 years, many research papers were focused on the efficacy of different commercial humates products on potato production. Data from humic acid (HA) trials showed that different cropping systems responded differently to different products in relation to yield and quality **[10]**, which also supports our results. However, the efficacy of humic products would seem to depend on many factors, including solar radiation, weather damage, soil type, crop species, yield level, and the absence or presence of other yield constraints (disease, pests, weeds, water stress) **[75]**. Moreover, many soils are rich in naturally occurring humic substances and plants may not benefit from additional application in these soils to the expected extent **[13]** and the source of the HS have a strong impact on whether plant growth is significantly improved **[45]**.

In our experiments, the biostimulatory effect and conventional nitrogen fertilization effect on the yield of potato tubers were examined. The open-field trials showed that the potato marketable yield was significantly affected in all observed variants compared to the control variant without treatment. The average yields of cultivated potato tubers are stated in (Table 3) as control, nitrogen, and Groundfix variant for their combinations. The lowest yield was measured within the control variant where no biostimulants or fertilizers were applied. In comparison with the Control variant, the increased marketable yield after the application of conventional nitrogen fertilizer alone was 149.53% higher. Potato plants inoculated with biostimulants, alone or in combination, significantly increased the marketable yield of tubers compared to the control (C), however, only a combination of Nitrogen fertilizer with both biostimulants has a higher marketable yield than fertilized variant alone with 195.16% increase above control, 18.29% increase above conventional variant and with the marketable yield of 47.61 $t.ha^{-1}$. Other biostimulant treatments were also significantly effective and increases were, respectively 115.19% for a combination of nitrogen with Groundfix (PGPB), 71.17% for a combination of Groundfix (PGPB) with Agriful (HS) and 33.78% for Groundfix (PGPB) alone.

Within similar experiments, biostimulant based on humic substances have shown a yield of 50 $t.ha^{-1}$ with 37% increase compared to the control. Bacterial biostimulant had 14% increase in the yield of about 40 $t.ha^{-1}$ **[76]**. In the study **[25]**, PGPR and HA mixed culture increased total potato tuber yield by 140% while conventional single treatment of 100% NPK fertilizer only led to an increase in potato production of 111% compared to the untreated and unfertilized control. Treatment with humic substances increased potato yield in comparison to chemical fertilizers also in the experiments of **[77]**, **[78]** and authors of **[10]** stated that in many of their studies application of humic substances resulted in a yield of potatoes increased by 11.4% to a maximum of 20.3%. Similarly, the bio-fertilization of potatoes with PGPB significantly increased the total yield by 21% above the untreated plants in **[79]** and by 1.8% in **[47]**.

Table 3 Average yield of potato tubers ($t.ha^{-1}$) depending on the observed experimental variants *; Nitra, 2020

Variant	Total yield	Marketable yield	Difference (%)
Control	18.25 ±0.48 *a*	16.13 ±0.47 *a*	100.00
N	42.58 ±0.91 *d*	40.25 ±0.82 *d*	249.53
N + G	36.46 ±1.46 *c*	34.71 ±1.39 c	215.19
N + G + A	50.67 ±1.19 *e*	47.61 ±1.07 *e*	295.16
G + A	30.56 ±1.53 *b*	27.61 ±1.48 *b*	171.17
G	23.38 ±0.92 *a*	21.58 ±0.72 *a*	133.78

Note: *Average ±standard deviation. The different letters listed with the mean values in the columns represent statistically significant differences between the observed varieties ($p < 0.05$).

Refractometric dry matter

The study showed (Table 4), that the refractometric dry matter content was significantly affected by N fertilization or biostimulant treatments in all observed variants except biostimulant combination without applied N. While the increase of concentrations of soluble solids in potato tubers was in all observed variants. The increase compared to the untreated variants was from 3% by the G + A variant to 34.5% by the N + G + A combined variant. These results are comparable to the results of the total and marketable yield of potato tubers as well as starch content.

Starch content

The Polarimetric Ewers method of starch content determination showed that used treatments of potato plants significantly increased (p <0.05) starch content in tubers in all observed variants (Table 4). The average tuber starch content after the application of biostimulants combination with N-fertilization was the highest, representing 15.85%, which means an increase of 13.6% compared to the control variant and an increase of 9.84% compared with the conventional cultivation without biostimulant. However, in contrast to these results, with the use of Groundfix alone the starch content in potato tubers was 13.83% which is less than both the control variant and the conventional variant.

Numerous studies reported a positive effect of biostimulants and an increase in vegetable quality after biostimulant application in many scientific papers. To compare our results, in **[40]** combined application of microbial inoculum and humic substances were the most effective variant, recording a 50% increase in shoot dry weight and a 43% increase in root dry weight compared to the control plants. In **[25]** tuber dry matter, starch and protein contents increased by 18.3%, 24.6%, and 48.6% respectively according to HS treatment. Similarly, in **[45]** shoot dry weight increases by 22 ±4%, and root dry weight increases by 21 ±6% in response to HS application. Plant growing pointers, plant nutritional status, and tubers excellence parameters (dry weight and other) responded positively to Humic acids application in experiments **[46]**. The results from hot pepper experiments indicate that fruit antioxidant activity, total phenolic, carbohydrate, capsaicin, carotenoids, total soluble solids, and titratable acidity significantly increased, but total flavonoid and ascorbic acid contents were not affected significantly by fulvic acid treatments applications **[55]** while similar results obtained also **[56]**. Fulvic acid improves fruit length, diameter, and yield of sweet pepper in **[76]** and vegetative growth, fruit yield, and quality of the three cultivars as compared with the control plants in the study **[58]**, moreover **[59]** confirmed increased maximum number of leaves plant, branches plant, plant height, stem diameter, number of fruits per plant, yield per plant and total yield. However, HA treatments had no significant effect on fruit firmness, fruit length, or diameter of pepper **[60]**. Cozzolino et al., **[26]** found a synergistic effect of the combination of HA with PGPB biostimulant, resulting in an improvement of potato parameters, especially P uptake. In **[71]** root application of PGPR strains significantly increased total soluble solids, total sugar, and reduced sugar, but decreased titratable acidity and had no important effect on the average strawberry fruit weight and pH. HA application had a significant effect on biological yield, grain yield, and harvest index of cereals in **[72]**, and PGPB and humic substances increased maize grain production by 65% under field conditions in **[12]**, similarly, root development of spring wheat inoculated with *A. brasilense* was significantly better that of noninoculated plants **[74]**.

Table 4 Average total dry matter, Refractometric dry matter and starch content depending on the observed experimental variants *, Nitra, Slovakia, 2020.

Variety/variant	Refractometric [°Bx]	Starch content [%]
Control	4.00 ±0.69 *a*	10.85 ±0.71 *a*
N	5.12 ±0.68 *bc*	14.43 ±0.47 *c*
N + G	4.86 ±0.23 *b*	13.89 ±0.83 *b*
N + G + A	5.38 ±0.18 *c*	15.85 ±0.78 *d*
G + A	4.12 ±0.38 *a*	14.09 ±0.53 *bc*
G	4.78 ±0.38 *b*	13.83 ±0.49 *b*

Note: *Average ±standard deviation. The different letters listed with the mean values in the columns represent statistically significant differences between the observed varieties (p <0.05).

Vitamin C

The application of biostimulants (Agriful and Groudfix) had a statistically positive effect on vitamin C content in potato tubers within variants, where PGPB and HS biostimulants or N fertilizer alone was applied. G + A combination increased vitamin C content by 16% and G + A with N combination increased vitamin C by 20% in comparison to the untreated (control) variant, with the max value of 18.00 mg.kg^{-1}. On the contrary, applying PGPB alone or with N fertilizer did not positively affect the accumulation of vitamin C in tubers as (Table 5) shown. These results indicate the importance of organic material, the especially potentially synergic effect of humic and fulvic acids on soil microbiota.

Total polyphenols

Based on the results of this study, it can be assumed that the applied plant growth-promoting bacteria as well as humic substances have a positive effect on total polyphenols content in the potato tubers while in most cases these results were significant. All monitored variants accumulated a higher content of total polyphenols in the tubers than the control variant without fertilization and treatment of the stand in the range of 3-14.5% increase compared to the control variant as can be seen in (Table 5). The maximum total polyphenols promotion was obtained by PGPB alone inoculation with 2235.5 mg GAE.kg^{-1} and a 14.5 % increase above control and a 9.4% increase compared to N fertilizer alone. PGPB an HS combination was second most fertile within results with 2150.50 mg GAE.kg^{-1} of total polyphenols and 13.1% increase above control and 5.5% difference with nitrogen variant.

Antioxidant activity of potato tubers

Biostimulant treatment did not show statistically significant differences in the AOA (antioxidant activity) of potatoes except for the Groundfix variant where 38.00% of AOA was measured, which represents a 13.4% increase compared to the untreated variant. Similar results were obtained variant where N alone was fertilized. According to results obtained by the DPPH method, the antioxidant activity showed differences between control and observed variants from a 3% decrease in the N+G+A variant to an increase of 13.4% in the case of variants N and G, as is shown in (Table 5).

Table 5 Average vitamin C, Total polyphenols and antioxidant activity content depending on the observed experimental variants *, Nitra, Slovakia, 2020.

Variant	Vitamin C [mg.kg^{-1}]	TPC [mg GAE.kg^{-1}]	AOA [%]	AOA [mg TE.kg^{-1}]
Control	15.00 ±1.39 *b*	1901.00 ±1.90 *a*	33.50 ±2.12 *a*	179.00 ±8.49 *ab*
N	17.40 ±1.51 *c*	2044.00 ±8.49 *bc*	38.00 ±1.41 *b*	199.00 ±5.66 *c*
N + G	14.40 ±1.20 *a*	2113.50 ±44.55 *cd*	33.00 ±0.10 *a*	176.00 ±0.21 *ab*
N + G + A	18.00 ±1.25 *c*	1958.50 ±68.59 *ab*	32.50 ±0.71 *a*	173.00 ±4.24 *a*
G + A	17.40 ±1.40 *c*	2150.50 ±37.48 *cd*	35.50 ±0.71 *ab*	188.00 ±4.24 *bc*
G	15.00 ±1.20 *b*	2235.50 ±68.59 *d*	38.00 ±0.12 *b*	201.00 ± 1.41 *c*

Note: *Average ±standard deviation. The different letters listed with the mean values in the columns represent statistically significant differences between the observed varieties (p <0.05).

The increase in tuber yield due to the applied biostimulants did not have a negative effect on their quality in this case, on the contrary, in most cases, we could observe its increase. These results are supported by the study of **[19]**. In their case, applied HS plant biostimulants showed beneficial effects on both productive and qualitative potato parameters. Among quality features, tuber size, contents of protein, vitamin C, starch, and phenols are the principal parameters influenced by plant biostimulants, while reducing the normal dose of fertilizer without reducing yield. Within other similar scientific papers, compared to our study combined application of microbial consortium and humic substances was the most effective, recording a 50% increase in shoot dry weight and a 43% increase in root dry weight compared to the control plants **[41]**, or shoot dry weight increases of 22 ±4% and root dry weight increases of 21 ±6% in response to HS application **[45]**. Potato plant growing pointers, plant nutritional status, and tubers excellence parameters (dry weight and other) responded positively to humic acid application rates **[46]**. However different results were obtained **[37]**, where soil application of humic acid had no effect on tuber size, total yield, or other chemical compositions of tubers but increased mineral contents in the soil and tubers and substantially decreased incidence of hollow heart, which supports the results of **[47]**. Humic acids (HA) provide the formation of the organominerals in soil; thus, they improve the nutrient concentration of tomato leaves and agricultural production **[48]** and improved iron uptake under salinity stress **[49]**. This is also reflected in the

better germination of tomato seeds **[50]** and the overall productivity and quality of plants **[51]**. The appropriate dose of HA treatment led to a significant increase in tomato plant height and fresh and dry weight together with marketable yield **[29]**, total production of fruits **[52]**, and vitamin C and total soluble solids (TSS) concentration as compared with control **[53]**. Ruiz and Salas **[54]** confirmed that significant increases in the yield and quality of the tomato fruit can be obtained by a combination of PGPB inoculation with appropriate organic materials used as fertilizers. Fulvic acid improves fruit length, diameter, and yield of sweet pepper in **[57]** and vegetative growth, fruit yield, and quality of the three cultivars as compared with the control plants in a study **[58]**, moreover **[59]** confirmed increased maximum number of leaves plant, branches plant, plant height, stem diameter, number of fruits per plant, yield per plant and total yield. The inoculated bell pepper plants always showed higher yield than the control ones in **[61]**, and in a study of **[62]** increased plant emergence, root and shoot length, and biomass and fruit yield but also caused an increase in available NPK content and sustain soil health. However, inoculation by beneficial bacteria did not affect marketable fruit yield or the pigments (chlorophylls, lycopene, and b-carotene) and carbohydrate contents in the pepper fruits, but flavonoids and anthocyanins were increased significantly by the addition of the bacteria **[63]**. Similar treatment had little effect on pod mineral content. chlorophylls, total carotenoid, and vitamin C contents in **[64]**. Data of **[65]** stated that all morphological characteristic parameters of eggplant plants (plant length, number of leaves and number of branches, and fresh and dry weight of leaves per plant) were improved by using HS biostimulator treatment compared to non-treated plants (control). Yield and its components of eggplant plants also followed the same trend with a similar result in the study **[66]**. Comparable great results were obtained from HS combination with NPK fertilizer **[67]**. Combining recommended rate and half the recommended rate of inorganic fertilizer with foliar fertilizer and PGPB improved the yield of eggplant **[68]**. Soil application of humic acid increased the dry weight of foliage, the number of tubers, yield per plant, and total yield of tubers with more average tuber weight and higher N, P, K, Total carbohydrates, and inulin contents also in **[69]** and similarly, inulin levels and yield were higher in the endophytic bacteria treatment under both conditions (normal and drought) but especially increased under drought stress in comparison to control **[70]**. In **[73]** results showed that the combinations of NPK and potassium humate increased onion bulb size, weight, and storage ability.

Pearson product-model correlation of observed parameters

The highest positive correlation was achieved between total polyphenols and antioxidant activity (+1.0), but also between total yield and total starch content of potato tubers (+0.77), total yield and vitamin C content (+0.62), and yield and refractometric dry matter (+0.63) (Figure 2**)**. These are important parameters for both the grower and the consumers, especially the fact, that despite the increase in yield, applied fertilizer or biostimulants did not influence the quality of tubers except in the case of the AOA and the total polyphenol content (-0.25). Based on our results and other studies in the discussion, the results are potentially attributed to the effect of nitrogen fertilization and biostimulating effect of applied PGPB and HS on the potato plants, especially synergic between those two.

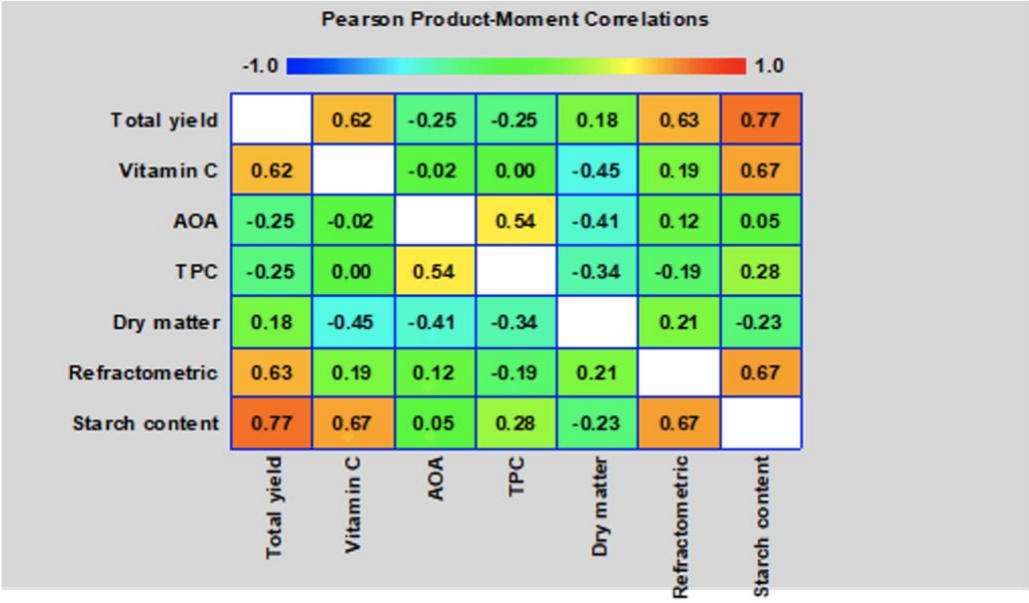

Figure 2 Pearson product-model correlation between observed parameters.

CONCLUSION

In recent years, there is a significant increase in the interest of farmers and scientific studies on biostimulant effects on cultivated plants' yield and quality. The presented results show that the observed biostimulants – Agriful and Groudfix had a significant effect on increasing the total yield of harvested potato tubers, but also a positive effect on the quality of harvested tubers was manifested. The total dry matter, refractometric dry matter, and starch content increased after biostimulant or nitrogen application, which can have a positive impact on the storability of the grown potatoes. In all observed variants total polyphenols and antioxidant activity increase compared to control, and a statistically significant increase in vitamin C content. We recorded the most positive changes in the case of variants with combined application of humic substances and PGPB, which we have substantiated with similar results in the extensive discussion. We believe that our similar results can lead to a better understanding of the biostimulating effect in different crops, soil, and climatic conditions, which can lead to more sustainable agriculture and healthy food production.

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The study of physicochemical and technological properties of boiled sausage recommended for the older adults

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ABSTRACT

A recipe for cooked sausages of the herodietic direction using protein hydrolysate in the amount of 3%, 5%, and 7% of the mass of raw materials is proposed. The recipe is based on "Beef Sausage". Organoleptic evaluation of the prototypes showed that when protein hydrolysate was added in an amount of 7%, a specific taste characteristic of by-products was present in the prototype. Based on the sensory evaluation results, it was decided to continue the study of samples with the addition of 3% and 5% protein hydrolysate. The results of the study of the physicochemical parameters of the prototypes with the addition of protein hydrolysate 3% and 5%, showed the values of the mass fraction of protein 16.65% and 19.29%, fat 9.85% and 12.25%, carbohydrates 2.85% and 3.07% respectively, indicating an increase in the amount of protein and a decrease in the proportion of fat compared to the control sample. A significant increase in the content of such essential amino acids as lysine and valine and interchangeable amino acids as arginine, glycine, and serine in the test samples confirms that the protein hydrolysate introduced into the prototype is rich and well-balanced in amino acid composition. To study the effect of protein hydrolysate on the quality of meat products, the moisture binding capacity was determined, which was 3% and 5% in the experimental samples with the addition of protein hydrolysate – 75.62% and 79.13%, which is 3.4% and 8.2% higher than that of the control sample, respectively. The sample with the addition of 5% hydrolysate (80.01) has the greatest moisture-retaining ability, which is 9% higher than that of the sample with 3% hydrolysate and 15.8% higher than the control indicator. The study results of the fat-holding capacity in the samples also showed positive dynamics.

Keywords: balanced nutrition, older person, aging, herodietic product, moisture-binding ability

INTRODUCTION

Currently, there is a progressive aging of the population worldwide. In 2000, there were about 600 million people over the age of 60 in the world; according to WHO forecasts, in 2025, the number of older adults will increase to 1.2 billion people, and in 2050 the expected number is 2 billion people **[1]**. In the Republic of Kazakhstan, there is an increase in the share of older adults in the age structure of the country's population. At the beginning of 2019, people over the age of 60 accounted for 11.6%, over the age of 65 7.5% of the country's total population **[2]**. According to the UN classification, a society in which the proportion of people over 65 years of age from the entire population of the country is 7% or more refers to aging. In this regard, it can be argued that our country is at the initial stage of demographic aging. From 2010 to 2018, life expectancy in our country increased from 68.3 years to 73.12 years. Despite the positive growth in life expectancy, it is worth noting that it does not correspond to the country's income levels. The life expectancy of the Economic Cooperation Organization countries, such as Chile and Turkey, with similar GDPs, is 80 years **[3]**. The analysis of food products presented on the market of our country has shown that the following areas are developing dynamically: the production of baby food products, therapeutic and preventive directions. However, their market share is

minimal in the range and direction of action on the human body. Herodietic products are mainly represented in products made from dairy raw materials, bakery products, and meat products in limited quantities.

A study of the nutrition of elderly people in the capital of Kazakhstan (Nursultan) shows that the diet mainly consisted of the following products: proteins and fats of animal origin and easily digestible carbohydrates. It is noted that the products had an excess of saturated fatty acids, a lack of polyunsaturated fatty acids (PUFA), a high level of consumption of simple carbohydrates, a deficiency in vitamins: D, A, B1, E, C, biotin, folic and pantothenic acids, and a lack of calcium and potassium **[4]**.

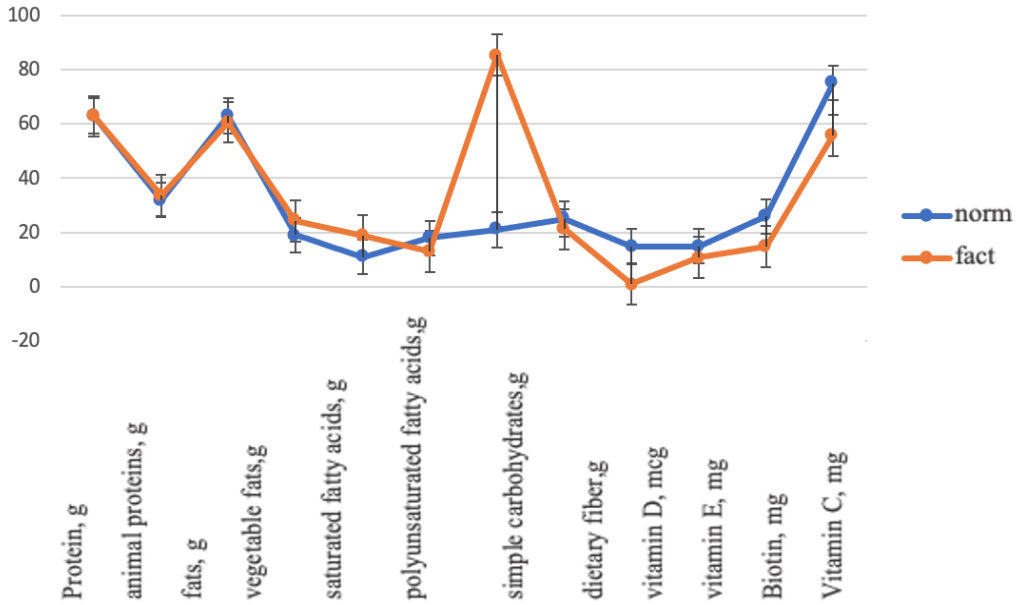

Figure 1 Daily intake of older adults in Nur-Sultan of food substances in comparison with recommended norms **[5]**.

The study revealed that respondents consume meat and meat products daily: 55.2% of respondents, 10% once a week, and 4.3% do not consume at all. In particular, beef was consumed by 31.4%, horse meat by 29%, mutton by 8.1%, and 8.6% consumed as food.

It was revealed that the low level of calcium, vitamin D, and potassium intake. At the same time, calcium in women is 528 mg/day, and in men, 549 mg/day with a normal requirement of 1300 mg/day. Vitamin D with a norm for women and men of 15 mcg/day; the actual intake was 0.92 and 1.15 mcg/day, respectively. Potassium at a rate of 5000 mg/day, the actual consumption was 2681 mg/day in women and 2835 mg/day in men.

Analysis of the antioxidant potential of raw materials of animal origin, the objects of the study were by-products: liver, brain, heart, aorta, and mesenteric lymph nodes of pigs. As a result, superoxide dismutase, catalase activity, antioxidant activity by the FRAP method, and the concentration of active products interacting with 2-thiobarbituric acid were determined. Liver tissues showed the greatest antioxidant activity of superoxide dismutase and catalase, which amounted to 1398.3 ±16.5 U/g of raw materials and 53.27 ±1.58 U/g of raw materials per minute. The antioxidant activity determined by the FRAP method was 4.10 ±0.16 mmol^{-eq}. dihydroquercetin/g of raw materials. The lowest antioxidant activity was observed in the aorta, which was 0.36 ±0.02 mmol^{-eq}. dihydroquercetin/g of raw materials. Scientists emphasized that by-products of the meat industry should be used as a source of biologically active components **[6]**.

The study showed a positive effect of β-alanine on the work efficiency of older adults when it is added to the diet as a dietary supplement **[7]**.

The technology of pates for the elderly with the use of raw meat with a high content of connective tissue has been developed. The technology provides for the inclusion of the following components in the recipe: beef trimmings – 35%, grade I beef – 27%, corn groats – 17.5%, oatmeal – 7.3%, carrots – 2.1%, ridge bacon – 10.2%, soybean oil – 0.9%. The above product is recommended for older adults suffering from or predisposed to cardiovascular and gastrointestinal diseases. The following recipe is recommended for older adults with impaired lipid metabolism: grade I beef – 36%, cattle head meat – 18%, buckwheat – 7.2%, carrots – 27.4%, ridge bacon – 10.5%, soybean oil – 0.9% **[8]**.

A boiled sausage for the elderly, "Zdravitsa" has been developed, including cumin, milk thistle oil, and lactulose. These biologically active food components contribute to improving digestion and removing toxins from the liver.

The use of raw meat materials for the production of products for the elderly is relevant and promising. The meat's raw materials contain biologically active substances, such as high-grade animal protein, minerals, vitamins, and fatty acids. These indicators determine the functional properties of raw materials necessary to prevent diseases of the musculoskeletal system (including osteoporosis, osteoarthritis), iron deficiency anemia.

Thus, the main components for products for the elderly that form their orientation are: connective tissue proteins, dietary fibers, vitamins, minerals, antioxidants, and phospholipids.

Scientific hypothesis

Development of technology for producing boiled sausage using protein hydrolysate is recommended for the elderly. Processing legs with a put joint (beef, lamb, horse meat) will increase the quality indicators in the finished boiled sausage. We expect a significant effect of collagen on the consistency of the finished product.

MATERIAL AND METHODOLOGY

Samples

The research objects were legs with a put joint (beef, mutton, horse meat).

Chemicals

All reagents used were of U.S.P. purity or higher. All solvents, including water, were used with the LC/MS label.

Instruments

The MOD MARS 6 microwave sample preparation system was used for sample preparation. The amino acid composition was determined using a high-performance liquid chromatograph "Agilent-1200".

Laboratory Methods

Laboratory studies of raw meat materials were carried out based on the NAO "S.Seifullin KATU" (Nur-Sultan, Kazakhstan). The following were investigated: the total chemical composition (moisture, fat, protein, ash) and amino acid composition **[9]**, **[10]**, **[11]**, **[12]**, **[13]**.

Description of the Experiment

Sample preparation: The objects of the study were legs with a put joint (horse meat, beef, lamb). The main stage of the tests was grinding meat products into minced meat. Grinding was carried out using a meat grinder. The diameter of the grate is 2 mm. Enzyme treatment and hydrolysis process. For experimental studies, samples of beef, horse, and lamb legs with a put joint were selected. The effect of enzyme preparations on connective tissue raw materials was studied using beef, horse, and lamb legs processed according to the traditional technology of processing meat and bone by-products at meat processing enterprises. Legs with a put joint were crushed with a band saw into discs 15-20 mm wide and weighing 50-85 g. 200 ml of distilled water was added to 100 g of legs, and the suspension was heated for 40-45 minutes at 95-98 °C. The released fat was separated. The fat-free legs were placed in a fermentation tank. As is known, to carry out effective hydrolysis of protein substrates, it is necessary to choose the optimal concentration of the enzyme correctly. Therefore, in the future, three enzyme concentrations were selected for the selection of the enzyme concentration, referring to the literature data: 1%, 2%, and 3% protease solution.

A total of 18 samples were selected for the study, in which 1%, 2%, and 3% protease solution was added, and 6 samples for each type of concentration, respectively. Hydrolysis was carried out until the collagen proteins were dissolved entirely, visually observing the samples for 24 hours, with a time interval of 6 hours. An external assessment of protein hydrolysis was performed every 6 hours, which showed the dynamics of protein dissolution from small flakes in the substrate to more extensive precipitation. Hydrolysis was carried out at a temperature of 40 ±5 °C for 24 ±2 hours at pH 8.0.

Number of samples analyzed: We analyzed 18 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 two times.

Design of the experiment: Physicochemical analyses of the meat mince samples were conducted to standard methods: Total nitrogen content was assayed by the Kjeldahl method using devices DK6, UDK129 (VELP SCIENTIFICA, Italy), an automated incinerator, and a distillation apparatus **[14]**. Moisture was determined by drying the sample in a metal bottle at a temperature of 105°C to constant weight **[15]**. Total fat was determined via the Soxhlet method **[16]**. The ash content was determined via the dry ashing method.

Figure 2 Samples of legs with a put joint.

Statistical Analysis

The data were analyzed using Statistica 12.0 (STATISTICA, 2014; StatSoft Inc., Tulsa, OK, USA). The values are presented as the mean ± SEM. The differences were considered to be statistically significant at $p \leq 0.05$. The data were analyzed by One-way ANOVA using free web-based software.

RESULTS AND DISCUSSION

Regarding the quantitative content of proteins, by-products of the II category are not inferior to meat. Consequently, producing various biologically active substances can be a valuable source of proteins.

Table 1 The yield of offal to the mass of meat.

The name of the indicator	Output of meat offal, %		
	cattle	mutton	horse meat
meat offal,	24.0 ±0.90	17.2 ±0.7	22.4 ±0.9
including legs with a put joint	3.37 ±0.07	3.6 ±0.05	3.59 ±0.05

The table shows the output of legs with a put joint from the total number of offal, which is 3.37% for cattle, 3.6% mutton, and 3.67 for horse meat. By-products with high nutritional value allow them to be used in the production of sausages, pates, canned food, and jellies.

Table 2 Chemical composition and energy value of wool by-products of category 2.

Title	Content				Energy value, kcal
	moisture	protein	fat	ash	
Mutton legs with a put joint	64.6 ±0.40	27.2 ±0.10	7.8 ±0.2	0.8 ±0.02	168.7
Horse legs with a put joint	68.3 ±0.40	26.7 ±0.14	3.8 ±0.2	1.2 ±0.02	139.4
Beef legs with a putty joint	65.7 ±0.40	26.7 ±0.11	6.5 ±0.2	1.2 ±0.03	161.3
Pig's legs with a putty joint	55.5 ±0.60	22.2 ±0.10	21.45 ±0.2	0.8 ±0.02	281.85

Analysis of the chemical composition of wool by-products of category 2 shows that the protein content in mutton legs with a put joint is 27.10-27.30% when both in horse and beef wool derivatives, the amount of protein is at the same level of 26.56-26.84%. This allows us to conclude that mutton, horse, and beef legs with a put joint can be used as raw materials for producing protein hydrolysates.

The fat content in raw materials is one of the important indicators in the production of protein hydrolysates since its content of more than 15-20% complicates the drying process and reduces the shelf life. The study showed that horse, lamb, and beef wool offal (legs with a put joint) have a relatively low-fat content with increased protein mass fraction. Thus, horse legs contain 3.8% fat, beef 6.5%, lamb contains 7.8%, and pork – 21.45%. It follows from this that the low-fat content in the studied samples will allow obtaining protein hydrolysate with optimal quality indicators and using it as an additive in the formulation of a herodietic product.

Data for the amino acid composition of the protein of meat raw materials and offal of category 2 are presented in Table 3.

Table 3 Amino acid composition of protein of meat offal of category II, g/100 g of protein.

Indicators	Mutton legs with a put joint	Horse legs with a put joint	beef legs with a putty joint
Valin	2.1 ±0.10	3.2 ±0.10	2.0 ±0.10
Leucine	6.5 ±0.25	7.5 ±0.25	6.3 ±0.25
Isoleucine	2.3 ±0.15	2.2 ±0.15	2.3 ±0.15
Lysine	6.4 ±0.25	6.7 ±0.25	6.4 ±0.25
Methionine	1.1 ±0.18	0.53 ±0.18	1.1 ±0.18
Threonine	3.8 ±0.10	3.8 ±0.10	4.4 ±0.10
Tryptophan	1.24 ±0.02	0.8 ±0.02	-
Phenylalanine	3.1 ±0.15	2.8 ±0.15	3.4 ±0.15
Alanin	9.9 ±0.36	9.4 ±0.36	3.6 ±0.36
Arginine	7.9 ±0.30	8.2 ±0.30	8.7 ±0.30
Aspartic acid	9.5 ±0.20	6.9 ±0.20	7.3 ±0.20
Histidine	1.3 ±0.03	1.70 ±0.03	1.1 ±0.03
Glycine	9.5 ±0.5	15.2 ±0.5	3.5 ±0.5
Glutamic acid	15.8 ±0.70	17.2 ±0.70	14.4 ±0.70
Proline	8.1 ±0.25	7.8 ±0.25	6.8 ±0.25
Serin	4.9 ±0.25	6.5 ±0.25	6.9 ±0.25
Tyrosine	2.7 ±0.15	4.7 ±0.15	5.5 ±0.15

The research results show a high content of glycine, alanine, glutamic acid, serine, and proline in the by-products of category 2, i.e., those amino acids that are mainly contained in collagen.

Thus, the conducted studies indicate a high potential for the possibility of using these raw materials and have all the prerequisites for producing meat products of the herodietic direction from them.

As a result of the analysis of existing technologies of herodietic meat products, the use of collagen-containing raw materials requires the pretreatment of wool offal. One of the processing options is the hydrolysis of meat and bone raw materials. In the hydrolysis of meat and bone raw materials, fat content is one of the main criteria. With the amount of 15-20% of lipids in the fetal joints, obtaining protein hydrolysates is difficult. In this regard, it is required to pre-degrease meat and bone raw materials.

Heat treatment is one of the widely used methods of extracting fat from bones. The heating of raw materials leads to the denaturation of proteins, which contributes to fat extraction from the bone. Under the influence of high temperatures, fat becomes fluid and less viscous.

When conducting experimental studies, horse, beef, and lamb legs with a put joint are stored at a temperature of 0-6 °C. They are transferred to degreasing no later than 8 hours after deboning. If necessary, these by-products can be stored at a temperature of -18 °C for no more than two months.

Taking into account the peculiarities of the preparation of components, the technological process was worked out, and a technological scheme for producing protein hydrolysate was proposed.

Samples of beef, horse, and sheep legs with a put joint were selected for experimental studies. Based on the research results and for more complete use of all the resources of meat raw materials, in the production of cooked sausages of the herodietic direction, it is proposed to use protein hydrolysate obtained from beef, horse, and lamb legs with a put joint.

The formulations of the control and experimental samples are presented in Table 4.

Table 4 Formulations of control and experimental samples.

Name of ingredients	Samples			
	Control "Beef Sausage"	Experience 1	Experience 2	Experience 3
Veneered beef of the first grade, kg	70	70	70	70
Fat veined beef, kg	15	12	11	10
Raw beef fat	12	10	10	10
Protein hydrolysate, kg	-	3	5	7
Dry purslane extract, kg	-	1	1	1
Table salt, kg	2.1	2.1	2.1	2.1
Sodium nitrite, kg	0.05	0.05	0.05	0.05
Granulated sugar, kg	0.19	0.19	0.19	0.19
Black pepper, kg	0.15	0.15	0.15	0.15
Cardamom or nutmeg, kg	0.2	0.2	0.2	0.2

The proposed ratio of ingredients provides the required structure, nutritional and biological value, and high functional and technological properties in the finished product.

An organoleptic evaluation of the prototypes was carried out (Figure 3).

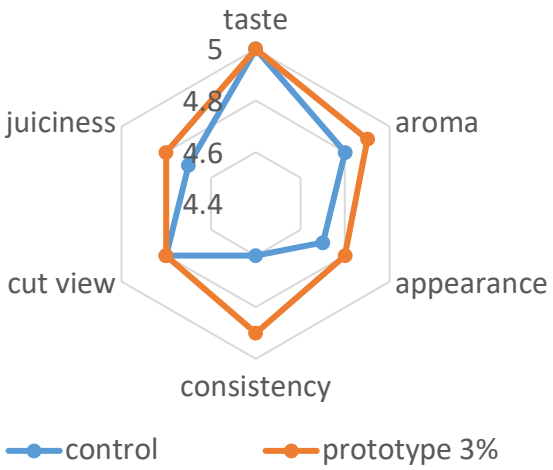

Figure 3 Sensory evaluation of finished products.

Organoleptic evaluation of the prototypes showed that the optimal amount of protein hydrolysate addition is 3% and 5%. Both samples had a good appearance, consistency, taste, and smell. When protein hydrolysate was added in 7%, a specific taste characteristic of by-products was present in the test sample. Based on the sensory evaluation results, it was decided to continue the study of samples with the addition of 3% and 5% protein hydrolysate.

The results of the study of the chemical composition of control and experimental samples are presented in table 5.

Table 5 Physico-chemical parameters of control and experimental samples.

Name of samples	Mass fraction, %			
	protein	fat	carbohydrates	moisture
"Beef sausage" (control sample)	18.93	14.90	3.07	56.86
Experiment 1 (with the addition of purslane 1%, protein hydrolysate 3%)	16.65	9.85	2.85	51.31
Experiment 2 (with the addition of purslane 1%, protein hydrolysate 5%)	19.29	12.25	3.07	51.10

In conditions of insufficient protein content in the body, the proteins contained in the tissues begin to hydrolyze. For this reason, it is very important to follow the recommended protein intake standards **[2]**. According to the WHO FAO recommendations, the protein intake rate is 65-100 g per day or 10-15% of the amount of protein consumed in food.

Taking into account the formalized requirements for the composition of herodietic products, the mass fraction of protein of a specialized product should be at least 10%.

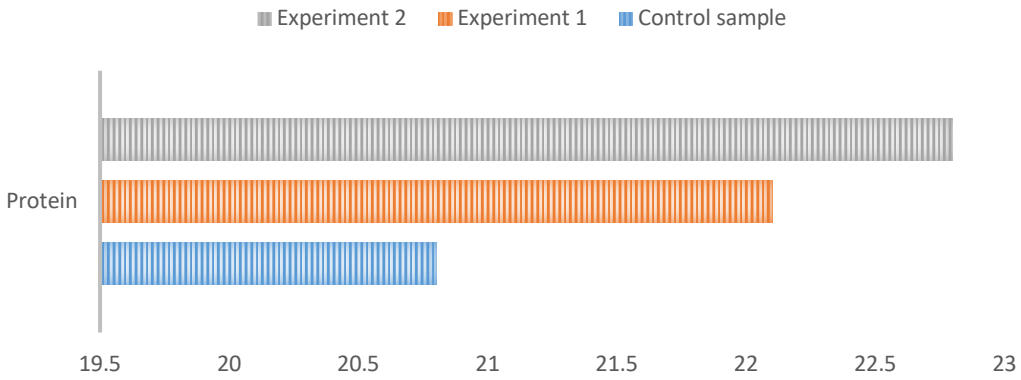

Figure 4 Effect of protein hydrolysate on protein indicators in finished products.

One of the main indicators of food quality is the biological value, reflecting the degree of compliance with the amino acid balance of the body's needs, necessary for the course of physiological processes in the body (Table 6).

Table 6 Amino acid composition of boiled sausages with the addition of protein hydrolysate.

Name of amino acids	"Beef sausage" (control sample), %	Experiment 1 (with the addition of purslane 1%, protein hydrolysate 3%)	Experiment 2 (with the addition of purslane 1%, protein hydrolysate 5%), %
	Mass fraction of amino acids, %		
Arginine	1.477 ±0.591	1.780 ±0.712	2.058 ±0.823
Lysine	2.685 ±0.913	2.671 ±0.908	2.743 ±0.933
Tyrosine	1.193 ±0.358	1.142 ±0.343	1.183 ±0.355
Phenylalanine	1.402 ±0.421	1.365 ±0.409	1.320 ±0.396
Histidine	0.895 ±0.448	0.801 ±0.401	0.617 ±0.309
Leucine+isoleucine	2.238 ±0.582	2.077 ±0.540	2.058 ±0.535
Methionine	0.925 ±0.314	0.861 ±0.293	0.857 ±0.291
Valine	1.939 ±0.776	2.077 ±0.831	2.572 ±1.029
Proline	2.089 ±0.543	2.522 ±0.656	2.058 ±0.535
Threonine	1.387 ±0.555	1.484 ±0.593	1.543 ±0.617
Serine	1.298 ±0.337	1.484 ±0.386	1.423 ±0.370
Alanine	2.238 ±0.582	2.671 ±0.694	2.229 ±0.580
Glycine	2.387 ±0.812	3.412 ±1.160	2.401 ±0.816

A significant increase in the content of essential amino acids such as lysine and valine and interchangeable amino acids such as arginine, glycine, and serine in the test samples confirms that the protein hydrolysate introduced into the prototype is rich and well-balanced in amino acid composition.

The most important functional properties of proteins include solubility; stabilizing dispersed systems (emulsions, foams, suspensions) and forming gels; adhesive and rheological properties (viscosity, elasticity); water-binding, fat-binding, texturing, and film-forming ability.

To study the effect of protein hydrolysate on the quality indicators of meat products, moisture-binding, moisture-retaining, and fat-retaining abilities were determined.

Table 7 Basic functional and technological properties of boiled sausages with the addition of protein hydrolysate.

Name of samples	Indicators, %			
	moisture binding capacity, %	moisture-holding capacity, %	fat-holding capacity, %	The yield of finished products, %
"Beef sausage" (control sample)	73.12 ±0.36	69.12 ±0.72	57.18 ±0.51	107
Experiment 1 (with the addition of purslane 1%, protein hydrolysate 3%)	75.62 ±0.84	73.37 ±0.91	59.13 ±0.83	110
Experiment 2 (with the addition of purslane 1%, protein hydrolysate 5%)	79.13 ±0.62	80.01 ±0.65	60.05 ±1.01	114

Protein hydrolysates of animal origin, in comparison with popular soy protein isolates, are characterized by an increased (two to three times) moisture-retaining ability, comparable fat-retaining ability, and significantly greater (4-8 times) strength of water-fat emulsion and can be used in sausage recipes instead of soy proteins.

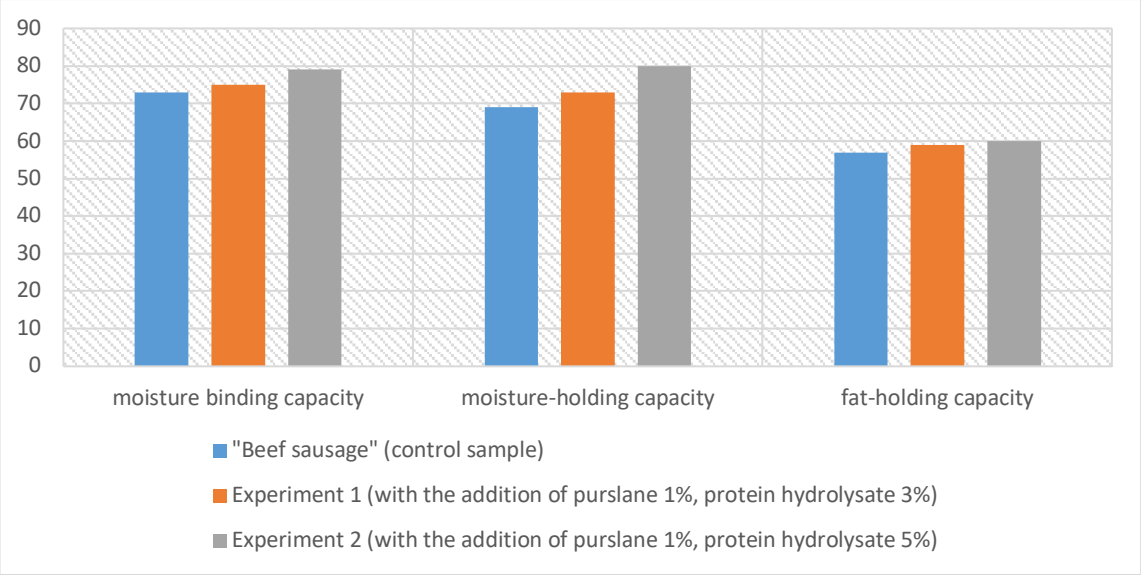

Figure 5 Functional and technological properties of the studied samples.

The addition of protein hydrolysate leads to an increase in the water-binding capacity in the test samples and thereby provides a high yield of products. The presented data indicate that the yield increases in experimental samples with the addition of protein hydrolysate in an amount of 5%. This is explained by the fact that protein hydrolysate binds a large proportion of moisture, having such important properties of natural meat additives as solubility, emulsifying and gelling abilities, and provides an increased yield of finished products.

The global population is becoming older, and this cohort still considers red meat (beef and sheep meat) an important staple in their diet. It is understood that the requirements from these red meats will vary as a person ages, often because of associated physiological changes, nutrition and health concerns, and reduced sensorial capacities. This creates an imperative to develop red meat products that appeal to and satisfy the demands of older adults. Based on these studies, it was apparent that these specifications may be delivered using available knowledge of intrinsic and extrinsic influences on red meat if considered within the context of elderly customers. That is, to deliver upon older adults' requirements for conventional red meat products, which are inexpensive, of consistent and perceivable high quality, and quell any associated health concerns for the consumer **[17]**. Red meat has a tougher texture compared with many other food products. Therefore consumption is often reduced among older adults. Acidic treatments had a positive effect on WBSF values (reduced the WBSF values from 23.35 N for control to 14.83 N), and texture parameters and a combination with apple fibre and rice starch may improve the health profile of a meat product with benefits for consumers, particularly for the older population. This study optimized and successfully validated a novel meat product with a softer texture (apple fibre 0.15%, rice starch 0.30%, and citric acid 0.16 M). The results obtained for the objective measurements of tenderness were confirmed

by consumers' tenderness results (p <0.05); moreover, texture-optimized beef samples were found to be more acceptable by older consumers compared with the control **[18]**. Meat processors have a role to play in enhancing the availability of appropriate foodstuffs for older people through developing targeted products that will meet this cohort's specialized nutritional and chemosensory needs. Meat intakes in the older population are commonly reduced because the relatively tough texture of the meat can impair mastication. In this study, beef steaks tenderized with papain and papain: bromelain (50:50) were demonstrated to produce more tender meat products, with a lower cook loss compared with tenderization with bromelain alone, which has relevance to the development of texture-optimized meat products that appeal to older adults with difficulty in mastication. This information could help meat processors develop strategies for optimizing texture-modified beef products within their businesses **[19]**. Among diverse and numerous food energy sources, beef is a valuable dietary source of high-quality bioavailable protein and is very suitable for the diet of the elderly population **[20]**. There is a need to develop softer-textured beef products for older consumers with masticatory issues, which might contribute to offsetting the decline in meat intake. The effect of citric acid on meat tenderness has been studied by several authors and was reported to have a beneficial effect on meat tenderness, contributing to the partial denaturation of proteins which might make it accessible for the absorption of different added ingredients **[21]**, **[22]**. On the other hand, it has been reported that dietary fibres, including fruit fibres and rice starch, could be used as functional ingredients in meat products **[23]**. It has been suggested that apple pulp represents a typical source of dietary fibre due to its high concentration of flavonoids and carotenes with superior quality compared with other fibres **[24]**. Meat and meat products are a good sources of bioactive compounds with positive effects on human health, such as vitamins, minerals, peptides, or fatty acids. Growing food consumer awareness and intensified global meat producers' competition put pressure on creating new healthier meat products. To meet these expectations, producers use supplements with functional properties for animal diets and as direct additives for meat products. In the presented work seven groups of key functional constituents were chosen: (i) fatty acids; (ii) minerals; (iii) vitamins; (iv) plant antioxidants; (v) dietary fibers; (vi) probiotics and (vii) bioactive peptides. Each of them is discussed in terms of their impact on human health and some quality attributes of the final products **[25]**. In 2 experiments, dark-cutting (DC) beef strip loins were used to test the effects of citric acid-enhancement pH on the visual and instrumental color of fresh and cooked steaks. In Exp. 1 and 2, each DC (mean pH = 6.57 and 6.65, respectively) and normal-pH, low USDA Choice (CH; mean pH = 5.48 and 5.51, respectively) strip loin was cut into 2 equal-length sections, and DC sections were injected to 111% of raw section weight with pH 3.5 to 5.0 (Exp. 1) or pH 2.0 to 3.5 (Exp. 2) solutions made by mixing citric acid in either 0.05% orthophosphate (PO) solution or tap water (HO) base solutions (Exp. 1) and 0.5% PO or 0.5% tripolyphosphate solution base solutions (Exp. 2) **[26]**. Rice starch (RS) and fructooligosaccharides (FOS) were studied as substitutes for phosphates (STPP) and dextrose (Dex) in cooked hams using response surface methodology (RSM). RS, STPP, Dex, and FOS were combined in 25 runs and applied to the Biceps femoris (BF) and Semimembranosus (SM) muscles. Muscles were injected (120% green weight), tumbled, netted, and steam cooked. Cook loss and yield were affected by STPP. Colour was predominantly affected by muscle type, and the ingredients studied, whereas texture was principally affected by STPP and RS. NMR and expressible moisture data showed higher free water retention in samples containing RS. This was visualized by light microscopy as starch gel pockets. Despite some reductions in yield, it is feasible to substitute STPP with RS and obtain a satisfactory quality product. However, higher levels of added FOS would be required to warrant a health claim **[27]**. Chicken breast dipped with citric acid (CA) was treated by sous vide processing and stored in a refrigerated state for 0, 3, 6, 9, and 14 d. A non-dipped control group (CON) and three groups dipped in different concentrations of citric acid concentration were analyzed (0.5%, 0.5CIT; 2.0%, 2CIT and 5.0%, 5CIT; w/v). Cooking yield and moisture content increased due to the citric acid. While the redness of the juice and meat in all groups showed a significant increase during storage, the redness of the citric acid groups was reduced compared to the control group (p <0.05). The findings indicated positive effects in sous vide chicken breast's physicochemical properties and storage ability at 2% and 5% citric acid concentrations **[28]**. Sensory characteristics and visual acceptability of cooked hams with rice starch (RS) and fructooligosaccharides (FOS) as substitutes for, respectively, sodium tripolyphosphate (STPP) and dextrose (De) were evaluated. Replacement of STPP with RS is associated with hams being less juicy, salty, and springy, but more adhesive and could negatively affect appearance. Still, replacement of Dex by FOS had minimal sensory influence. The relative importance of product appearance, pack labels, and price information cues in simulated purchasing decisions was also investigated. Consumer purchase choices were more influenced by product appearance than pack labels referring to additives or price. Including labelling information regarding the reduction or exclusion of phosphates may be more important than labels regarding a salt reduction. For the Irish consumers studied here, using phosphates in cooked hams sounds artificial, unhealthy, and unknown, whereas dietary fibre was perceived as healthy, natural, and improving the eating quality **[29]**. The effects of fat substitution (≤15%) with commercial encapsulated and unencapsulated fish oils on the technological and eating quality of beef burgers

over storage [modified atmosphere packs (80% O_2:20% CO_2); constantly illuminated retail display at 4 °C; for 15days] were studied using design of experiment (DOE). Burger formulations comprised beef shin (59.5%), salt (0.5%), and vitamin E (0.015%) combined with varying levels of beef-fat/fish oils depending on the treatment. Increasing amounts of encapsulated and unencapsulated fish oils in burgers increased polyunsaturated fatty acid content (p <0.001). Storage decreased (p <0.001) a* values, which agreed with oxymyoglobin data. Panellists scored the optimised burger formulation (p <0.05) lower than controls for overall acceptability **[30]**. Although Texture Profile Analysis (TPA) is useful for most solid foods, the misuse of TPA parameters for liquid foods has led to misunderstandings and confusion. Here, we warn of the risk of misuse of TPA parameters for liquid foods **[31]**. There is a rapid change in our overall lifestyle due to the impact of globalization. Every day hasty life has forced consumers to be dependent upon fast foods, which contain the meagre amount of dietary fibre. Non-starch polysaccharides, resistant oligosaccharides, lignin, substances associated with NSP and lignin complex in plants, other analogous carbohydrates, such as resistant starch and dextrins, and synthesized carbohydrate compounds, like polydextrose are categorized as dietary fibre. They are mostly concentrated in cereals, pulses, fruits and vegetables. It has been proclaimed that daily dietary fibre intake helps in the prevention of many nutritional disorders like gut-related problems, cardiovascular diseases, type 2 diabetes, certain types of cancer, and obesity **[32]**. Beef semitendinosus (ST) muscle was marinated for 24 h in 2% NaCl solution and 1.5% lactic, acetic, and citric acid solutions individually and in mixed marinades for the combination of NaCl and three kinds of weak organic acids, respectively. The effectiveness of marinades on beef ST muscle was investigated. Changes in denaturation characteristics of connective tissue collagen were examined using differential scanning calorimetry; microstructural changes of collagenous fibers were observed with scanning electron microscopy; textural properties of meat were studied by texture profile analysis **[21]**. Acidification of meat can improve texture; however it also increases susceptibility to lipid oxidation. The effect of injection and marination of citric acid to acidify and sodium carbonate or sodium tri-polyphosphate to increase the pH of beef on tenderness, microstructure, and oxidative stability was determined. The water-holding capacity and tenderness of beef semitendinosus muscle increased significantly at pH 3.52 upon adding citric acid. They returned to the level of an untreated sample after the pH was increased (pH ∼5.26) by sodium tri-polyphosphate **[22]**. Ingredients in Meat Products presents the most up-to-date information regarding the utilization of non-meat ingredients in the manufacturing of processed meat products in a wcomprehensive and practical way Emphasis has been placed on helping the reader attain a fundamental understanding of (i) the basic chemical and physical properties of each of these groups of ingredients, as we understand them today; (ii) how these properties affect their functionality in meat systems; and (iii) how to take advantage of the ingredients' functional properties to maximize their application in real-life situations **[23]**. This paper overviews the biophysical methods developed to access meat structure information. The meat industry needs reliable meat quality information throughout the production process to guarantee high-quality meat products for consumers. Fast and non-invasive sensors will shortly be deployed, based on the development of biophysical methods for assessing meat structure. Reliable meat quality information (tenderness, flavour, juiciness, colour) can be provided by many different meat structure assessments either by means of mechanical (i.e., Warner-Bratzler shear force), optical (colour measurements, fluorescence) electrical probing or using ultrasonic measurements, electromagnetic waves, NMR, NIR, and so on **[33]**. An excellent source of high biological value protein, vitamin B12, niacin, vitamin B6, iron, zinc, and phosphorus. A source of long-chain omega-3 polyunsaturated fats, riboflavin, pantothenic acid, selenium, and possibly also vitamin D. Mostly low in fat and sodium. Sources of a range of endogenous antioxidants and other bioactive substances include taurine, carnitine, carnosine, ubiquinone, glutathione, and creatine **[20]**. Longissimus muscle obtained from beef carcasses was used in this research. Initially, 0.596, 1.0%, and 1.5% lactic and citric acid solutions were prepared. The meat was marinated in these solutions (1:4 w/v) in polyethylene bags at 4 °C for 72 h. Bound water, pH, weight gain, cooking loss, and Warner Bratzler shear (WBS) were evaluated. Differential Scanning Calorimetry (DSC) was used to determine the bound water content in meat samples. The latent heat of melting (ΔHm) and bound water were found to be functions of the moisture content of marinated meat. There was a significant decrease in pH due to marination. The samples marinated with citric acid held less water than lactic acid. The WS values in control samples were higher than in marinated samples. Cooking loss was lower in samples marinated with lactic acid than citric acid maacid-marinateds **[34]**. The citric acid (CIT, 0.3%) was assessed for its ability to reduce the pink color defect in the ground, cooked (80 °C) turkey breast associated with nicotinamide hemochrome (NICHEME) and nitrosyl hemochrome (NITHEME). CIT incorporation in nicotinamide-treated (NIC, 1.0%) samples (CIT plus NIC) reduced (p <0.05) redness by 51% compared to the control and 63% compared to the NIC-only treatment. CIT addition in sodium nitrite-treated (NIT, 10 ppm) samples (CIT plus NIT) was similar (p >0.05) in redness to the control and reduced (p <0.05) the redness by 43% compared to the NIT- only treatment **[35]**. Confocal laser scanning microscopy (CLSM) and low field nuclear magnetic resonance (LFNMR) relaxometry were combined to characterize microstructural changes and water distribution in fresh and

cooked pork for 14 days. On day 1 (24 h postmortem) a few muscle fibres, which appeared swollen, were observed in both fresh and cooked meat. An identical microstructure was still apparent after 14 days. However, the number of muscle fibres showing distinguished characteristics increased throughout the aging period. Hence, it was apparent that during aging, the individual fibres swell and disintegrate at different rates **[36]**. Acetic, citric, and lactic acid were incorporated into restructured beef steaks to determine their effect on collagen. These steaks were analyzed for collagen solubility, total collagen content, shear force behavior, sarcoplasmic and myofibrillar protein extractability, thermal stability, and color. These results were compared to those obtained from control samples containing high and low collagen. Results indicate that these acids increased collagen solubility, total collagen content, and shear force values compared to the control samples. No differences (p >0.05) were found in sarcoplasmic protein extractability, although myofibrillar protein extractability declined. Acid treatment decreased the thermal stability of collagen. The Hunter Color values of the uncooked and cooked acid-treated steaks differed (p <0.05) from the controls **[37]**. Meat products manufactured from low-value cuts may be unacceptably tough because of the high connective tissue content of meats used in their manufacture. The effects of shin beef treated with lactic acid at two concentrations (0.5 mol/l and 0.05 mol/l) and citric acid (0.05 mol/l) on cook loss, colour, textural and sensory characteristics of frankfurters were investigated. The results were compared to two controls containing high and low-value beef. Results indicate that the acids had little or no effect on cook loss and, at the low concentrations, had no apparent tenderising effect compared to the control shin beef. However, a significant effect on tenderness (p <0.05) was observed for lactic acid at a higher concentration, but to the detriment of flavour and acceptability. The sensory attribute of juiciness increased due to the addition of the acids, while lactic acid at the lower concentration improved overall texture and acceptability. Instrumental texture analysis indicated that lactic acid had a tenderising effect particularly at higher concentrations. These results demonstrate that adding lactic acid can potentially improve the functionality of meat products for use in value-added meat products **[38]**.

CONCLUSION

The formulation of experimental samples of boiled sausage for herodietic purposes with the addition of protein hydrolysate has been developed. Organoleptic evaluation of experimental samples with the addition of protein hydrolysate 3%, 5%, 7% was carried out. The results of the study showed that when protein hydrolysate was added in an amount of more than 5%, a specific taste characteristic of offal was present in the experimental samples. Based on the sensory evaluation results, it was decided to continue the study of samples with the addition of 3% and 5% protein hydrolysate. The study of the amino acid composition of control and experimental samples showed a significant increase in the content of essential amino acids such as lysine by 2.1%, valine by 32.6%, and interchangeable amino acids such as arginine by 39.3%, glycine 0.5% and serine by 9.6%.

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The study of the effect of drinks based on extracts of herbal adaptogens on the functional status of athletes during physical activity

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ABSTRACT

Adverse environmental factors, stress, lack of sleep and rest, and heavy physical exertion, deplete the human body. In particular, the reserves of the main metabolites, water, and oxygen, are very limited. People, especially athletes, need to take special dietary supplements with adaptogenic properties to adapt to stressful extreme loads. In this study, the influence of using extracts of leuzea, ginseng, and *Eleutherococcus* on athletes' performance, endurance, strength, and emotional state is carried out. The studies were conducted on four groups of male athletes aged from 19 to 25 years. For three weeks, diagnostics of vital lung capacity, Stange, and Genchi tests are carried out, and data on the general impressions of athletes are collected. According to the research results, the use of adaptogens leads to an increase in physical performance. After the first week of the study, a positive effect on the human body are noticed: improve well-being and increased athletic performance. When using *Eleutherococcus*, there was a change in the work of the central nervous system (motor functions): tasks begin to be performed in an organized and accelerated manner without deterioration of well-being, but the volume of strength exercises remained the same. When using the drug leuzea, muscle strength was noted, which allowed to increase the load. There is a positive effect of phytopreparations on the body, namely on the functions of the cardiovascular, central nervous and endocrine systems. In 4 participants who took leuzea, the performance in power competitions improved by 18.5% compared to the control group. The intake of *Eleutherococcus* and ginseng is accompanied by an increase in the activity of neurotransmitter cells, i.e., the effect on the mesolimbic system. In addition, a study of hematological blood parameters and hormonal statuses at the beginning and end of the study was conducted with the subjects who took leuzea extract. So, the use of the drug leuzea leads to the following positive changes: a more significant increase in ESR, a more significant increase in hemoglobin, compared with the control group. The conclusion is made about the practicality of taking biologically active additives based on some plant adaptogens.

Keywords: herbal extract, adaptogen, sport, adaptive potential

INTRODUCTION

The main metabolites, water, and oxygen reserves in the human body are very limited. To maintain a high efficiency level in the body, it is necessary to constantly replenish metabolic reserves as they are spent. Proper and balanced nutrition ensures the entry into the body of all substances necessary for the normal course of metabolic reactions that form the basis of most vital functions and are responsible for human health **[1]**.

However, with heavy physical exertion, which every athlete has to face, traditional eating regimes do not allow covering the daily energy consumption. Therefore, athletes often experience a deficiency of individual nutrients, which manifests in difficulties in carrying out certain types of energy transformations or increasing the overall level of energy production. The consequence of this situation is increased fatigue in the body, as well as a decrease in the body's resistance to various adverse factors (sudden temperature changes, infections, stress, etc.) **[2]**, **[3]**. It is possible to solve the problem of relative nutritional deficiency in intense training and competition conditions by introducing a special diet and using special biologically active food additives containing vitamins, essential trace elements in bioavailable form, and other biologically active substances of natural origin **[4]**, **[5]**, **[6]**, **[7]**. To adapt to stressful loads, athletes need special biologically active additives with adaptogenic effects **[8]**, **[9]**.

Adaptogen preparations are made from raw materials of plant or animal origin. In addition, minerals can also serve as adaptogens. Functionally, adaptogen preparations are involved in optimizing the physiological processes of the body, in particular, normalizing metabolic processes **[10]**. This leads to the economical use of reserve substances and energy sources, strengthens the body's protective functions, and increases the body's ability to resist tissue destruction. Repeated use of adaptogens in violation of homeostasis caused by physical exertion leads to forming a structural trace **[11]**. In some cases, researchers of the effect of physical exertion on the body's physiological processes suggest using adaptogens of plant origin to maintain homeostasis **[12]**, **[13]**, **[14]**.

When using adaptogens of plant origin, microsomal enzymes and antioxidants are synthesized in the body, which contributes to the activation of the process of withdrawal from the body or utilization of xenobionts and free radical metals **[15]**, **[16]**, **[17]**. Herbal preparations are often made from Safflower leuzea, Rhodiola rosea, lemongrass, golden root, ginseng, *Eleutherococcus*, and other plants [18]. The relevance of the search for natural adaptogens is also dictated by the fact that they are quickly incorporated into the biochemical process and are not toxic even with prolonged use **[19]**.

Thus, the use of adaptogen drugs is necessary in cases where the body requires extremely high performance and the fastest recovery. At the same time, these drugs should be as harmless as possible and not have a negative effect on the body in the distant future. Such properties are mainly possessed by preparations made from objects of plant origin.

Research in this direction will make it possible soon to abandon the use of chemical medicinal and synthetic drugs in favor of natural ones.

This work aimed to assess the impact of the systematic use of beverages based on herbal extracts-adaptogens on the functional state of athletes.

Scientific hypothesis

The systematic use of adaptogens of natural origin in the form of beverages based on aqueous extracts will help improve the body's adaptive capabilities and increase athletes' physical performance.

MATERIAL AND METHODOLOGY

Samples

Extracts of herb adaptogens: safflower leuzea, ginseng, eleuterococus.

Chemicals

We used reagents of recognized analytical purity and distilled water. The work used the following chemicals: Ethanol, Sodium carbonate, and Ascorbic acid. All chemicals above were purchased by LenReactive LLC (Sants Petersburg, Russia) and were of analytical grade quality.

Animals and Biological Material

The study on athletes was conducted following the standards of the Helsinki Declaration of the World Association "Ethical principles of scientific medical research with human participation" and "Rules of Clinical Practice in the Russian Federation" (2003). All athletes gave their voluntary consent to participate in the study. Blood sampling was carried out on an empty stomach before training loads in the morning.

Instruments

Ultrasound dispersant UZD 1-0.063/22 (UZT, Krasnodar, Russia), immunochemical analyzer Immulite 1000 (DPS, New York, USA), complex for stress tests Cardiovit AT-104 Esp. (Schiller, Bern, Switzerland), bicycle ergometer ERG-911 BP (Schiller, Bern, Switzerland), biochemical blood analyzer Olympus AU 400 (Olympus Europa SE & Co. KG, Hamburg, Germany), hematology analyzer MEK 7222 (NihonKohden, Tokyo, Japan).

Laboratory Methods

The study of the antioxidant activity of leuzea, ginseng, and *Eleutherococcus* extracts was carried out by the spectrophotometric method according to Rzhepakovsky et al. (2022) **[20]** concerning ascorbic acid.

The study of the functional state of athletes was carried out by express analysis of the state of health with data processing in the program "Diamond" of the Youth Health Center (Vladikavkaz, Russia).

General physical performance was determined by the method of V. L. Karpman **[21]**. The main indicators of adaptation to physical activity were:

1. Heart rate. There is a linear relationship between the heart rate and the intensity of the load, so in sports practice, heart rate is often used as a criterion for assessing the intensity of training.
2. Blood pressure. Speed-power and strength sports increase blood pressure, and low-intensity cyclical (walking, slow running, swimming, skiing, rowing, cycling) – reduce.
3. The vital capacity of the lungs is an indicator reflecting the functional capabilities of the respiratory system.
4. Adaptive potential – the limit of a person's resistance to physical exertion shows how quickly the body adapts to a stressful situation.
5. The Rouffier index – reflects the adaptive capabilities of the cardiovascular system in response to a dosed load and simultaneously characterizes the overall endurance level.
6. The Skibinsky index – reflects the functional capabilities of the respiratory and circulatory organs and the body's resistance to hypoxia.
7. The Stange test is a functional test for assessing the state of the cardiovascular and respiratory systems, which consists in determining the maximum duration of arbitrary breath retention after inhalation.
8. The Genchi test is a functional test for assessing the state of the cardiovascular and respiratory systems, which consists in determining the maximum duration of arbitrary breath retention after exhalation.

Blood biochemical parameters were studied using the Olympus AU series biochemical analyzer (Germany). Hematological analysis was performed on a hematological analyzer MEK 7222 (Nihon Kohden, Japan). The level of cortisol and testosterone was determined on the immunochemical analyzer Immulite 1000 (DPS, USA). Functional diagnostic methods were carried out using the Cardiovit AT-104 Esp. The stress test complex is complete with the ERG-911 BP bicycle ergometer (Schiller, Switzerland).

Description of the Experiment

Sample preparation: The The extracts were obtained at room temperature using an ultrasonic dispersant. Water was used as an extractant. Ultrasonic extracts were treated using the device UZD 1-0.063/22. Ultrasonic exposure to solid plant raw materials was carried out with an intensity of 18 to 22 kHz for 3-5 minutes. Extracts were made in a ratio of 1:10. 10 g was poured into a chemical cup of dry crushed roots of Safflower leuzea, *Eleutherococcus*, and ginseng, and 100 cm^3 of distilled water was poured, after which the generator nozzle was immersed in this cup, and the raw materials were processed.

It should be noted that during ultrasonic treatment, the medium was heated to 60 °C, which does not lead to the inactivation of adaptogens. After the treatment was completed, the solution was filtered out. The prepared extracts are shown in Figure 1.

Figure 1 Snapshot of prepared adaptogen extracts. From left to right: ginseng, leuzea, *Eleuterococcus*.

Number of samples analyzed: 3.
Number of repeated analyses: 3.
Number of experiment replication: 1.
Design of the experiment: At Powders of ginseng, leuzea, and *Eleutherococcus* roots were purchased for the experiment. Drinks were prepared from plant powders by ultrasonic extraction, which athletes systematically used for 3 weeks.

The experimental athletes were selected in the age category of 19-25 years (mainly amateur athletes), men of average height and build (Table 1). The volunteers were randomly divided into four groups of 20 people each. The first group was a control group; athletes did not use any additives to their main diet. Athletes of the second group used ginseng extract, the third group - Eleuterococus, the fourth group - leuzea.

Thus, the athletes of groups 2-4 took extracts of three types in the prescribed volume (330 ml) daily before each training session for three weeks to identify the adaptive properties of the studied extracts. Athletes of the control group consumed 330 ml of water instead of prepared drinks.

Table 1 Anthropometric and functional indexes of experimental athletes.

Group	**Group 1**			**Group 2**			**Group 3**			**Group 4**		
Number of people	**20**			**20**			**20**			**20**		
The additive used	**Clean water**			**Ginseng Extract**			***Eleuterococcus* Extract**			**Leuzea Extract**		
	mean	min	min	mean	min	min	mean	min	min	mean	min	min
Age, years	21	19	25	22.2	19	25	21.6	20	23	21	19	24
Height, cm	177.4	172	183	176.6	172	180	176.8	174	180	175.8	169	182
Weight, kg	77.8	64	88	80.8	72	89	79.2	67	87	79.8	72	87
BMI	22.7	20.6	26.3	25.9	23.5	28.1	25.4	21.4	27.8	25.9	22.6	30.5
Heart rate	85.4	67	112	87	65	107	81.6	65	110	86.2	78	96
AD system, mmHg.	116.4	110	120	119	110	125	115	110	120	119	115	125
AD diast., mmHg.	73.8	65	85	74	60	85	74	70	80	79	70	80
Adaptive potential	2.23	1.9	2.7	2.37	2.1	2.7	2.25	2.0	2.6	2.4	2.3	2.5
Rufier Index	17	12	21	13.4	8	18	12.2	9	16	12.8	8	16
Skibinsky Index	1.76	0.73	3.1	1.48	0.79	2.42	1.64	1.23	2	1.91	1.5	2.7

Studies of the functional state of the experimental subjects were conducted in the health center of polyclinic No.1, Vladikavkaz (Russia), as well as in the laboratories of the North Ossetian State Medical Academy.

During the experiment, the dynamics of changes in the functional state of athletes were determined, as well as the main blood parameters were determined, according to which a conclusion was made about adaptation to physical exertion in the training cycle.

Statistical Analysis

Statistical processing of experimental data. The results were processed using the statistical package PASW Statistics 18, version 18.0.0 (SPSS Inc., USA). Verifying the normality of the signs distribution was carried out using the Kolmogorov-Smirnov criteria. The critical significance level when statistical testing hypotheses in the study was assumed to be 0.05.

RESULTS AND DISCUSSION

As can be seen from the graph (Figure 2), *Eleutherococcus* extract has the highest indicator of antioxidant activity (AOA index) – 871.5 mmol/l, which confirms the results of previous studies **[22]**. Leuzea extract was characterized by an antioxidant activity value of 832.9 mmol/l, which is 37.2% higher than ginseng extract. Nevertheless, all the prepared extracts were characterized by a relatively high AOA value on the ascorbic acid scale, indicating a high extractive yield during ultrasonic extraction.

The main and invariable property of adaptogens is an increase in the physical work performed **[23]**. The importance of adaptogens as regulators of homeostasis is significant. This is primarily due to increasing environmental pollution and urban stress, which lead to previously rare diseases **[24]**. Adaptogenic drugs enable a modern person to tolerate various external negative effects more easily, increasing his resistance to various extreme factors **[25]**.

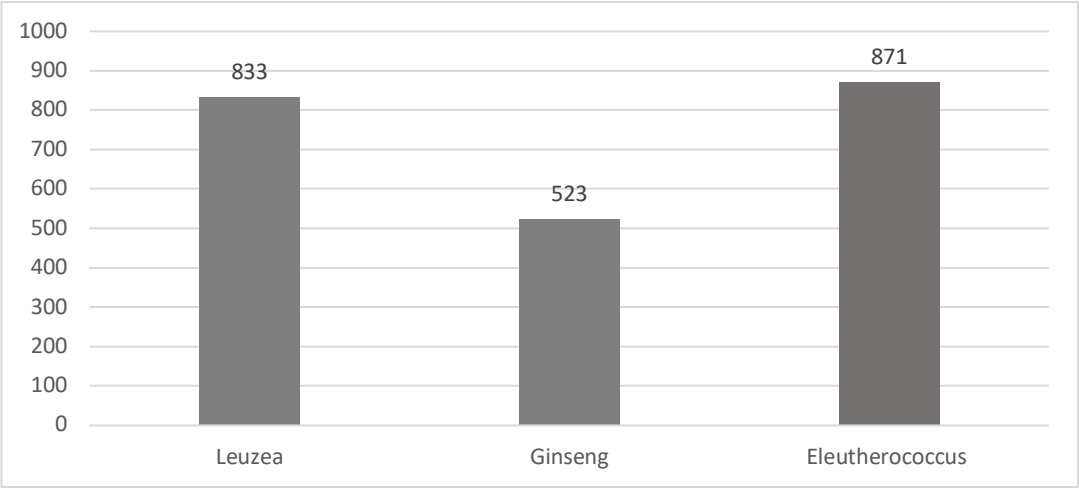

Figure 2 Results of studies of antioxidant activity of model systems, mg/l in terms of ascorbic acid.

To conduct experimental studies on the study of the functional state and adaptation to the physical exertion of amateur athletes, 4 groups of volunteers were organized among students of the North Ossetian State Medical Academy. The first group was a control group, i.e. athletes were subjected to physical exertion but did not take the studied extracts. The next three groups were differentiated by the type of extracts taken – ginseng, *Eleutherococcus*, and leuzea. Physical activity was organized daily throughout the study and took place at a submaximal intensity with a cycle of 3 minutes.

The results of a three-week cycle of studies of the main functional indicators of experimental amateur athletes are presented in Figures 3-5.

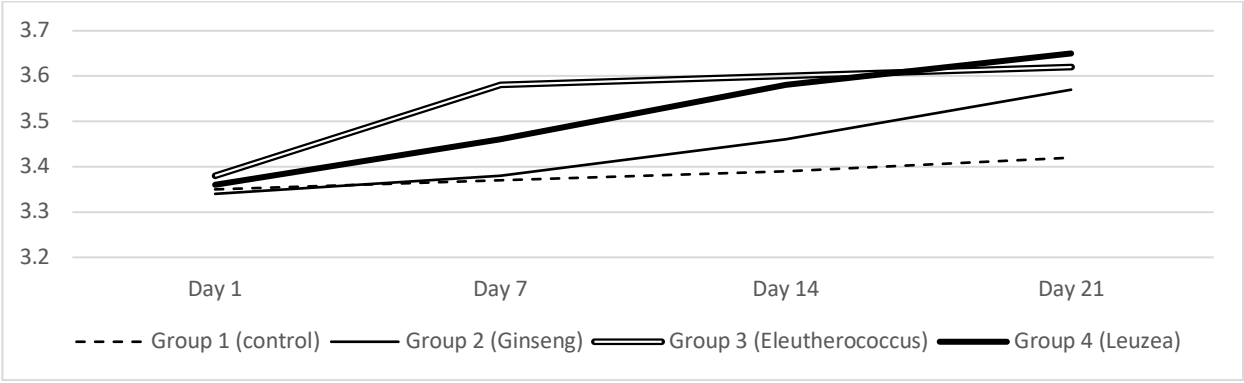

Figure 3 Dynamics of the vital capacity of the lungs of the studied athletes.

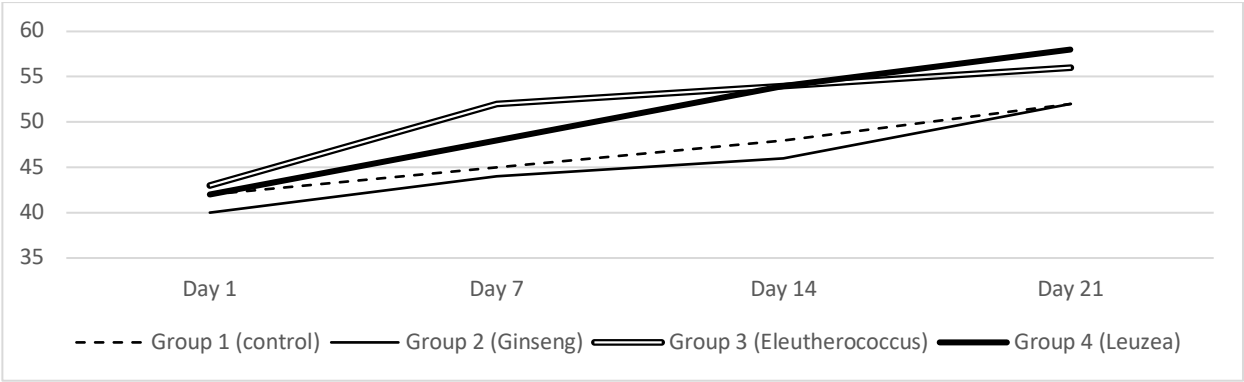

Figure 4 Dynamics of the Stange test of the studied athletes.

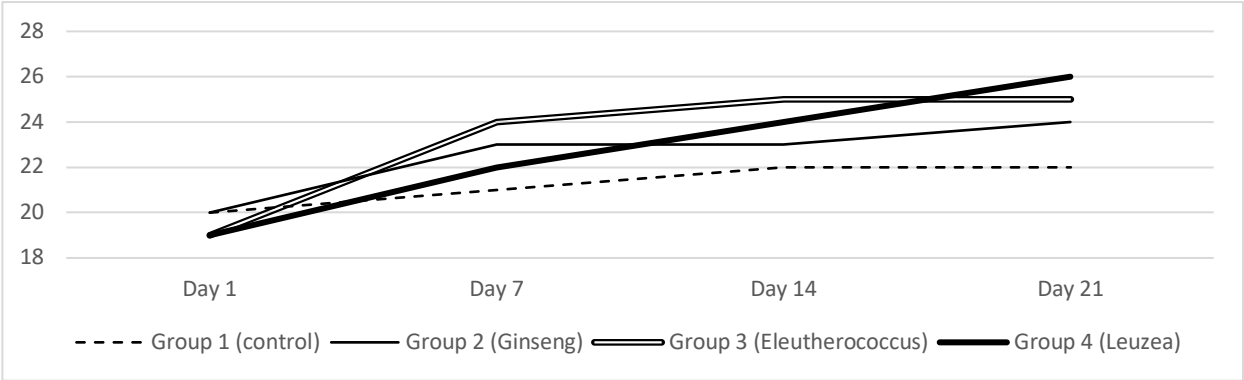

Figure 5 Dynamics of the Genchi test of the studied athletes.

The research results showed that even weekly use of adaptogens led to increased physical performance, which corresponds to reports of other researchers **[8]**, **[11]**, **[26]**. The positive effect on the human body was noticed after the first week of the study. Compared with the control group, there is an improvement in well-being, and an increase in athletic performance. The volunteers who took the *Eleutherococcus* drug showed a change in the work of the central nervous system (motor functions): tasks began to be performed in an organized and accelerated manner without deterioration of well-being, but the volume of strength exercises remained the same. In turn, muscle strength was noted when using the drug leuzea, which made it possible to increase the load. Even though the effect of the drug based on leuzea manifested itself only in the 2nd week of testing, the sum of the effects of its use was higher than that of other drugs. At the same time, reactions related to the metabolism of sex hormones were noted among the participants. Combining the data obtained, it is possible to note the positive effect of phytopreparations on the body, namely on the functions of the cardiovascular, central nervous, and endocrine systems. General indicators such as endurance, resistance to hypoxia, reaction speed, and muscle strength have also improved, which can be proved by **[27]**, **[28]**, **[29]**.

Several authors provide supporting material in favor of the anabolic action of adaptogens from the ginseng family **[30]**, **[31]**, **[32]**, **[33]**. Also, doubts are expressed about the presence of similar properties in herbal preparations **[34]**, **[35]**. At the same time, the anabolic effect is explained only by improving the energy supply of both working tissues and sex glands. And this may apply to most phytopreparations. Leuzea drugs have great potential, as they help in the fight against this phenomenon, preventing it. In 4 participants who took Leuzea, the performance of power competitions improved by 18.5% compared to the control group.

The property of leuzea preparations to accelerate the processes of protein synthesis and harmlessness make them a promising source of phytoecdysteroids, which, affecting protein metabolism, do not cause disturbances in the hormonal picture of healthy people.

The effect on the work of muscles is carried out through the anabolic effect of adaptogens on the body **[36]**, **[37]**, **[38]**. The intake of *Eleutherococcus* and ginseng is accompanied by increased activity of neurotransmitter cells, i.e. the effect on the mesolimbic system. This is also evidenced by the fact that the volunteers who took *Eleutherococcus* drug noted a good sleep throughout the study.

Figure 6 shows the growth dynamics of each studied indicator at the end date of the experiment.

The effect of adaptogen extracts on the functional indexes of athletes was studied between group 5 (control group) and group 6 (experimental group). At the same time, group 5 was formed from 12 people with average indicators based on group 1, and group 6 was formed from 12 people with average indicators based on group 2 taking leuzea extract. At the first stage of studies with adaptogen extracts, the normal values of blood pressure, heart rate, and blood pressure were determined, which made it possible to create two groups equal in average functional indicators – group 5 (control), which was offered to take 330 ml of water before a training session and group 6 (experimental), whose members took 330 ml of a prepared drink based on leuzea extract, which had the best effect on the functional abilities of experimental athletes. The duration of the studies at the second stage was also 21 days.

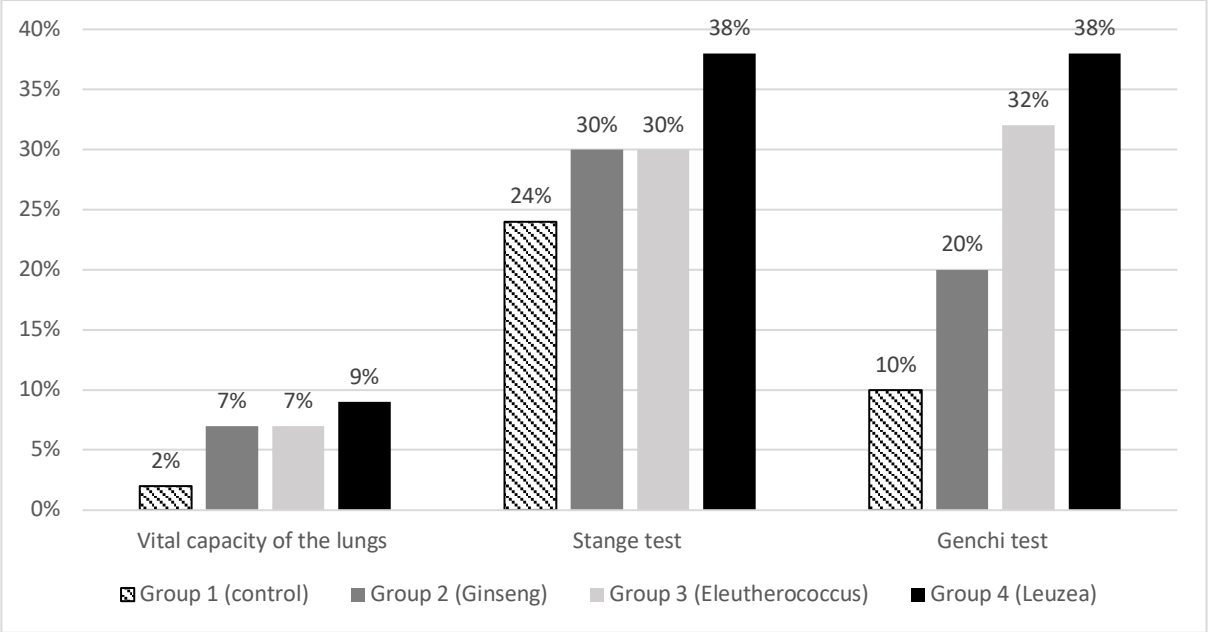

Figure 6 Relative growth of the studied indicators.

The research object at the second stage was hematological blood parameters since they can be used to determine the effect of physical stress on the state of the cardiovascular system. The results of the studies are presented in Figure 7.

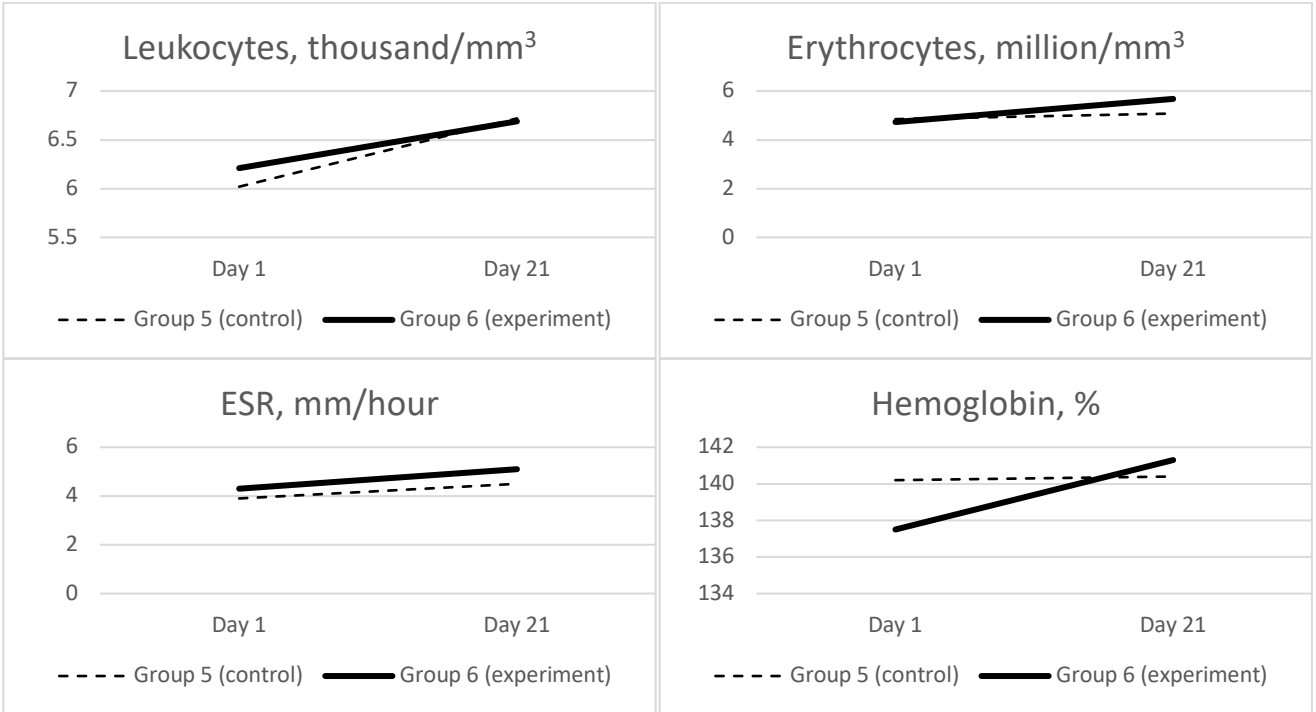

Figure 7 Results of the study of hematological parameters of the blood.

The level of erythrocytes in the blood of athletes of the control and experimental groups at the beginning of the experiment corresponded to the physiological norm and amounted to 4.85 ±0.16 and 4.73 ±0.17 million/mm^3, respectively **[39]**. Physical exertion contributed to an increase in the content of red blood cells in both groups; however, in the case of taking leuzea extract, the difference over three weeks of training was more significant.
In the control group, the ESR at the beginning of the experiment corresponded to 3.9 ±0.52 mm/h. At the end of the experiment, this value did not significantly change – 4.5 ±0.8 mm/h. There were no significant differences in this indicator at the beginning and end of observations and in the experimental group of subjects. Thus, during the 21 days of the training cycle, the ESR did not undergo significant changes in students of both groups, although some tendency to increase this indicator can be traced in both groups.

As expected, the increased number of erythrocytes in the tested athletes taking leuzea extract also increased the blood hemoglobin content from 136.3 ±0.95% to 141.3 ±0.80% by the end of the training cycle. At the same time, in the control group, who performed the same amount of physical activity but did not receive leuzea extract, the blood Hb content by the end of the training was almost equal to the initial one.

Following the change in the hematological parameters of the subjects' blood, changes were also found in the hemorheological characteristics of the blood of volunteer athletes. Samples were taken once after 21 days of training since the beginning of the research. The results of the studies are presented in Figure 8.

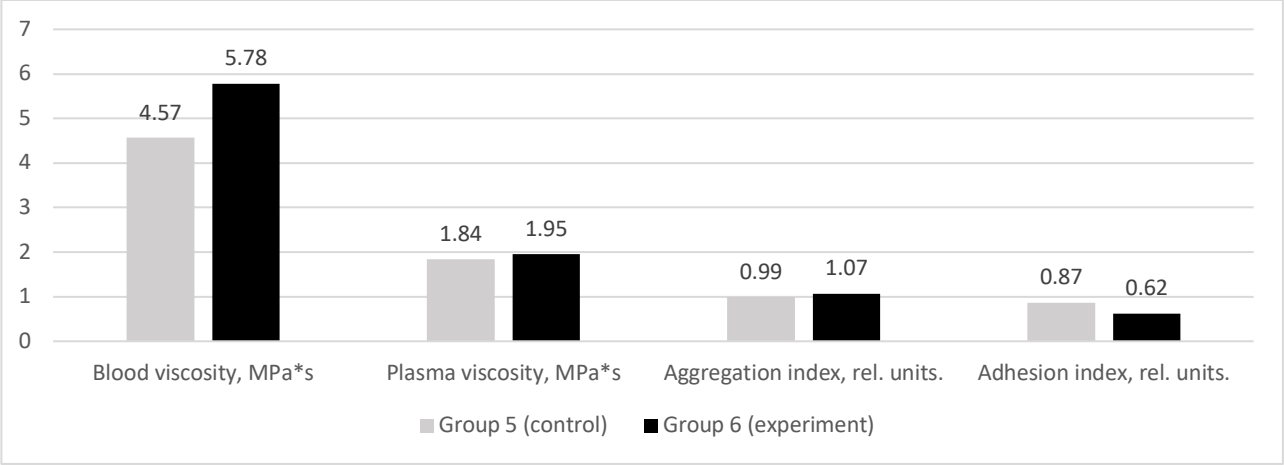

Figure 8 Results of studies of the hemorheological characteristics of the subjects' blood.

It is known that an increase in plasma protein content stimulates the process of aggregation **[40]**, **[41]**, **[42]**. In the study, the experimental group's erythrocyte aggregation index was 1.07 ±0.02 a. u., whereas, in control, its value was 0.99 ±0.01 a. u. Changes in the parameters characterizing the deformability of erythrocytes were less significant. Thanks to an integrated approach to the study of blood flow, it was possible to identify those intravascular factors that have the most significant impact on the state of the cardiovascular system during physical exertion. In the study, such factors were an increase in plasma viscosity, erythrocyte aggregation, and leukocyte adhesion. These changes cause an increase in blood viscosity in individuals with higher adaptive capabilities of the body **[43]**, **[44]**. In addition, the revealed hemorheological changes increase the efficiency of oxygen transport **[45]**. In addition, a slight decrease in protein concentration was observed in the control group, indicating the insufficiency of anabolic processes, as indicated by a decrease in testosterone synthesis (Table 2).

Table 2 Indicators of the hormonal status of athletes against the background of taking natural adaptogens.

Indicators	Group 5 (control)		Group 6 (experiment)	
	Day 1	Day 21	Day 1	Day 21
Cortisol mg/dl (norm 11.3-25)	16.4	18.6	16.5	19.5
Testosterone ng/dl (norm m:105-545)	436.3	241.0	358.3	266
Testosterone/cortisol (male) c.u.*10^2	0.028	0.011	0.022	0.015

Such changes indicate a lack of adaptive potential of the body and the presence of signs of overtraining. The study of hormonal status showed that the studied indicators in the analyzed groups had no statistically significant dynamics and were within the reference values. However, in the comparison group, there was a significant decrease in the testosterone/cortisol ratio index, which characterizes the tension of metabolic processes and increased catabolism. In contrast, the cortisol level practically did not change, which can be regarded as the initial stage of maladaptation **[46]**, **[47]**.

Thus, the results showed that for immediate adaptation to physical activity, the best option is using drinks based on eleuterococus extracts. With systematic training, the best adaptive potential was observed in people who took drinks based on leuzea extract.

Taking a drink based on leuzea extract directly affects the hematological parameters of the blood, increasing the level of red blood cells and, accordingly, hemoglobin, which is a factor of adaptation to physical exertion.

CONCLUSION

The results of the conducted studies have shown that using adaptogen extracts during the week significantly increases efficiency. The earliest effect was provided by the drug *Eleutherococcus* in the form of reducing fatigue and increasing the resistance of hypoxia. Athletes who take *Eleutherococcus* extract noted an improvement in well-being, an increase in athletic performance, low irritability, and sound sleep. At the same time, they also note the lack of dynamics of strength exercises. In turn, the drug leuzea makes it possible to significantly increase physical activity after two weeks; athletes note a significant increase in muscle strength. According to the results of the three-week experiment, data were obtained that in the control group the vital capacity of the lungs increased by 2%, the result of the Stange Test improved by 24%, and the result of the Genghi Test improved by 10%. At the same time, the group taking the ginseng drug shows the following dynamics: +7% – vital capacity of the lungs, +30% – Stange Test, +20% – Genghi Test. The group taking *Eleutherococcus*: +7% – lung capacity, +30% – Stange Test, +32% – Genghi Test. The group taking the drug leuzea: +9% – lung capacity, +38% – Stange Test, +38% – Genghi Test. In 4 participants who took Levzea, the performance in power competitions improved by 18.5% compared to the control group. According to the results of the experiments, it was the drug leuzea that showed the greatest effect. In addition, the use of levzea significantly increased the content of red blood cells in the blood (from 4.73 to 5.68 million/mm^3, that is, by 20.08%), compared with the control group (from 4.85 to 5.08 million/mm^3, that is, by 4.74%). Consequently, the hemoglobin level increased significantly (from 137.5% to 141.3%), compared with the control group (from 140.2 to 140.4). Thus, the results showed that for immediate adaptation to physical activity, the best option is to drink drinks based on *Eleutherococcus* extracts. With systematic training, the best adaptive potential was observed in people who took drinks based on leucea extract. Taking a drink based on levzea extract directly affects hematological blood parameters, increasing the level of red blood cells and, accordingly, hemoglobin, which is a factor of adaptation to physical exertion.

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The study of the nutritional and biological value of functional semi-finished fish products "fish balls"

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ABSTRACT

In the context of the problem of the organization of high-quality nutrition for consumers, the ways of its solution by expanding the range of products based on raw fish materials are considered. The necessity of creating combined semi-finished products with adequate substitution for plant components is justified, which allows increasing the amount of dietary fiber consumed and reducing the caloric content of the product, enriching minced fish with carbohydrates (polysaccharides and dietary fibers), amino acids, as well as macro- and microelements. Thus, a comparative analysis of the content of essential amino acids in the muscle tissue of fish in the inland waters of the Republic of Kazakhstan with some oceanic and marine fish showed that the content of amino acids such as leucine, lysine, threonine, phenylalanine is slightly higher. They are characterized by a high content of essential amino acids limiting the biological value, g/100 g of protein: lysine – 8.8-11.6; methionine – 2.1-3.1; tryptophan – 1.0-1.1. The data analysis shows that a higher pH value of fish meat corresponds to a higher elasticity value. The pH shift to the alkaline side of more than 7.5, although it promotes the release of myosin, reduces the elasticity of meat. In our study, we used minced fish from Carp, Pikeperch, Bream, and Pike. It was found that with the addition of 30% of the functional supplement of kelp, the moisture-retaining capacity of the fish semi-finished product was 48.6% and pH 6.67. With the addition of 30% of the functional pumpkin additive, the moisture-retaining capacity of the fish semi-finished product was 49.27% and pH 6.04. Developing semi-finished fish products with plant components makes it possible to obtain products of high biological value with a juicy consistency, which meets modern trends in healthy nutrition.

Keywords: balanced nutrition, fish, fish product, amino acid composition, moisture binding ability

INTRODUCTION

One of the ways to solve the problem of supplying the population with high-grade foods rich in proteins is to increase the production of fishery products, particularly commercial fish farming. Kazakhstan has a significant number of diverse ecologically clean reservoirs in which it is possible to produce environmentally friendly fish products (the total area of Kazakhstan's reservoirs, excluding the Caspian Sea, is about 5 million hectares). In the Republic of Kazakhstan, the export of fish products is among the important agricultural goods and ranks third in the ranking of the country's exports **[1]**. In the existing global trends in the production and turnover of food products, much attention is paid to developing and implementing preventive measures to ensure the safety of food products and the stable production of high-quality food products **[2]**. Fish product quality and safety are important for competitive advantage in the market **[3]**. Such stability is achieved only by applying a systematic approach that includes a detailed analysis and assessment of the nutritional value of finished fish products. Fish products are an important source of essential human nutrients: iodine and phosphorus, as well as protein and polyunsaturated fatty acids (eicosapentaenoic, docosahexaenoic, linoleic, linolenic, arachidonic) and fat-soluble vitamins. The nutritional value of fish products is high. Consumption of fish products by people with poor health

and different age groups, including children and, the elderly, pregnant women, is recommended. This connection shows the need to ensure guaranteed high-quality fish products **[4]**.

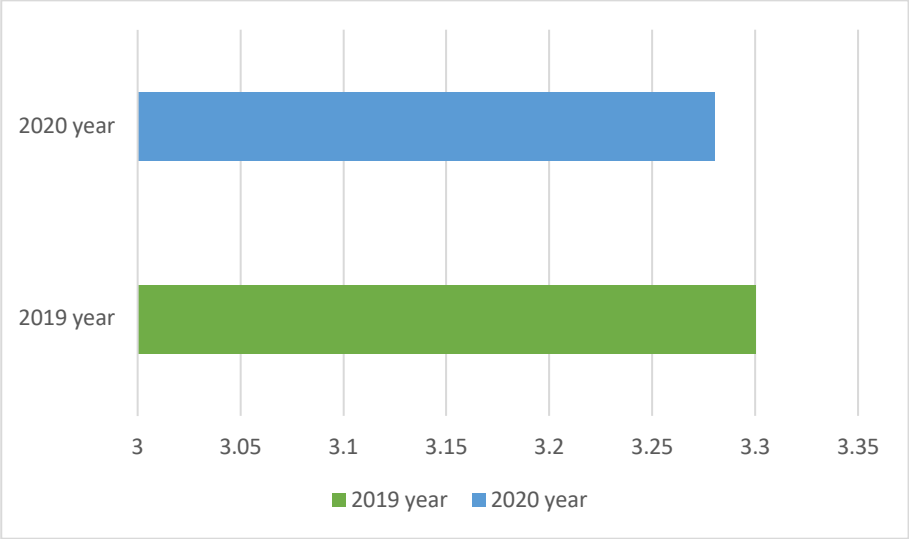

Figure 1 fish processing and production in Kazakhstan, USD.

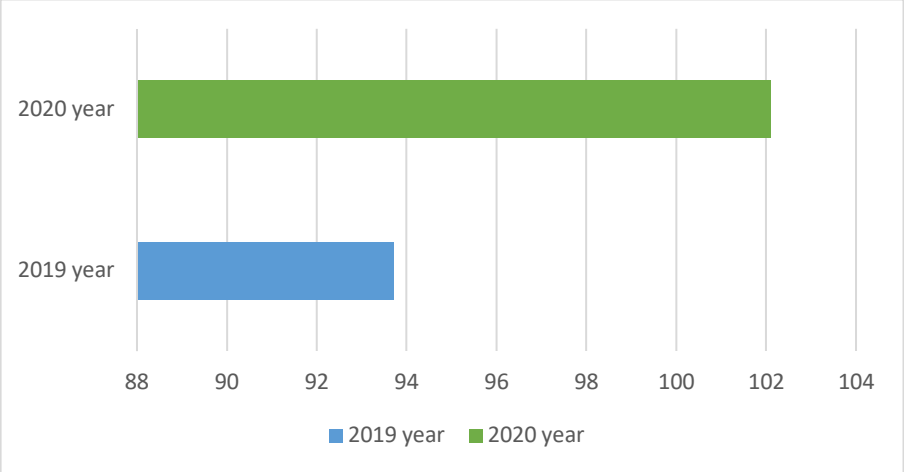

Figure 2 fish industrial production index in Kazakhstan, %.

Due to a large number of valuable nutritional nutrients (protein, fat, vitamins, etc.), fish products are susceptible to microbiological spoilage, leading not only to a rapid deterioration of organoleptic properties but also to the development of pathogenic microorganisms. In addition, the fish itself may contain dangerous parasites (varieties of trematodes, cestodes, scrapers, nematodes), toxins (tetrodotoxin, algotoxin, tyramine, putrescine, cadaverine, ichthyotoxin, etc.), heavy metals (primarily mercury) and pesticides. And in the process of production and storage, nitrosamines, benzopyrene, heavy metals, etc., may get into or form in fish products **[5]**. In recent decades, there has been a tendency in the fishing industry to increase the demand for fish products with a high degree of readiness **[6]**. First, this concerns the production of fish cutlets, which consumers actively buy in stores and public catering enterprises. The final heat treatment (heating, roasting, etc.) carried out outside the manufacturer (at home or catering establishments) does not imply the possibility of controlling the cooking modes of fish cutlets, which increases the risk of poisoning them in case of insufficient heat treatment. At the same time, quality claims will be addressed primarily to the manufacturer.

In this regard, scientific research aims to study semi-finished fish products' nutritional and biological value.

Scientific hypothesis

The development of the functional product from fish and vegetable raw materials can improve the nutritional value of the fish balls product. We expect an increase in the amino acid composition and mineral substances in the finished product after adding a functional supplement from kelp and pumpkin.

MATERIAL AND METHODOLOGY

Samples

Fish raw materials: carp, pike perch, bream, pike. Functional additives of vegetable origin: dried kelp and pumpkin.

Chemicals

All reagents used were of U.S.P. purity or higher. All solvents, including water, were used with the LC/MS label.

Instruments

The MOD MARS 6 microwave sample preparation system (MARS 6 Synthesis, CEM) was used for sample preparation. The amino acid composition was determined using high-performance liquid chromatography "Agilent-1200", with a separating column InfinityLab Poroshell 120 HILIC 1.9 microns.

Laboratory Methods

Laboratory studies of raw materials were carried out based on JSC "Almaty technological university" (Almaty, Kazakhstan). The total chemical composition: of moisture **[9]**, fat **[10]**, protein **[11]**, ash **[12]**, and amino acid composition **[13]** were determined.

Protein measurements were performed using the Kjeldahl method **[43]**. 5 g of homogeneous fillet with 20 mL of concentrated sulfuric acid and 8 g of catalysts were placed in a special container and then heated at 350 °C for 30 min. After mineralization, the sample was quantitatively transferred to a solution of NaOH at a concentration of 33%, sealed, and distilled off with steam. The resulting steam distillate was transferred to a container containing several drops of the Tashiro indicator. The titration was performed with a solution of 0.01 N sulfuric acid.

Total fat was measured by the Soxhlet method **[8]**. 4 g of the dried sample in a paper cartridge was placed in an extraction flask of a Soxhlet apparatus. Petroleum ether with a boiling point of 45 °C was used for the extraction. After multiple extractions, the weight of the test cartridge to constant weight was determined. The difference between the initial and final weight shows the percentage of fat.

Moisture was determined by the method of drying **[7]**. 5 g of the sample was placed in a container and dried for 1 hour at 150 °C.

Description of the Experiment

Sample preparation: The fish was cut after defrosting to study the mass composition.

Mass composition is the ratio of the mass of individual body parts and organs, expressed as a percentage of the mass of the whole fish. The study of mass composition is necessary for their rational use.

The cutting was carried out manually. When cutting fish, the heads, scales, skin, fins, insides, and black film were separated. Then fillets were cut from the spine while removing the spinal and rib bones. Each part was weighed, and its percentage ratio to the total weight of the fish was determined.

Number of samples analyzed: we analyzed 12 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 two times.

Design of the experiment: At the beginning of the experiment, we determined the content of moisture, protein, fat, ash, salt, and acidity. The amino acid composition, the water-binding ability of minced fish in combination with vegetable ingredients, and the mineral composition were studied. Based on the data obtained, determine the recipe for a semi-finished product from minced fish.

Statistical Analysis

Microsoft Excel and Statistica 15 produced the statistical data analysis. All experiments were carried out in triplicate and the results reported are the results of those replicate determinations with standard deviations. The Student t-test was used for the statistical analysis of the obtained results. Data are presented as mean ±standard error of the mean (SEM). The smallest acceptable difference for probes from the one sample was pointed at 5%. Probes with more differences were not considered.

RESULTS AND DISCUSSION

Analysis of the data on the mass composition of fish shows that the relative mass of pure meat (without skin) in the studied fish is 35-40% of the total weight. The terrain coefficient, defined as the ratio of the pulp part to other parts, is 0.58 for carp, 0.60 for walleye, 0.62 for bream, and 0.62 for pike. The averaged data on the mass composition of particle fish are presented in Table 1.

Table 1 Mass composition of fish, %.

Fish	Muscle tissue	Heads	Entrails, skin, scales, bones, fins	Losses
Carp	36.90 ±5.28^{c}	20.13 ±2.93^{b}	37.34 ±4.73^{c}	5.63 ±2.03^{a}
Pike perch	37.39 ±4.17^{c}	23.68 ±0.05^{b}	34.98 ±3.95^{d}	4.45 ±1.40^{a}
Bream	38.10 ±4.15^{d}	24.12 ±2.04^{b}	30.63 ±0.79^{c}	7.15 ±0.29^{a}
Pike	38.20 ±3.12^{c}	20.06 ±1.02^{b}	38.19 ±4.03^{c}	3.01 ±1.32^{a}

Note: $^{a-d}$ means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). All values are expressed as the mean ±SD (standard deviation).

In the production of semi-finished fish products, muscle tissue is of the greatest interest. A complex chemical composition characterizes muscle tissue. It includes many chemicals, among which water, proteins, lipids, and minerals predominate. The content of the main components varies quite widely depending on many factors.

The chemical composition was evaluated based on the average values obtained by analyzing average fish samples taken according to the method

The average chemical composition of muscle tissue is shown in Table 2.

Table 2 General chemical composition of fish muscle tissue, %.

Indicators	Bream	Perch
Water	75.29 ±1.80^{a}	78.50 ±0.70^{a}
Protein	17.5 ±0.12^{a}	18.8 ±0.10^{a}
Fat	4.80 ±1.30^{b}	0.88 ±0.35^{a}
Mineral substances	1.25 ±0.42^{a}	1.13 ±0.24^{a}

Note: $^{a-b}$ means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). All values are expressed as the mean ±SD (standard deviation).

The results of the analysis of the chemical composition of fish showed that the main components of muscle tissue - water, fat, and protein - are quantitatively dependent on each other. Fish with a high-fat content have less water and protein. The criteria protein/moisture, fat/protein, and fat/moisture are used to characterise the muscle tissue of fish. We calculated the above criteria based on the total chemical composition data (Table 3).

Table 3 Criteria for evaluating the qualitative indicators of the muscle tissue of particle fish, %.

Fish	Criteria		
	protein/moisture	fat/moisture	fat/protein
Carp	0.23 ±0.12^{b}	0.06 ±0.15^{a}	0.27 ±0.12^{b}
Pike perch	0.24 ±0.30^{b}	0.01 ±0.12^{a}	0.03 ±0.30^{a}
Bream	0.21 ±0.15^{b}	0.08 ±0.15^{a}	0.37 ±0.12^{c}
Pike	0.23 ±0.12^{b}	0.01 ±0.12^{a}	0.04 ±0.12^{a}

Note: $^{a-c}$ means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). All values are expressed as the mean ±SD (standard deviation).

For a complete characterization of the biological value, the amino acid composition of the muscle tissue of fish was studied (Table 4). Analysis of the amino acid composition data indicates a rich set of essential amino acids in the proteins of the studied fish. A comparative analysis of the content of essential amino acids in the muscle tissue of fish in inland waters of the Republic of Kazakhstan with some oceanic and marine fish showed that the content of amino acids such as leucine, lysine, threonine, phenylalanine is slightly higher in them (Table 4). They are characterized by a high content of essential amino acids limiting the biological value, g/100 g of protein: lysine 8.8-11.6; methionine 2.1-3.1; tryptophan 1.0-1.1.

Table 4 Amino acid composition of fish meat proteins, g/100 g of protein Amino Acids.

	Fish			
	carp	**walleye**	**pike**	**bream**
Valin	6.6 ±0.591[b]	5.3 ±0.712[a]	5.3 ±0.823[a]	6.4 ±0.823[b]
Isoleucine	5.1 ±0.913[a]	5.1 ±0.908[a]	5.1 ±0.567[a]	5.0 ±0.765[a]
Leucine	9.2 ±0.358[b]	7.6 ±0.343[a]	7.6 ±0.485[a]	9.1 ±0.498[b]
Lysine	11.6 ±0.421[b]	8.8 ±0.409[a]	8.8 ±0.498[a]	11.6 ±0.564[b]
Methionine	3.3 ±0.448[b]	2.1 ±0.401[a]	2.1 ±0.713[a]	3.1 ±0.583[b]
Threonine	5.9 ±0.582[b]	4.3 ±0.540[a]	4.3 ±0.481[a]	5.9 ±0.387[b]
Tryptophan	1.1 ±0.314[a]	1.0 ±0.293[a]	1.0 ±0.794[a]	1.1 ±0.987[a]
Phenylalanine	5.1 ±0.776[b]	3.8 ±0.831[a]	3.8 ±0.298[a]	5.0 ±0.639[b]
Total essential amino acids	47.9 ±0.582[b]	38.0 ±0.656[a]	38.0 ±0.743[a]	47.2 ±0.351[b]
Alanine	6.9 ±0.337[a]	7.1 ±0.593[a]	6.6 ±0.458[a]	6.7 ±0.769[a]
Arginine	6.0 ±0.555[b]	5.6 ±0.386[a]	5.6 ±0.489[a]	5.9 ±0.475[b]
Aspartic acid	10.9 ±0.812[b]	8.8 ±0.694[a]	8.8 ±0.845[a]	10.5 ±0.867[b]
Histidine	2.2 ±0.587[a]	2.2 ±0.160[a]	3.6 ±0.645[b]	2.2 ±0.476[a]
Glycine	3.7 ±0.912[a]	5.5 ±0.293[b]	5.5 ±0.948[b]	3.8 ±0.398[a]
Glutamic acid	16.6 ±0.811[b]	12.8 ±0.656[a]	12.8 ±0.526[a]	16.6 ±0.374[b]
Proline	3.1 ±0.448[a]	6.1 ±0.912[b]	6.1 ±0.185[b]	3.1 ±0.189[a]
Serine	5.0 ±0.451[b]	3.1 ±0.871[a]	3.1 ±0.367[a]	5.0 ±0.856[b]
Tyrosine	3.8 ±0.358[c]	2.8 ±0.784[b]	2.4 ±0.847[a]	3.7 ±0.769[c]
Cystine	-	1.5 ±0.654[a]	1.5 ±0.657[a]	-
Oxyproline	-	-	-	-
Total interchangeable amino acids	58.2 ±0.324[a]	55.5 ±0.293[a]	56.0 ±0.412[a]	57.5 ±0.385[a]

Note: [a-c] means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). All values are expressed as the mean ±SD (standard deviation).

For a reasonable combination of minced fish from various types of fish, studies of the functional and technological properties of minced meat are carried out.

During experimental studies, the elasticity of fish raw materials was studied, which can be judged by the content of myosin and the pH value. The data analysis shows that a higher pH value of fish meat corresponds to a higher elasticity value. The pH shift to the alkaline side of more than 7.5, although it promotes the release of myosin, reduces the elasticity of meat. Perch has the best elasticity (3.32-4.12). Changes in the elasticity of the same species of fish are probably due to the following factors: the age of the fish, the season and depth of their habitat, the shelf life, the method of processing raw materials. Fish meat's pH value also affects its moisture binding ability, which increases with increasing pH.

Based on the results of the analysis of the literature data, the possibility and expediency of expanding the range of minced fish products with vegetable raw materials have been established. Scientists have researched the combination of fish and duck meat to improve the nutritional value and the technological properties of finished products **[9]**. The theory of balanced nutrition allows us to study and justify the nutritional and biological value of many products based on the study of their chemical analysis **[10]**. studies of the chemical composition of human food products allow us to understand values and categories. Thus, the nutritional value of a product is the ratio of the product's chemical composition to match the basic form of a balanced diet **[11]**. The functional properties of the finished product directly depend on the chemical composition of the raw materials. fats, proteins, carbohydrates in the composition of food products characterize the sensory qualities of the finished product **[12]**. The compatibility of the components of the finished product and the compatibility of the chemical composition of all components inside the product is a key point in developing new food products **[13]**. The most important and initial work in developing new food products is studying raw materials' nutritional value and chemical composition **[14]**. The table analysis shows that the freshwater fish studied belongs to low-fat protein raw materials. The crude protein content in the carp muscles was 18.60-18.78%, depending on the catch season. The protein concentration in the carp meat was slightly lower and amounted to 17.60-17.70%. High levels of protein in muscle in both marine and freshwater aquaculture have been identified by several researchers **[15]**. Scientists from different countries are researching the chemical analysis of fish raw materials. So, some results confirm the increased influence of fish protein in the human diet **[16]**, **[17]**, **[18]**. A comparative analysis of fish meat with poultry meat showed that the ratio of protein and fat is almost the same **[19]**, **[20]**. Studies have also been conducted to replace fish meat with poultry meat. studies have shown that this combination positively affects the

final product **[21]**, **[22]**. The muscle tissue's moisture and protein content determine the finished product's consistency, taste, and yield. The moisture content also significantly impacts the structure of functional groups of protein molecules, their stabilization and spatial configuration, and thus the functional and technological properties of the meat system as a whole **[23]**, **[24]**. Calculating the coefficients of chemical composition and food saturation gives an understanding of the saturation of the finished product **[25]**. complex heterogeneous food systems include minced meat, especially fish meat. The technological properties of a product directly depend on the chemical composition of the carcass **[26]**. Protein and its properties in finished food products largely depend on the addition of sodium chloride to it. The method of salting and its quantity directly affect the structural and mechanical characteristics of food products **[27]**. The muscle tissue of fish and the ability to bind moisture depends on several indicators, namely temperature, acidity, and degree of dispersion **[28]**. The minced fish's size and the grinding grate's diameter are extremely important in choosing optimal indicators of immobilized moisture **[29]**. The moisture binding ability takes an active part in maintaining the freshness of fish. it also greatly impacts the output and quality of finished products **[30]**. According to some scientists, an increase in the moisture binding ability increases the adhesive qualities of minced fish, the elasticity of the resulting minced fish, the shear stress decreases, and the functional properties improve **[31]**. Introducing up to 3% salt into minced meat improves the rheological characteristics of minced meat, increasing the solubility of proteins **[32]**. Some scientists say adding less than 1% of table salt to minced meat is not recommended. This can damage raw materials and, subsequently, finished products **[33]**. Functional groups of proteins have properties to attract free water, increasing hydration. and as a consequence, the moisture-binding ability. This is due to the addition of table salt **[34]**. The higher emulsifying ability of the muscle tissue of the crucian carp is due to the values of the coefficients Cw and PWF, as well as the content of bound moisture. The EC and SE of minced meat are higher when NaCl is added. The values of these indicators are consistent with the indicators of WHC and WBC of minced fish. They are due to the increased content of water-soluble and salt-soluble fractions of muscle tissue proteins of hydrobionts **[35]**. For this reason, only mobile and flexible protein macromolecules can form adsorption layers at the interface of the two phases and form a helical gel structure in a continuous phase **[36]**. According to Wang **[37]**, the emulsifying ability of minced poultry meat is 75%. The stability of the emulsion is about 70%, which, when combined with multicomponent functional mixtures based on animal proteins **[38]**, **[39]**, **[40]**, **[41]**, **[42]**, will effectively develop meat products with a combined composition of raw materials.

Introducing a vegetable component into minced fish will make it possible to obtain a new product – fish semi-finished products with functional properties. Experimental production of fish semi-finished products, "fish balls" for functional purposes was carried out in the educational and scientific center for meat processing of JSC "Almaty Technological University". Minced fish from pike perch, bream, and roach were the main raw materials. As functional ingredients, dried kelp and pumpkin. To carry out the planned studies, 10% to 30% of kelp and crushed pumpkin were added to the minced meat instead of minced fish. Physico-chemical and functional-technological indicators evaluated the resulting combined minced meat, in particular, the moisture binding ability and pH of minced meat were determined compared to the control. Minced fish without additives was used as a control. When vegetable additives are added to minced fish, the chemical composition of minced fish is replenished with dietary fibers, as evidenced by an increase in fiber content (Table 5, Figure 3).

Table 5 Physico-chemical parameters of the fish semi-finished product.

Sample	Mass fraction, %			Fiber content, %
	fat	protein	moisture	
Control sample	25.21 ±0.02^{c}	9.62 ±0.01^{b}	46.91 ±0.01^{d}	2.21 ±0.01^{a}
Prototype with 10% kelp replacement	24.75 ±0.05^{c}	9.76 ±0.02^{b}	54.10 ±0.02^{d}	3.14 ±0.01^{a}
Prototype with 30% kelp replacement	33.97 ±0.05^{c}	9.84 ±0.02^{b}	56.09 ±0.02^{d}	5.44 ±0.01^{a}
Prototype with 10% pumpkin replacement	23.72 ±0.05^{c}	9.72 ±0.02^{b}	46.33 ±0.01^{d}	2.92 ±0.01^{a}
Prototype with 30% pumpkin replacement	30.46 ±0.05^{c}	9.33 ±0.02^{b}	53.72 ±0.01^{d}	4.31 ±0.01^{a}

Note: $^{a-d}$ means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). All values are expressed as the mean ±SD (standard deviation).

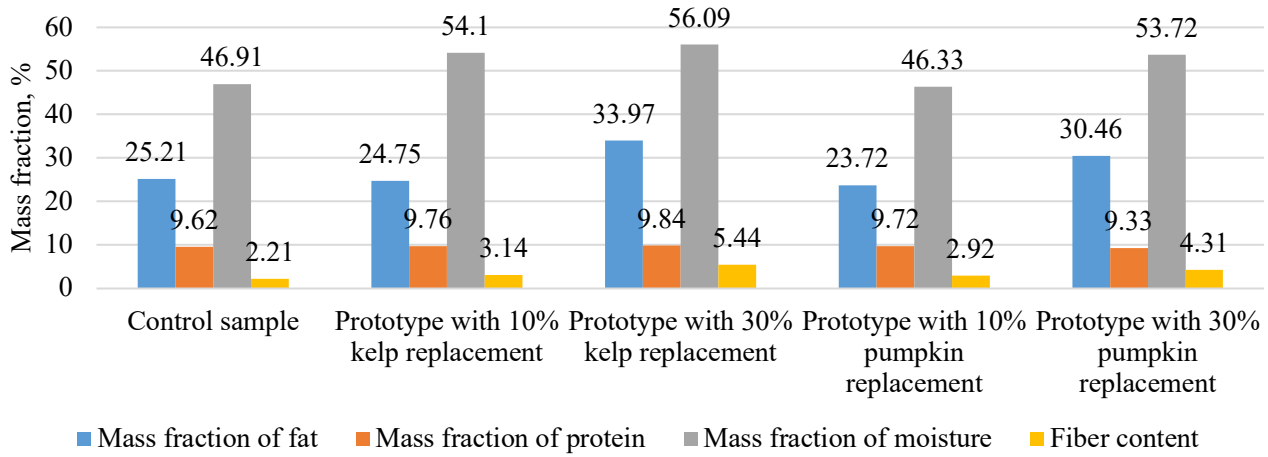

Figure 3 Dynamics of changes in physical and chemical parameters of the fish semi-finished product.

As a result of the study of functional and technological parameters of minced fish, the dependence of moisture binding ability and pH on the amount of application of functional ingredients was established. The ability of minced meat to bind and retain water and its stability during heat treatment varies depending on the morphological composition, pH, protein, fat, moisture, and dietary fiber content in minced meat and their ratio.
The content of muscle and connective tissue in raw materials and dietary fiber in minced meat significantly (p <0.05) affect (the moisture binding ability of) the functional properties of minced meat (Table 6).

Table 6 Functional and technological indicators of the fish semi-finished product.

Indicators	Control sample	Prototype			
		kelp		pumpkin	
		10%	30%	10%	30%
Moisture binding ability, %	49.27 ±0.59^{a}	59.54 ±0.65^{b}	62.48 ±1.19^{c}	50.66 ±1.06^{a}	59.84 ±1.02^{b}
Moisture-holding capacity, %	45.51 ±0.96^{a}	46.72 ±0.89^{a}	48.60 ±0.73^{c}	47.34 ±0.96b	49.27 ±0.84^{d}
Fat-holding capacity, %	69.06 ±1.04^{d}	50.82 ±0.76^{b}	40.68 ±0.85^{a}	60.68 ±1.15^{c}	50.73 ±0.91^{b}
pH	6.7 ±0.1^{c}	6.66 ±0.01^{b}	6.67 ±0.01^{b}	6.24 ±0.01^{a}	6.04 ±0.01^{a}

Note: $^{a-d}$ means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). All values are expressed as the mean ±SD (standard deviation).

The maximum moisture binding ability and pH are noted when functional ingredients are added to minced fish in 30%; moisture binding ability was 48.6% and pH 6.67 when replaced with kelp and 49.27 and 6.04, respectively, when replaced with pumpkin. The pH increases until a certain maximum value is reached, at which the maximum protein solubility is observed, affecting the hydrophilicity of fish proteins. Therefore, it causes an increase in the moisture binding ability of the combined stuffing system. The increase in these indicators is associated with introducing carbohydrates and dietary fibers contained in plant components into minced meat and their participation in forming protein-polysaccharide complexes with increased emulsifying and stabilizing ability and influencing the stabilization of the structure and the content of strongly bound moisture. Accordingly, combined minced meat's increased moisture binding ability is also associated with swelling processes.
The effect of introducing vegetable additives on the mineral content and amino acid composition of the fish semi-finished product was also studied (Table 7).

After analyzing the data presented in Table 7, it can be concluded that introducing plant components increases the product's nutritional value. In samples 1 and 2, due to the introduction of kelp, the mineral composition, and amino acid composition content increase. In the samples, an increase in iron content, a vital trace element for the human body, is noted.

Table 7 Dynamics of changes in the content of minerals, and amino acid composition of fish semi-finished products, depending on the proportion of vegetable additives.

Indicators	Control sample	Prototype			
		Prototype with 10% kelp replacement	Prototype with 30% kelp replacement	Prototype with 10% pumpkin replacement	Prototype with 30% pumpkin replacement
Mineral elements, mg/100 g					
Potassium	281.25 ±4.22^{a}	-	487.88 ±7.32^{d}	312.55 ±5.99^{c}	299.88 ±4.48^{b}
Magnesium	35.14 ±0.74^{b}	-	75.60 ±1.53^{c}	32.83 ±0.49^{a}	29.98 ±0.31^{a}
Iron	0.58 ±0.005^{a}	1.81 ±0.03^{b}	5.21 ±0.06^{c}	0.60 ±0.008^{a}	0.74 ±0.01^{a}
Sodium	55.32 ±0.61^{b}	107.43 ±1.61^{c}	194.72 ±4.09^{d}	49.79 ±0.55^{a}	47.12 ±0.46^{a}
Calcium	53.75 ±0.59^{c}	57.85 ±0.98^{d}	49.63 ±0.55^{b}	50.86 ±0.76^{b}	43.95 ±0.48^{a}
Zinc	1.2 ±0.01^{b}	1.43 ±0.03^{c}	1.2 ±0.01^{b}	1.11 ±0.01^{b}	0.91 ±0.02^{a}
Iodine	0.038 ±0.0003^{a}	0.188 ±0.012^{b}	0.78 ±0.015^{c}	0.034 ±0.0007^{a}	0.028 ±0.0005^{a}
Phosphorus	267.22 ±2.94^{c}	302.35 ±4.54^{d}	323.55 ±14.24^{e}	244.90 ±5.14^{b}	200.25 ±3.60^{a}
Mass fraction of amino acids, %					
Arginine	0.44 ±0.18^{a}	0.49 ±0.20^{b}	0.54 ±0.22^{c}	0.60 ±0.24^{d}	0.46 ±0.18^{a}
Lizine	0.50 ±0.17^{d}	0.49 ±0.17^{d}	0.35 ±0.12^{a}	0.45 ±0.15^{c}	0.40 ±0.14^{b}
Tyrosine	0.14 ±0.04^{a}	0.11 ±0.03^{a}	0.17 ±0.05^{a}	0.13 ±0.04^{a}	0.16 ±0.05^{a}
Phenylalanine	0.26 ±0.08^{a}	0.23 ±0.07^{a}	0.17 ±0.05^{a}	0.20 ±0.06^{a}	0.22 ±0.06^{a}
Histidine	0.07 ±0.03^{a}	0.10 ±0.05^{a}	0.13 ±0.06^{a}	0.20 ±0.10^{b}	0.19 ±0.09^{a}
Leucine+isoleucine	0.39 ±0.10^{c}	0.38 ±0.10^{c}	0.24 ±0.06^{a}	0.32 ±0.08^{b}	0.33 ±0.09^{b}
Methionine	0.21 ±0.07^{b}	0.20 ±0.07^{b}	0.15 ±0.05^{a}	0.20 ±0.07^{b}	0.17 ±0.06^{a}
Valine	0.41 ±0.16^{e}	0.39 ±0.16^{d}	0.22 ±0.09^{a}	0.32 ±0.13^{b}	0.36 ±0.14^{c}
Proline	0.28 ±0.07^{b}	0.30 ±0.08^{c}	0.15 ±0.04^{a}	0.20 ±0.05^{b}	0.27 ±0.07^{b}
Treoline	0.33 ±0.13^{c}	0.32 ±0.13^{c}	0.15 ±0.06^{a}	0.22 ±0.09^{b}	0.26 ±0.11^{b}
Serine	0.24 ±0.06^{b}	0.28 ±0.07^{c}	0.16 ±0.04^{a}	0.22 ±0.06^{b}	0.23 ±0.06^{b}
Alanine	0.42 ±0.11^{c}	0.45 ±0.12^{c}	0.26 ±0.07^{a}	0.37 ±0.10^{b}	0.40 ±0.10^{c}
Glycine	0.44 ±0.15^{c}	0.39 ±0.13^{b}	0.20 ±0.07^{a}	0.27 ±0.09^{a}	0.43 ±0.15^{c}

Note: $^{a-e}$ means within the same row with different uppercase letters differing significantly among different meat samples (p <0.05). All values are expressed as the mean ±SD (standard deviation).

Figure 4 Semi-finished fish products "fish balls".

CONCLUSION

Thus, a comparative analysis of the content of essential amino acids in the muscle tissue of fish in the inland waters of the Republic of Kazakhstan with some oceanic and marine fish showed that the content of amino acids such as leucine, lysine, threonine, phenylalanine is slightly higher. They are characterized by a high content of essential amino acids limiting the biological value, g/100 g of protein: lysine 8.8-11.6; methionine 2.1-3.1; tryptophan 1.0-1.1. The data analysis shows that a higher pH value of fish meat corresponds to a higher elasticity value. The pH shift to the alkaline side of more than 7.5, although it promotes the release of myosin, reduces the elasticity of meat. The maximum moisture binding ability and pH are noted when functional ingredients are added to minced fish in an amount of 30%. Moisture binding ability was 48.6% and pH 6.67 when replaced with kelp and 49.27 and 6.04, respectively, when replaced with pumpkin. The development of semi-finished fish products with the use of plant components makes it possible to obtain products of high biological value with a juicy consistency, which meets modern trends in healthy nutrition.

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The effect of functional bars on the biochemical parameters of blood during physical exertion

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ABSTRACT

Currently, the fast pace of life, changes in consumers' habits, and the trend toward a healthy lifestyle around the world create the need for new healthy foods for immediate consumption. In this regard, the production of snacks with high nutritional value, as well as giving them functional properties through the use of various types of raw materials, seems promising. The choice of components following the physiological needs of various population groups in snack production allows getting new specialized food products, which improves people's quality of life and health. The most promising is the production of functional snack bars by combining vegetable and dairy raw materials. It is promising to use mare's milk as a dairy raw material. The clinical efficacy of the new fruit-protein bars based on mare's milk (FBBs) was tested on 25 volunteers aged 25-35 years for 60 days. The control group consisted of 15 people who received two KDV fruit and nut bars for 60 days, the nutritional and energy value of which was comparable to the snacks studied. Before and after taking snacks, blood biochemical and immunological parameters were evaluated. The results indicate a positive effect of new snacks based on mare's milk on performance indicators, the state of cellular immunity, blood parameters, biochemical parameters, and antioxidant status. There is a decrease in fat mass, intracellular and extracellular fluid levels, and a reducing the number of final and intermediate lipid peroxidation products. The blood level of haemoglobin, erythrocytes, platelets, hematocrit, and all classes of immunoglobulins increased. Specialized functional protein bars, enriched with dry mare's milk, can be recommended to various categories of the population experiencing intensive physical activity and psychoemotional stress to increase their adaptive capabilities and performance.

Keywords: specialized nutrition, functional protein bars, mare's milk, physical activity, adaptive capabilities

INTRODUCTION

To date, there is an increase in the use of snacks (ready-to-eat light meals intended for a "snack") around the world, which is caused by the acceleration of the pace of life and changes in the culture of consumption of various goods **[1]**. The choice of snack products is becoming more and more diverse every year: these are crispy crackers made of wheat and rye bread, sweet and salty straws, all kinds of nuts and seafood that have undergone appropriate processing, instant porridge from various kinds of cereal, potato chips and other mouth-watering delicacies. The largest segment of the Kazakhstan snack market is the category "crackers", which accounts for 51% of the market in physical terms, as well as chips – 25% and nuts – 24% **[2]**, **[3]**.

In the rational and balanced nutrition system, a person needs two or three snacks during the day between main meals. Each meal should have an individual combination of proteins, fats, carbohydrates, and dietary fiber with the specified parameters of caloric content and antioxidant activity **[4]**, **[5]**, **[6]**. In turn, most snack products are characterized by high-calorie content and minimal content of vitamins and trace elements. They often have increased levels of oil and sugar, which causes obesity, hypertension, etc. Despite this, the market of snack products is actively developing and has a high investment attractiveness **[1]**.

The COVID-19 pandemic has seriously changed the population's eating behavior. Consumers are paying more attention to the general state of health, and people are looking for products with clean labels, which means that

they are more and more selective in choosing products. Demand is rapidly shifting away from fatty-spicy foods towards healthier, sugar-free, and low-calorie snacks packed in small portions. This is an important trend that stimulates the growth of the industry. In this regard, many scientific research works aim to increase snack products' nutritional value and give them functional properties with various raw materials (grain, fruit and vegetable, dairy, nuts, probiotics, etc.) **[1]**.

A review of the global and domestic market of functional snack products indicates the prospects for developing this direction in Kazakhstan. The global functional snacks market volume in 2021 was estimated at 85.6 billion US dollars. The cumulative annual growth rate (CAGR) is expected to be 6.6% from 2022 to 2030 **[1]**. In Kazakhstan, more and more people adhere to a healthy lifestyle, encouraging them to gradually switch from traditional chocolate bars to healthier alternatives containing cereals, fruits, and muesli. The domestic market for functional snacks is not yet large compared to the traditional one, but it is growing steadily. The annual consumption of snacks and fruit and vegetable-based bars has approached a 10% increase, and we expect this trend to continue **[7]**.

Snacks with different functional properties are prospects for improving the quality of life and health of the population of Kazakhstan. The selection of various components by the physiological needs of various groups of the population in the production of snacks allows you to get new specialized food products.

It is known that by the Technical Regulations of the Customs Union TR CU 027/2012 "On the safety of certain types of specialized food products, including dietary preventive and dietary therapeutic nutrition", all specialized food products are divided into the following groups:

- food products for baby food;
- food products for athletes' nutrition;
- food products for dietary preventive nutrition;
- food products for dietary therapeutic nutrition;
- food products for pregnant and lactating women.

The design of specialized snack products requires a scientifically based approach to selecting ingredients, considering the target consumers' needs. The choice of the composition of specialized snacks should be determined by several factors: age needs at the group level for food and biologically active substances (micronutrients), physical activity, or energy consumption (associated in the case of sports nutrition - with a sport, under difficult working conditions – with the peculiarities of harmful production, for dietary preventive or dietary therapeutic nutrition – with the nature of the disease). However, the key factor in selecting a scientifically based composition of specialized snacks is the features of the diet, allowing for the achievement of maximum efficiency of new products. When scientifically substantiating the composition of certain types of specialized snack products, it is necessary to consider the anthropometric characteristics of target consumers (Figure 1).

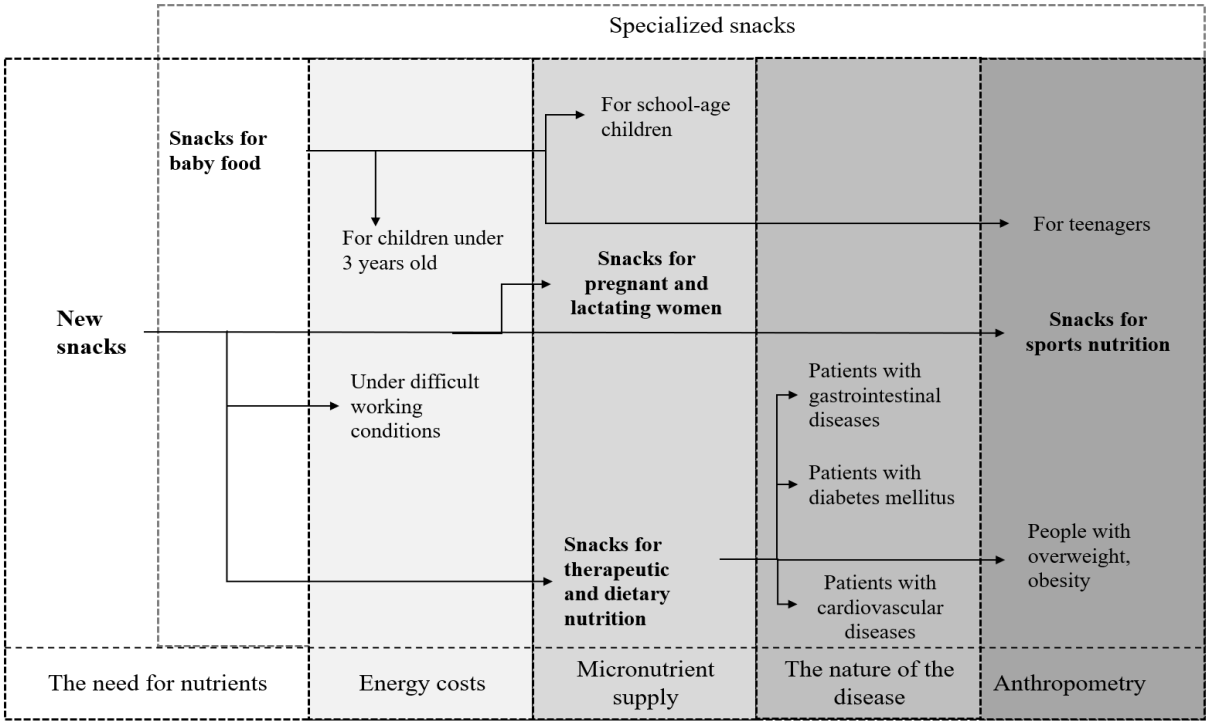

Figure 1 Specialized snacks for feeding target groups of consumers.

Snacks for sports nutrition are often presented in the form of bars. At the same time, research aimed at developing new products for sports nutrition indicates the prospects of using plant raw materials.

One of the promising directions for the development of the range of snack bars is the development of bars based on grain, dairy, and fruit and vegetable raw materials, which corresponds to the principles of healthy nutrition **[8]**, **[5]**, **[9]**, **[10]**. Developing technologies and recipes for new bars will make it possible to obtain high-quality products enriched with useful biologically active substances and low energy value. If necessary, such bars can be stored for 12 months or more. Expanding the range of snack bars also makes it possible to increase the possibilities of using local dairy, grain and fruit, and vegetable raw materials.

Bars from dairy, grain and fruit, and vegetable raw materials are rich in dietary fibers (pectin, hemicellulose, cellulose), minerals, macronutrients (potassium, sodium, magnesium, calcium, phosphorus), trace elements (iron, zinc, copper, manganese), the functional properties of which meet the requirements of preventive nutrition. New types of snacks can be used as biologically active food additives, as they have increased nutritional and biological value and good consumer and technological properties **[8]**, **[5]**, **[9]**, **[10]**.

Recently, dry mare's milk obtained by freeze-drying has become much more widely used for technological purposes since it has a higher content of the main biologically active ingredients than native milk **[11]**, **[12]**. Mare's milk is characterized by high lactose, low fat, and protein content, especially casein. The uniqueness of the mare's milk is also due to the high level of low molecular weight peptides and lysozyme, which is one of the main antibacterial proteins of milk and plays an important role in the formation of nonspecific immunity **[13]**, **[14]**.

According to literature data, low-molecular-weight peptides of mare's milk with a molecular weight of up to 15 kDa can maintain body homeostasis, showing antibacterial, antiviral, antioxidant, and regenerative activity **[15]**, **[16]**.

The production of specialized bars with new functional properties through the introduction of mare's milk will allow the market to release products that positively affect biochemical and immunological indicators, performance indicators, and the state of the antioxidant status of the body.

Scientific hypothesis

Snacks in the form of fruit-protein bars (FBBs) based on mare's milk positively affect biochemical and immunological indicators, performance indicators, and the state of the body's antioxidant status. We expect increased haemoglobin, erythrocytes, and immunoglobulins level and a decrease in malondialdehyde's and diene conjugates' levels.

MATERIAL AND METHODOLOGY

Samples

In clinical trials, fruit and protein bars based on mare's milk were used.

Instruments

The volunteers' body composition was studied on the InBody 770 analyzer (South Korea).

Biochemical blood tests of volunteers were performed on the ARCHITECT c8000 analyzer, manufactured by Abbott.

A full blood count (FBC) was completed on a UNICEL DXH-800 haematological analyzer, manufactured by Beckman Coulter.

Laboratory Methods

The InBody 770 analyzer was used to determine: the total amount of water, proteins, minerals, the ratio of muscle and fat mass, fat content, body mass index (BMI), weight, and skeletal muscle mass.

The immuno-chemiluminescent method was used in the study of blood biochemical parameters. Biochemical and immunological parameters were evaluated in the blood of the subjects before and after taking snacks: total protein, albumin, globulins, albumin-globulin ratio, the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, iron in serum, immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM) **[17]**.

The colorimetric method was used to determine the lipogram (Cholesterol, HDL, LDL, triglycerides, atherogenicity coefficient) **[18]**.

The level of glucose, pyruvic, and lactic acid was determined to assess the direction of bioenergetic processes. The state of lipid peroxidation (LP) was determined by the serum levels of diene conjugates (DC), malondialdehyde (MDA), as well as catalase activity **[19]**, **[20]**.

According to Mancini, the level of immunoglobulins in blood serum was determined by radial immunodiffusion **[21]**.

A detailed general blood test (erythrocytes, haemoglobin, leukocytes, platelets, ESR, reticulocytes, hematocrit, ferritin) was analyzed using accepted clinical research methods **[17]**, **[22]**.

Description of the Experiment

The clinical efficacy of FBB was assessed on 25 volunteers aged 25-35 years, who received two FBBs daily for 60 days. The recipe for the FBB snacks is given in Table 1. The energy value of bars per 100 g of product is proteins – 7.4 g, fats – 5.8 g, carbohydrates – 68.0 g, caloric content – 365.0 kcal.

Table 1 FBB recipe for 100 g of product.

Ingredients	Quantity, g
Dates	31.66
Sunflower seeds	14.01
Dried apples	13.15
Cranberry	12.38
Dry protein mixture:	10.0
Mare's milk	6.5
Fruit additives (dry currant berries)	1.0
Low molecular weight peptides from mare's milk	1.0
Soy Protein Isolate	0.5
Pectin	0.1
Maltodextrin	0.23
Inulin	0.1
Bacterial starter culture	0.5
Vitamin and mineral premix	0.05
Fucoidan	0.02
Raisin	9.78
Pumpkin Seeds	8.25
Almond	0.77

FBB photos are provided in Figure 2.

Figure 2 Fruit-protein bars (FBBs) based on mare's milk.

The control group consisted of 15 people who received two KDV fruit and nut bars for 60 days, the nutritional and energy value of which was comparable to the snacks studied. Fruit and nut bars of the "KDV" company (Konditerskij Dom Vostok - Confectionery house Vostok) contain 20.0 g of protein, 17.0 g of fat, and 47.0 g of carbohydrates, with a calorie content of 370 kcal.

The composition of the fruit and nut bars of the "KDV" company: chocolate glaze (granulated sugar, cocoa butter equivalent (refined deodorized vegetable oils (palm, shea), emulsifier – sunflower lecithin), cocoa mass, cocoa powder, cocoa butter, emulsifiers (soy lecithin, E476), vanilla flavor), dried apricots (apricots, preservative

E220), whey protein concentrate, dried grapes (raisins), roasted crushed peanut kernels, granulated sugar, dried bananas, passion fruit granules (passion fruit juice concentrate, corn starch), starch molasses, acidity regulator - citric acid, vegetable extract "Guarana extract" (natural guarana extract, maltodextrin, stabilizer -gum arabic, natural caffeine), flavor "Apricot", roasted crushed hazelnut kernels, vegetable extract "Apricot extract".

The basic volunteers' diet was balanced by the main nutrients and amounted to 2760 kcal (Table 2). In terms of calories, the additional nutrition of the experimental and control groups was identical. The caloric content of additional nutrition due to the bars was about 730-740 kcal in the experimental and control groups.

Table 2 The content of nutrients in the volunteers' diet.

Nutritional substances	The content of nutrients in the diet of volunteers
Energy, kcal	2760
Protein calories, %	15
Total Protein, g	104
Animal proteins, g	58
% animal proteins in total	55.8
Fat calories, %	32.7
Total fat, g	100
Vegetable fat, g	46.2
% vegetable fat in total	46
Saturated Fatty Acids (SFA), g	25.1
MUFAs, g	22.1
PUFAs, g	24.8
Cholesterol, mg	439
Carbohydrate calories, %	52.2
Total carbohydrates, g	360
Starch, g	221
Mono- and disaccharides, g	51
Dietary fiber, g	38.6
% of sugars from total carbohydrates	42
Vitamin A (RE), mcg	1319
Vitamin E, mg	26
Vitamin B1, mg	1.5
Vitamin B2, mg	1.72
Vitamin B3 (niacin), mg	18.6
Vitamin C, mg	123
Folate (folic acid), mcg	187
Calcium, mg	957
Magnesium, mg	457
Phosphorus, mg	1756
Iron, mg	25
Zinc, mg	10
Selenium, mcg	29.7
Iodine, mcg	127

Both the persons of the experimental and control groups were monitored for 60 days, receiving the recommended products daily as part of the diet. The composition of the FBB can have a positive effect on human endurance, so it was decided to introduce physical activity into the program of the experimental group: running, swimming, and cycling. The control group was not subjected to physical exertion. Before and after taking FBB, the body composition and biochemical and immunological parameters of the volunteers' blood were evaluated. Also, throughout the 60-day follow-up, volunteers were interviewed about their perception of products, the effect of products on the state of the gastrointestinal tract, and general well-being. All persons in the control and

experimental group agreed to participate in the studies. Blood sampling was performed on an empty stomach in the morning (9:00 am).

Statistical Analysis

The results were statistically processed using the XLSTAT program, calculating the arithmetic mean of the parameter, the mean square deviation, and the error of the arithmetic mean. The differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

The clinical efficacy of FBB was tested on a group of 25 volunteers aged 25-35 years. The analysis of body composition in the experimental group of individuals who received two FBB daily (Table 3) showed a significant decrease in the total water content in the body, as well as intracellular fluid from 25.0 ±0.4 l to 23.2 ±0.5, ($p \leq 0.05$). The protein content after taking the bars increased significantly from 10.2 ±0.5 to 11.5 ±0.4 kg. Although the difference was only 1.3 kg, this is considered a good result, which indicates the normalization of protein metabolism. The content of minerals in the body of volunteers significantly increased from 3.3 ±0.1 kg to 4.1 ±0.2 kg.

Table 3 Analysis of the body composition of the experimental group (n = 25) before and after FBB (M ±m) consumption.

Indicators	Before reception	After reception
Body weight	73.1 ±1.3	74.4 ±1.4
Total water content	39.0 ±0.6	36.2 ±0.7*
Intracellular fluid	25.0 ±0.4	23.2 ±0.5*
Extracellular fluid	14.0 ±0.2	13.0 ±0.3
Protein content	10.2 ±0.5	11.5 ±0.4*
Mineral content	3.3 ±0.1	4.1 ±0.2*
Body fat content	19.9 ±0.7	15.5 ±1.1*
Non-fat body mass	53.6 ±1.0	56.7 ±0.8*
Skeletal muscle mass	29.0 ±0.6	31.6 ±0.7
BMI	25.9 ±0.5	21.4 ±0.6*
Body fat percentage	27.2 ±0.9	20.8 ±1.0

Note: * – the differences are statistically significant to the data in the control group $p \leq 0.05$.

As a result of the 60-day intake of bars, there is a decrease in fat mass and the level of intracellular and extracellular fluid. Fat mass decreased by 23.5% from 19.9 ±0.7 kg to 15.5 ±1.1 kg. In studies, the fat-free body weight significantly increased in volunteers after 60 days of food intake from 53.6 ±1.0 kg to 56.7 ±0.8 kg, indicating a partial replacement of metabolically inactive tissues with active, particularly skeletal muscles and muscles of internal organs. The mass of skeletal muscles in volunteers after taking the product increased by an average of 9.0% – from 29.0 ±0.6 kg to 31.6 ±0.7 kg, which also indicates an increase in the degree of endurance of the body to physical exertion. According to Table 2, a significant decrease in body mass index (BMI) is important in volunteers from 25.9 ±0.5 to 21.4 ±0.6, ($p \leq 0.05$).

A significant decrease in the volunteers' total body water content, including the content of the intracellular and extracellular fluid, as well as an increase in protein levels, fat-free body weight, and skeletal muscle mass after using mare's milk bars indicates normalization of protein metabolism and synthesis of new structural proteins and tissues, replacement of metabolically inactive tissues with active components. A significant increase in the levels of mineral substances in the body of volunteers indicates the correction of violations of trace element metabolism and fluid metabolism.

Changes in body composition indicators in the control group after taking KDV fruit and nut bars were insignificant (Table 4).

Protein content increased by 7.6%, minerals – by 10.5%, fat – by 9.6%, and skeletal mass increased by only 2.8%. There is an increase in the total water content by 2.9% with an increase in the amount of intracellular fluid and a decrease in the extracellular fluid content. All of the above indicators were not reliable. The percentage of fat and body mass index increased.

Table 4 Analysis of the body composition of the control group (n = 15) before and after consumption of KDV fruit and nut bars (M ±m).

Indicators	Before reception	After reception
Body weight	72.5 ±1.0	76.6 ±1.2
Total water content	38.5 ±0.7	39.6 ±0.9
Intracellular fluid	27.0 ±0.5	28.8 ±0.8
Extracellular fluid	11.5 ±0.2	10.8 ±0.4
Protein content	11.8 ±0.6	12.7 ±0.6
Mineral content	3.8 ±0.3	4.2 ±0.4
Body fat content	21.8 ±0.8	23.9 ±1.5
Non-fat body mass	52.6 ±0.9	53.9 ±1.3
Skeletal muscle mass	25.3 ±0.5	26.0 ±0.7
BMI	26.7 ±0.6	27.8 ±0.7
Body fat percentage	30.1 ±0.9	31.2 ±1.2

The results of biochemical studies are presented in Table 5.

Table 5 Changes in biochemical blood parameters of the experimental group (n = 25) before and after taking FBB (M ±m).

Indicator Unit of measurement	Before reception	After reception
Total protein g/l	70.42 ±1.01	75.65 ±0.75*
Albumins %	45.70 ±0.72	49.70 ±0.55*
Globulins %	24.72 ±1.00	25.95 ±10.95
Albumin-globulin coefficient	1.85 ±0.10	1.92 ±0.21
Blood sugar mmol/l	5.60 ±0.21	5.20 ±0.14
Cholesterol mmol/l	4.05 ±0.40	3.80 ±0.35
Triglycerides mmol/l	0.83 ±0.08	0.84 ±0.09
HDL mmol/l	1.55 ±0.10	1.80 ±0.10
LDL mmol/l	2.7 ±0.20	1.9 ±0.20*
Atherogenicity coefficient	1.62 ±0.21	1.11 ±0.10
Iron mmol/l	19.60 ±0.20	22.5 ±0.43*
Total iron binding capacity mmol/l	48.5 ±3.20	58.6 ±4.30
Ferritin ug/l	215.36 ±20.31	454.29 ±31.44*

Note: * – the differences are statistically significant to the data in the control group $p \leq 0{,}05$.

As can be seen from the data presented in Table 3, after taking bars from volunteers after 60 days, there was a significant increase in total protein and albumin in the blood. The albumin index increased by an average of 4% compared to the data before taking the bars. The globulin index also increased by an average of 5% after taking the product.

The data obtained indicate the beneficial effect of bars on the state of protein metabolism in general and confirm the results of increasing the level of proteins in the body.

In the control group, there were practically no significant changes in the above indicators. There was a slight decrease in total protein and albumin in the blood serum, but it should be noted that these shifts were also not reliable.

Along with changes in the protein composition of blood serum indicators, after taking FBB, a decrease in blood sugar, total cholesterol, low-density lipoproteins, and atherogenicity coefficient was revealed by 7.1; 6.1; 26.9, and 31.5%, respectively. A decrease in low-density lipoproteins and atherogenicity coefficient against the background of a general decrease in cholesterol should be considered a positive result of the effect of FBB on lipid metabolism. This may be due to an increased intake of PUFA, anti-oxidant vitamins, and reduced content of animal fats in the diet with a high atherogenicity index (Table 3).

An increase in the blood level of iron and the iron-binding ability of blood serum, as well as a significantly increased level of ferritin, indicate the anti-anemic nature of FBB. This is due to the increased content of macro- and microelements, readily available proteins, vitamin C, folic acid, and B vitamins in the bars, which favorably affect the processes of hematopoiesis.

A decrease in the activity of transamination enzymes in the blood serum of the experimental group after 60-day consumption of FBB (Table 6). Thus, the activity of alanine aminotransferase and aspartate aminotransferase after taking the products decreased by 18.2 and 25.7%, respectively, but these changes were insignificant.

Table 6 Enzymatic activity in blood serum in the experimental group (n = 25) before and after taking FBB (M ±m).

Indicator Unit of measurement	Before reception	After reception
Alanine Aminotransferase (ALT) U/l	30.29 ±3.54	24.79 ±2.34
Aspartate Aminotransferase (ACT) U/l	41.49 ±9.43	30.81 ±2.57

Note: * – the differences are statistically significant to the data in the control group $p \leq 0,05$.

In the control group (n = 15), there was also no significant change in the activity of the above serum enzymes, but a decrease in the above enzymes by 4.2 and 6.3% were also detected for ALT and AST, respectively.

The assessment of hematological blood parameters before and after taking the products by the control group revealed certain shifts in the blood formula in comparison with the data before taking them (Table 7). As can be seen from the data presented in Table 7, volunteers had an unreliable increase in the blood level of hemoglobin, erythrocytes, platelets, and hematocrit after taking the products.

Table 7 Enzymatic activity in blood serum in the experimental group (n = 25) before and after taking FBB (M ±m).

Indicator Unit of measurement	Before reception	After reception
Hemoglobin, g/l	146.10 ±4.34	149.61 ±3.63
Platelets, 10^9, g/l	261.51 ±8.46	266.45 ±8.25
Red blood cells, 10^{12}, g/l	4.75 ±0.09	4.88 ±0.09
Hematocrit, g/l	0.42 ±0.01	0.46 ±0.009

The data obtained indicate an increase in the hematopoietic function of the volunteers' bodies against the background of taking FBB. This is due to the presence in the bars of high-grade protein, iron, vitamin C, folic acid, and B vitamins that stimulate the processes of hematopoiesis.

It is known that any physical activity accompanied by certain psycho-emotional stress leads to the initiation of free radical oxidation of lipids in the body **[23]**. With an increase in physical and psychological stress, the processes of lipid peroxidation (LP) intensify **[24]**, which can be demonstrated by increasing the level of primary and final products of lipid peroxidation and changing the activity of key enzymes of the antioxidant system **[25]**.

Pro- and antioxidant defence systems in a healthy body control the level of free radical formation and maintain the antioxidant status at a certain stationary level. Depending on the state of the body, and the influence of adverse environmental factors, the antioxidant defence (AD) system can regulate the formation and destruction of free radicals **[26]**.

Activation of LP is observed only at maximum loads, which is manifested in an increase in the content of LP products in the blood serum **[26]**, as well as a decrease in the functional activity of the antioxidant defence system **[27]**.

Considering the above, along with the assessment of biochemical parameters of blood, we studied the state of the LP-AD system in blood serum against the background of taking specialized food. The data obtained are shown in Table 8.

Table 8 Dynamics of changes in indicators of lipid peroxidation and enzymes of antioxidant protection systems in blood serum in the experimental and control groups before and after taking specialized foods (M ±m).

Period		DC mmol/l	MDA mmol/l	Serum catalase mmol/min/l
The experimental group (n = 25)	before reception	72.0 ±4.2*	42.6 ±3.3*	0.41 ±0.03*
	after reception	41.3 ±3.5	15.6 ±1.9	0.25 ±0.04
Control group (n = 15)	before reception	74.8 ±5.5	45.5 ±4.3	0.39 ±0.05
	after reception	64.3 ±3.4	36.1 ±3.4	0.29 ±0.02

Note: * – the differences are statistically significant to the data before and after taking the products $p \leq 0.05$.

As seen from the data given in Table 7, there was a significant increase in serum levels of malondialdehyde (MDA) and diene conjugates (DC) in the control group (persons who are not subjected to physical exertion) before taking specialized foods. Catalase activity also significantly increased 2.7 times.

Consumption of FBB for 60 days against the background of physical activity contributed to a decrease in the serum of the experimental group of final and intermediate lipid peroxidation products. In particular, the level of MDA and DC decreased by 38.7 and 24.6%, respectively, compared with the data before taking FBB. Catalase activity also decreased by 39.0%, compared with the results obtained at the beginning of the experiment.

The results of changes in the indicators of the LP-AD system indicate a specialized product's favorable effect on the body's antioxidant status.

The excessive accumulation of lipid peroxidation products revealed during experimental studies may indicate a decrease in the activity of the antioxidant defense system.

The results obtained, related to the assessment of physical performance in the experimental group and the indicators of the LP-AD system in the blood, differ from the results of the control group who did not exercise.

An equally important characteristic in assessing the impact of specialized products on the human body is the assessment of their immune status, which characterizes the state of the body's defence system **[30]**. Thus, after exhausting physical exertion, according to the available literature data, there is an increase in the number of leukocytes **[28]**, **[29]**, **[30]**. At the same time, there are a decrease in leukocyte functional activity and NK-cell depression **[31]**, and the number of T-lymphocytes and the level of immunoglobulins reduces **[32]**. Dopsaj et al. noted that people undergoing extreme physical activity could suffer from short-term immunosuppression and increased infection risk **[33]**.

Evaluation of the cellular and humoral components of the immune system allowed us to identify several specific changes after taking FBB by the experimental group (Table 9).

Table 9 Changes in immunological parameters in the experimental group (n = 25) before and after taking the special product (M ±m).

Indicator Unit of measurement	Before reception	After reception
Immunoglobulin G (IgG) g/l	10.87 ±0.57	11.11 ±0.50
Immunoglobulin A (IgA) g/l	2.10 ±0.18	2.28 ±0.16
Immunoglobulin M (IgM) g/l	1.22 ±0.11	1.78 ±0.12*

Note: * – the differences are significant when compared with the data before and after taking FBB.

The baseline data for IgG, IgA, and IgM immunoglobulins in the control group who did not experience physical activity were 9.01 ±0.78; 1.78 ±0.14; 1.01 ±0.10 g/l, respectively. The results obtained indicate that the state of humoral immunity is related to the intensity of physical exertion on the one hand, and on the other hand, the role of enriching the daily diet of volunteers with missing nutritional factors that determine resistance and endurance to increased physical exertion.

An increase in the level of all classes of immunoglobulins characterizing the state of humoral immunity indicates a good adaptation of the immune system. Repeatedly in scientific works, there has been a connection between a decrease in the indicators of humoral factors of immunity against the background of intense physical activity **[34]**, **[35]**. This led to an increase in the development of acute diseases **[36]**. It can be considered a syndrome of immune dysfunction **[37]**.

The contradictory information available in the scientific literature regarding the effect of physical activity on the immune system depends on the body's individual reaction, the type of physical activity, age, and other factors and conditions in which the state of immunity was assessed **[38]**. Scientific evidence indicates that intense physical

activity has a depressing effect on the immune system **[39]**. Thus, Suzdalnitsky et al., studying the mechanisms of the development of immunodeficiency under intense loads, observed an increase in disorders in the cellular, humoral, and secretory links of immunity **[40]**. The most significant instability of humoral immunity as a consequence of various types of stress, including increased physical and psycho-emotional stress, was also noted in the works of other authors. The reduction of immunological resistance under intense psychophysical stress is based on disorders of neuroendocrine regulation, macro-, and micronutrient insufficiency, metabolic changes in the internal environment, and intoxication from foci of chronic infection **[41]**. It was noted that short-term stressors suppressed cellular immunity while maintaining humoral immunity. Meanwhile, chronic stressors were associated with suppressing both cellular and humoral indicators **[42]**.

The most significant immune disorders are observed with physical and psychoemotional loads **[43]**, hypothermia **[44]**, and disruption of adaptive mechanisms. Suzdalnitsky et al. stated that the human immune system should go through the phases of mobilization, compensation, decompensation, and recovery when adapting to physical exertion **[45]**. The earliest reflection of the breakdown of adaptation processes to physical and psychoemotional stress violates immunity **[46]**. Researchers agree that it is advisable to include dietary supplements in the diet of people subjected to constant physical exertion **[47]** and psychoemotional stress **[48]** to increase their physical endurance and mental stability.

When performing aerobic exercise (1000 meters) in the experimental group, after using FBB, there was a decrease in the concentration of lactic acid in the blood plasma by 23.1% compared to the initial data. After a two-month intake, against the background of this stress test, the volunteers showed a decrease in lactic acid levels by 40.7%, compared with the initial data. The content of pyruvic acid has not changed significantly. On average, the decrease in the level of pyruvate was in the range of 6.7-10.7% before and after taking FBB against the background of a stress test (Table 10). It should be noted that the changes in the concentration of lactate and pyruvate in the blood serum were not insignificant in the control group.

Table 10 Changes in the level of lactic and pyruvic acids before and after taking bars in blood plasma after a stress test (M ±m).

Indicator Unit of measurement	Control group (n = 15)		An experienced group (n = 25)	
	Before reception	After reception	Before reception	After reception
Lactate (mmol/l)	2.8 ±0.3	2.5 ±0.2	2.6 ±0.2	2.0 ±0.3
Pyruvate (ml/l)	0.032 ±0.01	0.030 ±0.03	0.030 ±0.01	0.028 ±0.02

The changes obtained indicate the activation of oxidative processes in all subjects after taking specialized products, while the changes in the experimental group were expressed to a significant extent.

Regular consumption for 60 days of FBB contributed to improving the performance and the anaerobic and aerobic links of the volunteers' energy system.

The results of the clinical evaluation of the effectiveness of FBB on the biochemical and immunological parameters of the volunteers' blood indicate an increase in antioxidant and immune statuses against the background of taking bars. The noted positive changes in blood parameters (the content of ferritin, serum iron, and the iron-binding ability of blood serum) indicate the antianemic properties of the product and its normalizing effect on the hematopoietic functions of the body.

Taking into account the beneficial effect of FBB on performance indicators, indicators of cellular immunity, and antioxidant status, this specialized nutrition can be recommended to various categories of the population experiencing increased physical activity to improve performance.

CONCLUSION

The results of the clinical evaluation of the effectiveness of FBB indicate their positive effect on performance indicators, the state of cellular immunity, blood parameters, biochemical parameters, as well as the antioxidant status of the body. There is a decrease in fat mass, intracellular and extracellular fluid levels, and a reducing the number of final and intermediate lipid peroxidation products. The blood level of hemoglobin, erythrocytes, platelets, hematocrit, and all classes of immunoglobulins increased. Considering the above, FBB with increased nutritional and biological value, enriched with unique ingredients, including dry mare's milk, can be recommended to various categories of the population experiencing intensive physical activity and staying in extreme environmental conditions to increase their adaptive capabilities and performance. The expansion of the range of snack products and the production and distribution of specialized snacks are seen as a promising direction and have social significance since it allows for improving the quality of life and health of people.

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The quality characteristics of biscuits made with plantain and purple rice flour as substitutes for wheat flour

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ABSTRACT

Biscuits are wheat flour-based manufactured food products. Another option is to locate a flour substitute, such as plantain flour or purple rice. This study aims to establish the ideal ratio of purple rice flour and plantain flour based on the quality attributes of biscuits. This study employed a one-factor, Completely Randomized Design (CRD) with five treatment levels and three replications. The observational data were analysed using ANOVA with the DNMRT further test at a 5% significant level. The treatment in this study compared purple rice flour and plantain flour to prepare biscuits. The ratio of purple rice flour to plantain flour had a very significant ($p < 0.01$) effect on water content (3.56%), ash content (2.11%), fat content (25.18%), crude fiber content (17.85%), protein content (4.72%), and carbohydrate content (61.49%), but no significant effect ($p > 0.01$) on antioxidant activity (55.83%). Except for protein, all treatments meet the SNI's requirements for biscuit quality. Based on the organoleptic test of taste, aroma, texture, and colour preferred by panellist with score of A (90-10) 3.52%, B (80-20) 3.97%, C (70-30) 4.42%, D (60-40) 5.03%, and E (50-50) 5.52% were obtained. The best-quality biscuits were in treatment E. (comparison of purple rice flour and plantain flour 50:50).

Keywords: biscuit, flour, plantain fluor, purple rice fluor, subsitution

INTRODUCTION

Biscuits are wheat flour-based manufactured food products. According to **[1]** biscuits are items made by baking dough made from wheat flour with or without the use of approved food additives. Biscuits are a type of snack that is commonly enjoyed in society. This is a dry product with a low water content. According to **[2]**, based on industry association data, biscuit consumption is expected to rise by 55-58% in 2012, owing to an increase in domestic consumption. Biscuits are enjoyed by people of all ages, including infants and adults, although in varying forms **[3]**.

The majority of biscuits on the market are made with wheat flour as the primary ingredient. Biscuits are made with wheat flour that has a low protein level. Non-wheat flour is now being researched for usage in the production of biscuits, particularly gluten-free biscuits **[4]**. As a result, several efforts are being undertaken to replace wheat flour with flour derived from local resources, such as tubers, seeds, and fruits, including purple rice and plantains.

Rice (*Oryza sativa* L.) is a food crop grown in underdeveloped nations as a staple diet or source of carbohydrates **[5]**. Rice comes in many different kinds: white, brown, black, and purple.

Purple rice with coloured grains has long been a unique and traditional dish in many cultures for desserts and medical purposes **[6]**. Today, the benefits of pigmented rice are generally known, and it is employed in commercial food production as well as dietary supplements, cosmetics, and medications **[7]**. Coloured rice is high in phenolic compounds. Flavonoid chemicals are one type of phenolic compound that has antioxidant properties **[8]**.

Because wheat flour is used as the principal basic ingredient in many processed food products in Indonesia, the country's reliance on wheat flour imports is growing. This can be decreased by using locally grown foods

such as plantains. One of the wild plantains is the plantain (*Musa balbisiana*), which is diploid **[9]**. While the plantain plant (*Musa balbisiana*) has numerous advantages, one is that its sap contains antioxidant chemicals, one of which can lessen the incidence of Alzheimer's **[10]**. Plantains have a rather high starch content, over 90% **[11]**. Plantain is suited for processing flour due to its high starch content. Plantain can be used as flour when the fruit is not mature, and the skin colour is still green due to the high starch and non-starch polysaccharides **[12]**. The benefit of processing into flour is that it has a longer shelf life and is more practical when used to produce other food products. Plantains and seeds have a relatively high mineral content (in ppm), including calcium, magnesium, potassium, sodium, manganese, and phosphorus **[13]**.

Figure 1 and 2 represents the plantain flour and purple rice powder used in our research. The final product of the biscuit is present in Figure 3.

Figure 1 Plantain flour.

Figure 2 Purple rice powder.

Scientific hypothesis

This research investigated the effect of utilizing plantain flour as an alternative raw material to wheat flour in the production of biscuits. The addition of plantain flour will greatly reduce reliance on wheat flour while also enhancing the economic worth of plantains. It can also determine the influence of the ratio of purple rice flour to plantain flour on the quality of biscuits and comparing purple rice flour and plantain flour in manufacturing biscuits that consumers enjoy. This hypothesis is supported by research conducted by **[9]** on the ratio of wheat flour (30% plantain flour) which is the most preferred by the panelists, and it is also known that the resistant starch content of plantain flour is higher (39.35%) than other types of bananas, implying that plantain has a great opportunity to be processed into functional products, one of which is the production of biscuits.

MATERIAL AND METHODOLOGY

Samples

This study was conducted at Ekasakti University's Agricultural Product Technology Laboratory in Padang. The study was carried out during March and April of 2021. Purple rice from Kenagarian Kasang, Padang Pariaman City, and Pasar Raya Padang City plantains were the main raw materials used in this study. The researchers developed purple rice flour and plantain flour.

Chemicals

ROFA Laboratorium Centre provided all reagents, which were of analytical grade (Indonesia):
1. Protein analysis, 1.25% concentrated sulfuric acid (H_2SO_4), and Aquades, 30% d NaOH, Methyl Indicator, Methyl red 0.2%, Methyl blue 0.2%, selenium mix, H_3BO_3%, HCl 0.1N are the materials for chemical analysis.
2. Fat content analysis using n-hexane.
3. Crude fiber content, ethanol, sulfuric acid (H_2SO_4), 1.25% NaOH, and 10% potassium sulfate analysis (K_2SO_4).
4. DPPH 45 ppm antioxidant test in methanol. Scales, stoves, cauldrons, basins, trays, spoons, sieves, mixers, knives, blenders, sieves, ovens, mixers, cake pans, and moulds are all needed to make biscuits. Margarine, eggs, honey, skim milk, salt, and vanilla are also included.

Animals and Biological Material

Animal and special biological materials were not used in this research.

Instruments

All tools were of analytical grade and were purchased from ROFA Laboratorium Centre (Indonesia). The tools for chemical analysis are:
1. Protein analysis, 500 ml Kjeldahl flask, distillation apparatus, 50 ml burette, 5 ml measuring pipette, 50 ml Erlenmeyer, dropper pipette, 250 ml beaker, and fume hood.
2. Antioxidant test, UV-VIS spectrophotometer.
3. Organoleptic test by 30 untrained panellists after being chosen through discrimination, descriptive and affective tests.

Laboratory Methods

In this investigation, the treatments were the following ratios of purple rice flour to plantain flour (%): A = 90:10; B = 80:20; C = 70:30; D = 60:40; E = 50:50. The recipe for purple rice flour and plantain flour biscuits refers to [14] in [15].

Description of the Experiment

Sample preparation: The sample preparation for biscuits can be seen in the following Table 1. Table 2 shows the ingredient formulation for the production of biscuits.

Table 1 Standard formulations for making biscuits.

No	Material type	Percentage (%)
1	Flour	50
2	Egg Yolk	20
3	Honey	10
4	Margarine	10
5	Skimmed Milk	10
6	Baking soda	0.2
7	Salt	0.2

Note: Source: **[14]** in **[15]**.

Table 2 Biscuit formulation in 200 g of ingredients.

No	Material type	Unit	Treatment				
			A	B	C	D	E
1	Purple rice flour	g	90	80	70	60	50
2	Plantain flour	g	10	20	30	40	50
3	Egg yolk	g	40	40	40	40	40
4	Honey	g	20	20	20	20	20
5	Margarine	g	20	20	20	20	20
6	Skimmed Milk	g	20	20	20	20	20
7	Baking soda	g	0.4	0.4	0.4	0.4	0.4
8	Salt	g	0.4	0.4	0.4	0.4	0.4

Note: Source: **[14]** in **[16]**.

Number of samples analyzed: We analyzed Purple rice flour 350 g, Plantain flour 150 g, Egg yolk 200 g, Honey 100 g, Margarine 100 g, Skimmed Milk 100 g, Baking soda 2 g, and Salt 2 g samples.

Number of repeated analyses: All measurements of instrument readings were performed five times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was three times.

Design of the experiment: The researcher made the biscuits themselves:

a. Production of modified rice flour **[17]**

- Purple rice washed with running water
- Drain and dry in the sun for 8 hours
- Smoothing with a blender and then sifting 60 mesh
- Get purple rice flour

b. Production of modified plantain flour **[16]**

- Plantains
- Peeling the plantain skin and then soaking it in citric acid for 5 minutes
- Washing with clean water
- Slicing plantains and then drying in the sun for 7 hours for 3 days
- Smoothing with a blender and then sifting 60 mesh
- Get plantain flour

c. Biscuit making **[14]** in **[15]**

- Mixing I
- Margarine, egg yolks, and honey, mixing with a hand mixer for ± 10 minutes
- Mixing II
- Purple rice flour and plantain flour, according to the treatment, baking soda, skim milk, and salt was mixed using a high-speed mixer for 2 minutes
- Thin dough with a thickness of 2 cm
- Printing with a diameter of 3 cm
- Baking in the oven (150°C, ± 10 minutes)
- Biscuits

We have used the following methods for physicochemical analysis: water content **[18]**, protein content **[18]**, ash content **[18]**, crude fiber content **[18]**, fat content **[18]**, an antioxidant with DPPH method **[19]** and organoleptic test **[20]**.

Statistical Analysis

Microsoft Excel and SPSS Version 34 produced the statistical data analysis. The design used in this study was a one-factor Simple Completely Randomized Design (CRD) with 5 treatment levels and 3 replications. Observational data were analyzed using Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) advanced test at a 5% significance level. The data from this research were entered into SPSS 26.0. (SPSS Analytics Partner) and then the data were evaluated using ANOVA (Analysis of Variance) and the Tukey–Kramer test to determine the significant differences.

RESULTS AND DISCUSSION

Water Content

The study of diversity revealed that the water level of the produced biscuits differed considerably (p >0.01) depending on the ratio of purple rice flour to plantain flour. Based on the further DNMRT test at the level of = 0.01, all treatments demonstrated a very substantial difference in the water content of the biscuits. Table 3 shows the average water content of biscuits. The biscuits' water content ranged from 3.56 to 5.01%. The water content of the biscuits revealed a decrease in yield while increasing the amount of plantain flour. Plantain flour has a low water content since biscuits are baked at 150 °C, allowing the baking process to evaporate and limiting the quantity of water in the biscuit dough **[21]**. Treatment A (90:10 comparison of purple rice flour and plantain flour) had the highest water content of 5.01%. Treatment E (50:50 comparison of purple rice flour and plantain flour) had the lowest water level of 3.56%. The water content will decrease if less purple rice flour is used and more plantain flour is used, and vice versa.

According to **[22]**, rice flour has a water content of 13%, while plantain flour has a water content of 7.46% **[9]**. According to the research, the more plantain flour used in making biscuits, the lower the water content of the biscuits. Except for treatment A, which did not meet the Indonesian National Standard **[23]** for biscuits, the water level of the biscuits produced was a maximum of 5%.

Each treatment's water content varies because the water's relationship with food ingredients varies; the water content in food can be divided into bound water and free water **[24]**. The amylose concentration and soaking temperature of rice seeds alter water absorption **[25]**. Rice (non-waxy rice) is classified into three types based on its amylose content: low amylose (20%), medium amylose (20-25%), and high amylose (> 25%). (Arraullo, et al 1976). If the amylose concentration is low, water absorption and swelling will rise at temperatures exceeding 65°C **[26]**.

Table 3 Average water content of biscuits.

Comparison of purple rice flour with plantain flour (%)	Water Content (%)		Standard Deviation
A = 90:10	5.01	a	0.05
B = 80:20	4.70	b	0.02
C = 70:30	4.35	c	0.01
D = 60:40	3.82	d	0.02
E = 50:50	3.56	e	0.04
KK: 3.86%			

Note: The numbers in the same column followed by different lowercase letters show a significant difference in the DNMRT follow-up test at the level of $\alpha = 0.01$. KK is the coefficient of diversity.

Ash Content

The diversity analysis revealed that the ratio of purple rice flour to plantain flour had a significant (p0.01) effect on the ash content of the final biscuits. Based on the DNMRT additional test, all treatments revealed a very significant difference in the ash content of the biscuits at the level of = 0.01. Table 4 shows the average ash content of biscuits.

Table 4 Average ash content of biscuits.

Comparison of purple rice flour with plantain flour (%)	Ash content (%)		Standard Deviation
A = 90:10	1.70	a	0.01
B = 80:20	1.83	b	0.04
C = 70:30	1.95	c	0.01
D = 60:40	2.04	d	0.02
E = 50:50	2.11	e	0.05
KK: 3.93%			

Note: The numbers in the same column followed by different lowercase letters show a very significant difference in the DNMRT follow-up test at the level of $\alpha = 0.05$. KK is the coefficient of diversity.

The ash content of biscuits ranged from 1.70 to 2.11%. The higher the ash content of the biscuits, the more plantain flour is used, and the less purple rice flour is used. This is due to the high mineral concentration of plantain flour, which causes the ash content to rise. Ash is classified as a mineral element or an organic compound **[27]**.

The maximum ash level of biscuits was discovered in treatment E (50:50 comparison of purple rice flour with plantain flour), which was 2.11%, while the lowest ash content was observed in treatment A (90:10 comparison of purple rice flour with plantain flour), which was 1.70%. The ash content rises as less purple rice flour is used and more plantain flour is used, and vice versa.

All treatments had at least 1.6% biscuit ash content, which met the Indonesian National Standard **[28]**. This is because plantain flour has more ash than purple rice flour. Plantain flour has an ash content of 5.3% **[9]**, whereas rice flour has an ash content of 1.0 **[22]**.

Food has ash as one of its constituents. This component comprises minerals such as potassium, phosphorus, sodium, and copper. Mineral elements in the body combine with organic molecules or free ions; mineral elements operate as building blocks and regulators. The body's mineral content must be within ideal ranges **[29]**. The higher the ash content of the biscuits, the more plantain flour is used. As a result, biscuits made with a lot of plantain flour have more minerals.

Fat Content

The diversity analysis revealed that the ratio of purple rice flour to plantain flour was very significant (p <0.01) in the fat content of the manufactured biscuits. Based on the DNMRT additional test, all treatments revealed a very significant difference in the fat content of the biscuits at the level of = 0.01. Table 5 shows the average ash content of biscuits.

Table 5 Average fat content of biscuits.

Comparison of purple rice flour with plantain flour (%)	Fat level (%)		Standard Deviation
A = 90:10	16.66	a	0.10
B = 80:20	19.19	b	0.03
C = 70:30	21.18	c	0.00
D = 60:40	23.10	d	0.00
E = 50:50	25.18	e	0.48
KK: 1.24%			

Note: The numbers in the same column followed by different lowercase letters show a very significant difference in the DNMRT follow-up test at the level of α = 0.01. KK is the coefficient of diversity.

The fat content of biscuits ranged from 16.66% to 25.18%. The more plantain flour used in the production of biscuits, the higher the fat content. This is because plantain flour has a larger fat content than purple rice flour. Treatment E (50:50 comparison of purple rice flour and plantain flour) had the highest fat content (25.18%). Treatment A (comparison of purple rice flour with plantain flour 90:10) had the lowest fat content value of 16.66%. The less purple rice flour used and the more plantain flour used, the lower the fat level, and vice versa.

The fat content of all treatments biscuits exceeded the maximum fat content limit in the Indonesian National Standard **[23]**, which is a minimum of 9.5%. This is because the fat in biscuits is obtained by adding butter, eggs, and cream milk to the biscuit dough formulation **[30]**, and plantain flour has a larger fat content than purple rice flour. Plantain flour has a fat content of 0.6% **[9]**. On the other hand, rice flour has a fat content of 0.5%. This statement follows the findings that increasing the use of purple rice flour in the production of biscuits reduces the fat level of the product.

Crude Fiber Content

The diversity analysis revealed that the ratio of purple rice flour to plantain flour had a highly significant (p <0.01) variation in the crude fiber content of the biscuits prepared. Based on the DNMRT additional test, all treatments revealed a very significant difference in the crude fiber content of the biscuits at the level of = 0.01. Table 6 shows the average crude fiber content of biscuits.

Table 6 Average content of crude fiber of biscuits.

Comparison of purple rice flour with plantain flour (%)	Crude Fiber Content (%)		Standard Deviation
A = 90:10	8.38	a	0.17
B = 80:20	10.08	b	0.03
C = 70:30	12.74	c	0.00
D = 60:40	14.50	d	0.00
E = 50:50	17.85	e	0.01
KK: 4.91%			

Note: The numbers in the same column followed by different lowercase letters show a very significant the difference in the DNMRT follow-up test at the level of α = 0.01. KK is the coefficient of diversity.

Biscuits had a crude fiber content ranging from 8.38 to 17.85%. The more plantain flour is used to make biscuits, the higher the crude fiber content. This is because plantain flour contains more crude fiber than purple rice flour. The maximum crude fiber content of biscuits was discovered in treatment E (50:50 comparison of purple rice flour and plantain flour), which was 17.85%, while the lowest crude fiber content was identified in treatment A (90:10 comparison of purple rice flour and plantain flour), which was 8.38%. This assertion is based on the study's findings that crude fiber content is inversely related to water content; the higher the crude fiber content, the lower the water content produced; and vice versa, the higher the crude fiber content, the higher

the water content produced. The less purple rice flour and plantain flour used, the higher the crude fiber content, and vice versa.

The crude fiber content of biscuits produced for all treatments exceeded the 0.5% maximum allowed in the Indonesian National Standard **[23]**. This is because plantain flour contains more crude fiber than purple rice flour. Plantain flour has 13.71% crude fiber **[9]**. The more plantain flour is used in the production of biscuits, the higher the crude fiber content of the biscuits. Crude fiber is made up of cellulose, pentose, and other ingredients. This crude fiber component has no nutritional value but is critical in facilitating the digestion process in the body **[32]**.

Protein Content

The study of diversity revealed that the protein composition of the biscuits made differed significantly ($p < 0.01$) between purple rice flour and plantain flour. Based on a subsequent DNMRT test at the threshold of = 0.01, all treatments exhibited a significant difference in biscuit protein content. Table 7 shows the average protein content of biscuits.

Table 7 Average protein content of biscuits.

Comparison of purple rice flour with plantain flour (%)	Protein Content (%)		Standard Deviation
A = 90:10	7.78	e	0.14
B = 80:20	6.75	d	0.00
C = 70:30	5.59	c	0.14
D = 60:40	5.00	b	0.00
E = 50:50	4.72	a	0.14
KK: 5.11%			

Note: The numbers in the same column followed by different lowercase letters show a significant difference in the DNMRT follow-up test at the level of $\alpha = 0.01$. KK is the coefficient of diversity.

The protein content of biscuits ranged from 4.72 to 7.78%. The more purple rice flour used to produce biscuits, the higher the protein level produced. This is due to the low protein content of plantain flour; the highest protein content of biscuits is found in treatment A (comparison of purple rice flour with plantain flour 90:10), which is 7.78%, while the lowest protein content is found in treatment E (comparison of purple rice flour with plantain flour 50:50), which is 4.72%. The less purple rice flour used and the more plantain flour used, the lower the protein contents, and vice versa.

The protein level of all treatment biscuits did not match the Indonesian National Standard **[23]**, which was 9%. This was due to the increased protein content of purple rice flour to plantain flour. Rice flour has a protein content of 7.59% **[33]**, while plantain flour has a protein content of 4.8% **[9]**. The more purple rice flour used in biscuit production, the higher the protein level of the biscuits.

Antioxidant Activity

The diversity analysis revealed no significant difference ($p > 0.01$) in the antioxidant content of the biscuits formed when purple rice flour was compared to plantain flour. Table 8 shows the average antioxidant content of biscuits.

Table 8 Average antioxidant content of biscuits.

Comparison of purple rice flour with plantain flour (%)	Antioxidant Activity	Standard Deviation
A = 90:10	85.83	10.41
B = 80:20	80.00	14.31
C = 70:30	70.66	19.50
D = 60:40	61.83	5.77
E = 50:50	55.83	0.29
KK: 9%		

Note: The numbers in the same column followed by different lowercase letters show a significant difference in the DNMRT follow-up test at the level of $\alpha = 0.01$. KK is the coefficient of diversity.

Biscuit antioxidant activity levels ranged from 55.83 to 85.83%. The less plantain flour used in biscuit production, the higher the antioxidant level of the biscuits produced. The increased usage of purple rice flour in the production of biscuits raises antioxidant activity. This is due to the presence of 67.64% antioxidant chemicals in purple rice flour **[34]**. In a comparison of antioxidants studied by **[35]**, levels of antioxidant activity in brown rice flour were found. The Mandel Handayani variety brown rice flour had the highest antioxidant activity concentration of the two types, ranging from 92.286 to 92.972%, whereas the Segreng Handayani variety had a range of 79.207 to 89.870%.

Treatment A (comparison of purple rice flour with plantain flour 90:10) had the greatest antioxidant levels of biscuits, at 85.83%, while treatment E (compare of purple rice flour with plantain flour 50:50) had the lowest antioxidant levels, at 55.83%. The antioxidant activity decreases as purple rice flour is used less and plantain flour is used more, and vice versa.

It is not specified in the SNI for biscuits for antioxidant activity because antioxidants are very important to investigate to establish the antioxidant content of the blend of purple rice flour and plantain flour in biscuits. This purple rice flour contains antioxidant chemicals that are beneficial to the body. The antioxidant activity of biscuits containing more purple rice flour will be enhanced. The antioxidant activity of all biscuit treatments was 70.83%. as stated by **[34]**. Purple rice has a level of antioxidant activity of 67.64%.

Carbohydrate Content

According to the results of the diversity study, the ratio of purple rice flour to plantain flour made a very significant difference (p 0.01) in the carbohydrate content of the biscuits created. Based on the DNMRT additional test at the level of = 0.01, all treatments exhibited a very significant variation in biscuit carbohydrate content. Table 9 shows the average carbohydrate content of biscuits.

Table 9 Average carbohydrate content of biscuits.

Comparison of purple rice flour with plantain flour (%)	Carbohydrate levels (%)		Standard Deviation
A = 90:10	71.72	a	0.73
B = 80:20	69.28	b	0.08
C = 70:30	66.91	c	0.12
D = 60:40	64.27	d	0.01
E = 50:50	61.49	e	0.03
KK: 2.58%			

Note: The numbers in the same column followed by different lowercase letters show a very significant difference in the DNMRT follow-up test at the level of α = 0.01. KK is the coefficient of diversity.

Biscuit carbohydrate content ranged from 61.49 to 71.72%. The more plantain flour used in biscuit baking, the lower the carbohydrate content produced. This is because purple rice flour has more carbs than plantain flour. Treatment A (comparison of purple rice flour with plantain flour 90:10) had the highest carbohydrate amount (71.72%), whereas treatment E (comparison of purple rice flour with plantain flour 50:50) had the lowest carbohydrate content (61.49%). The carbohydrate amount will decrease as less purple rice flour is used and more plantain flour is used, and vice versa.

The findings of this study support the assertion above that biscuits made with purple rice flour contain more carbohydrates, resulting in a greater carbohydrate content. The carbohydrate content of biscuits prepared for treatment A was at least 70% of the Indonesian National Standard **[23]** for biscuits.

This figure is slightly lower than the minimum SNI standard for biscuits, which specifies a minimum carbohydrate content of 70%. Plantain flour has a reduced carbohydrate percentage, namely 47.6-49.8% [9]. Even though plantain flour biscuits did not meet the minimum carbohydrate content level in **[23]** biscuits. Humans get the majority of their calories from carbohydrates. Carbohydrates also influence the properties of food items such as flavour, colour, and texture. Furthermore, carbohydrates are beneficial in the body because they inhibit the breakdown of excess body protein, and mineral loss, and aid in fat and protein metabolism **[36]**.

Organoleptic Test

The organoleptic test was performed using sensory assessment, which included sampling the taste and aroma of the biscuit and analyzing its texture and colour. The test was conducted using biscuits prepared according to the treatment formulation. By being put to the test by 30 untrained panellists.

a. Flavour

The essential factor in consumer acceptance of a product is its taste. Taste is distinct from smell in that it involves all five tongue senses. Several elements can influence taste, including chemical substances, temperature, concentration, and interaction with other flavour components [**36**]. Table 10 displays the panellists' ratings on the flavour of the biscuits.

Table 10 Biscuit taste test value.

Comparison of purple rice flour with plantain flour (%)	Flavour value (%)	Description
A = 90 : 10	3.56	different (tasteless)
B = 80 : 20	4.88	somewhat similar (almost like banana)
C = 70 : 30	4.24	somewhat similar (almost like banana)
D = 60 : 40	5.00	similar (banana flavour)
E = 50 : 50	5.60	similar (banana flavour)

Notes: taste scores include 7 = very much similar; 6 = very similar; 5 = similar; 4 = somewhat similar; 3 = different; 2 = very different; 1 = very much different.

Table 10 demonstrates that treatment E (50:50 comparison of purple rice flour and plantain flour) received the highest rating from the panellists for biscuit taste, with 5.60%. (similar). The panellists' lowest rating of the biscuit taste was 3.56% (different) in treatment A (comparison of purple rice flour with plantain flour 90:10).

The data gathered revealed that the higher the addition of purple rice flour, the lower the panellist acceptance rate. This is due to the slightly bland flavour of purple rice flour, which affects the biscuit taste. Adding plantain flour and other ingredients can improve the taste of the biscuits. However, based on the panellists' acceptance data, it can be determined that the panellists have accepted the combination of purple rice flour with plantain flour on a scale of 5 to 5.60, indicating that the panellists already enjoy the taste of the cookies.

Food products, in general, do not have a single flavour but a blend of several integrated flavours. Taste is the sensation of salty, sweet, sour, or bitter flavours created by substances dissolved in the tongue **[37]**.

b. Aroma

According to **[36]**, the five senses of smell greatly influence scent. There are four types of aromas that the nose may detect: aromatic, sour, rancid, and burnt. The scent also influences food products' delicacy and taste, consisting of three components: smell, taste, and stimulation **[38]**. Table 11 displays the panellists' ratings on the scent of biscuits.

Table 11 Biscuit aroma test value.

Comparison of purple rice flour with plantain flour (%)	Aroma Value(%)	Description
A = 90 : 10	3.36	different (not typical banana aroma)
B = 80 : 20	3.88	different (not typical banana aroma)
C = 70 : 30	4.44	somewhat similar (almost like banana)
D = 60 : 40	4.84	somewhat similar (almost like banana)
E = 50 : 50	5.44	similar (banana aroma)

Notes: taste scores include 7 = very much similar; 6 = very similar; 5 = similar; 4 = somewhat similar; 3 = different; 2 = very different; 1 = very much different.

Table 11 demonstrates that treatment E (50:50 comparison of purple rice flour and plantain flour) received the highest rating from the panellists for biscuit scent, with 5.44%. (similar). The panellists' lowest estimate of the biscuit scent was 3.36% (different) in treatment A (comparison of purple rice flour with plantain flour 90:10).

The inclusion of purple rice flour decreased fragrance reception. This is because purple rice flour does not have a significant scent. The aroma of the biscuits is derived from raw materials and other additives used during baking.

c. Texture

The water quantity, fat content, and number of carbohydrates and proteins all influence the appearance of meals. Texture changes can be induced by water or fat content loss, emulsion breakdown, or protein hydrolysis **[39]**. Table 12 displays the panellists' ratings of biscuit texture.

Table 12 shows that treatment E (comparison of purple rice flour and plantain flour 50:50) had the greatest rating for biscuit texture, 5.52% (similar), whereas treatment A received the lowest rating for biscuit texture (comparison of purple rice flour and plantain flour). The panellists' acceptance rating is 3.52% (dislike) on a scale of dislike to like (90:10).

Table 12 Value of biscuit texture test.

Comparison of purple rice flour with plantain flour (%)	Texture Value(%)	Description
A = 90 : 10	3.52	different (rough)
B = 80 : 20	3.96	different (rough)
C = 70 : 30	4.48	somewhat similar (rather rough)
D = 60 : 40	5.20	similar (soft)
E = 50 : 50	5.52	similar (soft)

Notes: taste scores include 7 = very much similar; 6 = very similar; 5 = similar; 4 = somewhat similar; 3 = different; 2 = very different; 1 = very much different.

The more plantain flour used, the softer the finished product and the greater the panellist acceptance rate. Adding purple rice flour was responsible for the low level of panellist acceptance of treatment A (comparison of purple rice flour and plantain flour 90:10). The biscuits would harden or solidify. Food's water, fat, protein, and carbohydrate content heavily influence its texture. The texture is a pressure sensation that can be felt with the mouth (when biting, chewing, and swallowing). Texture sensing can detect wetness, dryness, hardness, smoothness, roughness, and oiliness **[40]**.

d. Colour

Colour is vital in fulfilling human tastes, according to **[41]**. Colour assessment is done by examining the product firsthand using each panellist's sense of sight. Table 13 shows the panellists' ratings of the hue of the biscuits.

Tabel 13 Nilai uji warna biskuit.

Comparison of purple rice flour with plantain flour (%)	Colour Value (%)	Description
A = 90 : 10	3.64	do not like (light yellow)
B = 80 : 20	4.16	kinda like (yellow)
C = 70 : 30	4.52	kinda like (yellow)
D = 60 : 40	5.08	like (dark yellow)
E = 50 : 50	5.52	like (dark yellow)

Notes: taste scores include 7 = very much similar; 6 = very similar; 5 = similar; 4 = somewhat similar; 3 = different; 2 = very different; 1 = very much different.

Table 13 demonstrates that treatment E (comparison of purple rice flour and 50:50 plantain flour) received the highest colour assessment of 5.52%. (similar). Because the panellists find the dark yellow colour more appealing. Treatment A (comparison of purple rice flour and plantain flour 90:10) received the lowest rating of 3.64% (different). Because panellists find light skin to be less appealing.

Colour evaluation is accomplished through direct visual inspection of the product with each panellist's sense of sight. Many elements influence a product's quality, but before other factors are examined and assessed, the colour component visually emerges first in deciding panellists' product acceptability **[36]**.

Figure 3 Final product of biscuit.

CONCLUSION

A comparison of purple rice flour and plantain flour on the quality of antioxidant-rich biscuits revealed that purple rice flour had a significant effect on water, ash, crude fibre, fat, protein, and carbohydrate content but had no effect on antioxidant activity. Treatment E has 3.56% water content, 2.11% ash content, 25% fat content, 17.5% crude fiber content, 4.72% protein content, 55.83% antioxidant activity, and 61.49% carbohydrate content (comparison of purple rice flour and plantain flour 50:50). To limit the consumption of wheat flour, it is suggested that the community and biscuit entrepreneurs develop antioxidant-rich biscuit goods using purple rice flour and plantain flour.

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The authors declare no conflict of interest.

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

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The study of the cytotoxic effect of disinfectants

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ABSTRACT

The toxicity of individual disinfectants has been studied in vitro using human cell cultures (HT-29 (epithelial-like cells of colon adenocarcinoma), HEK 293 (human embryonic kidney cells)) to create a model for assessing the toxicity of residual amounts of disinfectants that can enter milk for a person. Standard tests have been used to assess cell viability and amount: methyl tetrazolium (MTT) test, neutral red cell staining (NRP), and sulforhodamine B (SRB) test. Disinfectants have a dose- and time-dependent cytotoxic effect on human cell cultures. IC50avg (concentration of the drug that suppresses a certain cell function by 50%) of disinfectants based on the effect on cell cultures (average value) is Biodez – 117.29 ±14 µl/l, Blanidas – 389.25 ±20.83 µl/l, Virkon-S – 343.04 ±28.04 µl/l, Neochlor – 473.82 ±30.16 µl/l, Phan – 56.71 ±7.05 µl/l, Chlorination – 343.28 ±27.26 µl/l, Chlorinated lime – 117.35 ±9.44 µl/l. Mean toxic doses for cell cultures are lower than the mean lethal dose (based on literature data) for rats and mice by gastric administration. The novelty is that determining the cytotoxicity of disinfectants in vitro using human cell cultures can significantly reduce the number of animals for establishing LD_{50} during the registration procedure of new agents, making it possible to make preliminary conclusions about the toxicity of substances at the stage of chemical screening, preliminary hygienic regulation, identify target organs of toxic influence.

Keywords: toxicity, cell culture, in vitro, disinfectant, genotoxicity

INTRODUCTION

Any new chemical compound, regardless of its intended purpose, shall be characterized in terms of its possible toxicity and biological activity. In addition, toxicological testing of drugs in preclinical trials shall ensure obtaining a reliable toxicological assessment **[1]**. Since Ukraine joined the European Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes on May 2, 2017, it is now clear that conducting all types of toxicological research on animals is not appropriate. The list of limitations for using laboratory animals for preliminary evaluation of disinfectants includes ethical issues, but economic and time costs are not less important, which significantly increase the cost and length of research **[2]**. In vitro methods are widely accepted in toxicology and widely used for the screening and classifying of chemicals **[3]**. Meanwhile, there is a sufficient number of standardized methods, in particular, using cell cultures of different organ natures, which allow obtaining adequate information for assessing the impact of xenobiotics on cell metabolism **[4]**, **[5]**, **[6]**. The variety of test systems is extraordinary, and more than 170 commercial organizations offer in vitro toxicity testing services covering mechanisms such as genotoxicity, hepatotoxicity, cardiotoxicity, and immunotoxicity **[7]**, **[8]**.

As for the dairy industry, scientists are developing methods to evaluate the quality of equipment disinfection **[9]**. In particular, the adenosine triphosphate bioluminescence method to determine bacterial contamination of surfaces and a test procedure for evaluating the cleanliness of milking equipment **[10]**.

Studies of structure-cytotoxicity relationships in many analogues of compounds can provide more detailed insights into the mechanisms of their action at the molecular and cellular levels **[11]**.

Various model systems have been created and improved to study all types of toxicity at the cellular level **[12]**, **[13]**.

We have investigated basic toxicity, which corresponds to a greater extent to acute toxicity at the microorganism level. Many markers for assessing the degree of cell damage in culture have already been developed, but it is obvious that the violation of even one of the cellular functions inevitably entails a negative impact on the overall viability of the monolayer after a certain time **[14]**. This facilitates the task of researchers significantly, as it involves, at least at the first evaluation stage of an in vitro toxicological study, the use of a limited set of cell cultures (most often standard, common lines) and a few simple indicators of cell viability **[15]**, **[16]**, **[17]**.

All of the above fully applies to disinfectants and their toxicity tests.

An integral component of safe food technology is the observance of good hygienic practices (GMP) during the entire process chain, an important component of which is the sanitation of process equipment **[18]**.

Modern disinfectants used for sanitizing milking and technological equipment of milk processing enterprises are multicomponent. They include not only active substances of disinfectants but also surfactants, stabilizers, etc. **[19]**.

The use of such means allows you to combine cleaning and disinfection in one operation and reduce the duration of sanitary treatment and water consumption. It is worth noting that it is necessary to carry out sanitary treatment of milking and technological equipment under technological instructions. In case of violated regimes of the final cleaning of disinfectants from the surfaces of technological equipment, the probability of xenobiotics entering milk and dairy products increases significantly **[20]**.

As a result of the use of detergents and cleaning disinfectants during technological processes related to the sanitation of food production equipment, unregulated by-products can be formed due to the interaction of chemical and organic substances, get into food products and cause food poisoning in people **[21]**. Metabolites of disinfectants can have carcinogenic and non-carcinogenic effects, in particular, affect endocrine disorders in the body. Understanding the consequences of such impacts on public health is of urgent importance to society and public officials responsible for the safety of drinking water and food **[22]**. By-products from the use of chlorine-containing disinfectants in the dairy industry inhibit iodine absorption in humans and contribute to the formation of metmyoglobin, which is a high risk, especially for children **[23]**.

In the available literature over the past 5 years, 9 groups and 36 types of such substances have been identified. Moreover, there is a statement that these compounds can be more toxic than the disinfectant itself. Such compounds in drinking water are of particular concern to scientists, as new disinfectants have recently emerged that require detailed investigation into the formation of toxic metabolites **[24]**.

Compliance with the rules of sanitary processing of equipment used for the manufacture of food products is very important for the safety of the final product since the residues of detergents and disinfectants can affect both the health of a consumer and the control tests for the presence of other harmful substances in the products. Thus, the residual amounts of such substances affect the reactions of the tests (BRT MRL; Delvotest SP-NT MCS; Eclipse 100) during the screening of antibiotics in goat milk **[25]**.

This problem in Ukraine is practically unexplored due to the lack of effective methods for determining micro concentrations of disinfectants in milk.

Improvement of methodological approaches during veterinary and sanitary control of food safety indicators and study of the possible negative impact of small doses of foreign chemical substances, including disinfectants and detergents and disinfectants, on human health are of important scientific and practical importance. Preventing residual amounts of disinfectants, detergents, and disinfectants in milk as a result of technological processes of its production and the corresponding control of the presence of these agents in milk is an urgent issue and requires in-depth scientific research and justification.

The paper aims to determine the cytotoxicity of low concentrations of individual disinfectants in vitro as a model for determining residual amounts of disinfectants that may enter milk during its production technology.

Scientific Hypothesis

We hypothesized that disinfectants with chemicals in their composition show dose-dependent cytotoxicity to human cell cultures. Their toxicity depends on the chemical nature of the disinfectant components and correlates with LD50 values obtained from tests with laboratory animals.

MATERIAL AND METHODOLOGY

Samples

Disinfectants – Biodez R (the active substance is polyhexamethylene guanidine hydrochloride - 20.0%). Manufacturer: Production and Scientific Enterprise "Ukrzoovetprompostach", Private Limited Company, Ukraine. According to GOST 12.1.00776, in terms of parameters of acute toxicity, the agent "Biodez" corresponds to the IV class – a low-hazardous substance, when applied to the skin and the III class – a moderately dangerous substance, when inhaled. The drug does not have cumulative, mutagenic, or carcinogenic properties.

Blanidas brand A. Manufacturer: Lysoform Medical LLC, Ukraine. The product's composition, the content of active and auxiliary substances, %: 1-Bromo-3-chloro-5,5-dimethyl hydantoin – 20-22% (the active substances), sodium tripolyphosphate, surfactants, corrosion inhibitors, table salt – up to 100.00. The content of active chlorine-bromine is at least 19.5%. Toxicity and safety of the product: according to the parameters of acute toxicity when injected into the stomach, when inhaled (in the form of vapor) and when applied to the skin, it belongs to the IV class of low-hazard substances. In its native form and the form of concentrated solutions (2.5%), it irritates the mucous membrane of the eyes and upper respiratory tract. In the recommended concentrations, it does not show skin-irritating properties or irritate the eyes.

Virkon-S. Manufacturer: Bayer. Composition: the active substance is potassium peroxymonosulfate, and auxiliary substances: sodium chloride, sulfamic acid, malic acid, sodium hexametaphosphate, sodium dodecylbenzene sulfonate, amaranth dye, flavouring with the smell of lemon. According to the level of toxicity, it belongs to moderately dangerous compounds (LD_{50} for white mice after oral administration is 3680 mg/kg of animal weight). In the recommended concentrations, it does not irritate the skin, slightly irritates mucous membranes, and does not cause sensitization.

Neochlor - (the active substance is sodium hypochlorite, and the product's initial content of active chlorine is from 7-9% (concentrate). The product's composition also includes detergent, anti-corrosion, stabilizing, antimicrobial, and flavouring additives). Manufacturer: Ukrainian Research and Production Center for Disinfection Problems CJSC (Ukraine). The agent "Neochlor" (a concentrate) belongs to the III class of dangerous (moderately dangerous substances); irritates the skin and mucous membranes; has weak cumulative properties; does not show a sensitizing effect; has no mutagenic properties.

Chlorination (dichloranthin, 5,5-dimethyl hydantoin is a chlorine-containing disinfectant of the third generation, active chlorine is not less than 13.5%); Manufacturer: "Farmakos" Scientific and Production Limited Liability Company (Ukraine). The product's composition, the content of active and auxiliary substances, and mass. %: 1,3-dichloro-5,5-dimethyl hydantoin (dichloranthin) – 21.5-23.5 (active ingredient); 5,5-dimethyl hydantoin – 12.5-16.5; dispersant – 9.0-12.5; anionic surfactants – 3.2-5.0; corrosion inhibitor up to 10.0; filler to 100.0. The mass fraction of active chlorine is at least 14.1%. Toxicity and safety of the product: chlorination belongs to moderately dangerous substances (hazard class 3) when ingested and inhaled into the body and low-hazard substances when applied to the skin (hazard class 4). In the conditions of inhalation action in the form of vapors, according to the degree of volatility, it belongs to low-hazardous substances. In dry form and concentrated solutions, it irritates the mucous membrane of the eyes and upper respiratory tract. In the concentrations recommended for cleaning and disinfection, it does not show skin-irritating properties and does not irritate the mucous membrane of the eyes. The product does not have skin resorptive and sensitizing, carcinogenic, mutagenic, or embryotoxic (according to the active substance) properties.

Phan (the active substance is didecyldimethylammonium chloride (at least 5%), auxiliary components (including surfactants and inorganic acid). Manufacturer: OU "BALTIACHEMI", Estonia. According to the parameters of acute toxicity when injected into the stomach, it belongs to class 3. In inhalation exposure and application to the skin, it belongs to the IV class of hazards (low-hazardous substances according to DSTU 12.1.007-76). It has no teratogenic or carcinogenic effect.

Clarified solution of perchloric lime. Chlorine lime is a mixture of hypochlorite, chloride, and calcium hydroxide. It belongs to the so-called mixed salts. Depending on the production method, chlorinated lime is produced in two grades: A and B. Moderately toxic. LD50 for rats – 850 mg/kg.

Animals, Plants and Biological Materials

Standardized cell cultures were obtained from the Cell Line Bank of the RE Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology, National Academy of Sciences of Ukraine.

Instruments

The research used disinfectants registered in Ukraine according to the established procedure that has different active substances belonging to different groups of chemical disinfectants and is allowed for use in Ukraine

Laboratory Methods

The research was carried out at the Central State Testing Laboratory of the State Production and Consumer Service in the Kyiv region and the city of Kyiv, the laboratory of industrial toxicology and occupational hygiene

of the Institute of Occupational Hygiene of the Academy of Medical Sciences of Ukraine, which the National Accreditation Agency of Ukraine accredits following DSTU requirements.

Cytotoxic properties were evaluated by three main tests, which took into account the viability of cells and their number – methyl tetrazolium test (MTT) **[26]**, staining cells with neutral red (NRP) **[27]** and the sulforhodamine B (SRB) test **[28].**

The use of in vitro toxicity tests is stipulated in Directive 2010/63/EU, which revises Directive 86/609/EEC on protecting animals used for experimental and other scientific purposes, adopted on September 22, 2010. The Directive is firmly based on the Three Rs principle to replace, reduce and improve the use of animals used for scientific purposes [**29**].

The studies were part of the research topic of the veterinary and sanitary examination department of the National University of Bioresources and Nature Management of Ukraine "Scientific assurance of the production of livestock products according to the Codex Alimentarius" (state registration number 0109U003215).

Description of the Experiment

Laboratory animals were not used.

Sample preparation: Cell cultures were stored in a frozen state. Cell cultures were cultured in vials. A new number of cells was thawed after two months of cultivation. Disinfectants were prepared before the test according to the producer's instructions. Distilled, deionized sterile water was used as solvent. Subsequently, to obtain the required concentration applied directly to the well, the working solution of disinfectants was added to the medium for cells so that the solvent in the first wells was not more than 2%.

Number of samples analyzed: 7 disinfectants in different concentrations were tested (10000, 5000, 2500, 1250, 625, 312,160, 80, 40, 20 mg/kg).

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: They were cultured in complete nutrient medium DMEM (“SIGMA”, USA) containing 4 mmol/L of L-glutamine, 10% of fetal calf serum (“SIGMA”, USA), 40 μg/ml of gentamicin in a humidified atmosphere with 5% CO_2 at a temperature of 37 °C. The medium was changed every 2 days. Transplantation of cells was carried out with the help of Versen’s solution when the cells formed a continuous monolayer on the substrate (4-5th day of growth).

To study the sensitivity of cells to disinfectants, the cell suspension was placed on 96-well tablets at a concentration of 5×10^3-1×10^4 cells/well in 100 μl of nutrient medium. After 24 hours, a solution of the studied disinfectants at 20-100000 mg/kg was added to the cells and cultured at 37 °C in a humidified atmosphere for 24-48 hours.

Afterwards, changes in the metabolic state of the cells were assessed by:

1.a decrease in total mitochondrial dehydrogenase activity in the microculture tetrazolium test (MTT – photometric method) reflects inhibition of cellular respiration intensity. The tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is taken into cells reduced in a mitochondria-dependent reaction to yield a formazan product. The product accumulates within the cell, because it cannot pass through the plasma membrane. The product is liberated on solubilisation of the cells and can readily be detected and quantified by a simple colourimetric method. The cells' ability to reduce MTT indicates mitochondrial integrity and activity which, in turn, may be interpreted as a measure of viability and/or cell number **[26]**.

The activity of lysosomes, which easily capture and accumulate neutral red dye in living cells (neutral red test) **[27]**.

Protein synthesis, the amount of which in cells was determined by the sulforhodamine B (SRB) assay **[28]**. According to the methods, dyes were added to the wells of the plate, and incubated in the thermostat for a certain time, and the optical density of the good contents was determined using a Multiscan Microplate spectrometer (Sweden). Empty wells and wells with cells in which no xenobiotic was added were used as controls and controls with solvent, i.e. distilled deionized water (2%) in the cell culture medium.

Live cells were stained because (depending on the method) formazan dye accumulates in the cell, the Neutral Red dye accumulates in the lysosomes, and Sulforhodamine B dye binds to the protein components of the cell.

After 24-48 hours, the studied compounds were added to the growth medium in different concentrations, and the cells were incubated under standard conditions for 24 hours, after which the cells were stained with one of the methods (with tetrazolium blue, with sulforhodamine B, and with neutral red).

Statistical Analysis

Statistical differences between experimental and control results were tested by one-way analysis of variance (ANOVA) and the student-Newman-Keuls test. Values of $p < 0.05$ were considered statistically significant. Statistical processing was performed in Microsoft Excel 2016 values were estimated using mean and standard deviations and subsequently evaluated in the statistical program XL Stat. In hypothesis testing, if the *p*-value is lower than a significant level, in the case of XL Stat software by Addinsoft (version 2019.3.2), it is 0.05, the null hypothesis was rejected and alternative hypothesis was confirmed.

RESULTS AND DISCUSSION

Determination of the toxicity of disinfectants in vitro was carried out on HT-29 and HEK 293 cell cultures. Cell cultures have a stable, homogeneous cell morphology, are well attached to the surface of the bottom of the wells and are also characterized by good growth potential. We also considered that the digestive organs and kidneys are of particular importance in the processes of xenobiotic excretion. In scientific works **[29]**, **[30]**, **[31]**, similar series of experimental studies are described, which were conducted in a later period and were focused on the study of the metabolism of various substances. As it is known, the reason for the toxic effect of xenobiotics on living systems is their ability to disrupt the course of basic biochemical processes (for example, protein biosynthesis, respiration, energy exchange, and substance metabolism).

It shall be noted that the content of disinfectants, detergents, and disinfectants in food products is not allowed by both national regulations and EU legislation (Ministry Order 2646:2019; DSTU 3662:2018 **[32]**. Directive (EU) No. 853 (2004), but their residual amounts in food products can cause negative effects on human health **[33]**. The scientific works **[34-37]** described a series of experimental studies, the purpose of which was to determine the residual content of disinfectants and detergents and disinfectants in food products using various methods and techniques.

The results obtained by us regarding the determination of the cytotoxic effect of disinfectants on the HT-29 cell culture (intestinal epithelium model) (Table 1) show that a clarified solution of perchloric lime caused the destruction of the monolayer and close to 100% cell death in concentrations that correspond to the concentrations of the working solutions of disinfectants and detergents (5000-10000 µl/l) solutions of Biodez, Blanidas, Vircon-S, neochlor, (Figure 1). A series of similar experimental studies were described in scientific works **[38]**, **[39]**, **[40]**, but the researchers used 293-T human kidney (embryonic) with different concentrations that correspond to the concentrations of working solutions of disinfectants and detergents and disinfectants (4500-9000 µl/l)

Biodez demonstrates significant cytotoxicity for HT-29 cell culture. Even at a concentration of 20 µl/l, cell survival is only 66.38 ±2.44% of cells ($p < 0.001$). It has almost the same cytotoxic effect on HT-29 and Phan cells. According to our research, 59.83 ±7.98% of the cells of this line survive at a concentration of 20 µl/l. Blanidas, Neochlor at a concentration of 20 µl/l do not cause cell death (the cell culture monolayer does not differ from the control) (Figure 2).

Table 1 Cytotoxic effect of disinfectants on cells of the HT-29-line, M ±m, n = 18.

	Cell viability indicators, %									
Concentration, mg/kg	**10000**	**5000**	**2500**	**1250**	**625**	**312**	**160**	**80**	**40**	**20**
Biodez	–	4.21 ±0.5*	8.44 ±2.11*	23.47 ±2.65*	24.49 ±5.20*	35.71 ±7.12*	37.90 ±3.25*	44.8 ±2.44*	**55.71 ±1.22***	66.28 ±2.44*
Blanidas	8.41 ±1.35*	26.17 ±2.03*	26.17 ±2.03*	24.30 ±5.09*	28.04 ±5.09*	42.99 ±1.02*	**65.33 ±5.09****	99.07 ±1.02	-	-
Virkon-S	2.88 ±1.75*	4.07 ±2.13*	5.75 ±3.32*	18.67 ±3.03*	32.83 ±9.57*	**44.69 ±6.55***	73.1 ±9.34	82.68 ±3.62	71.42 ±5.67$	-
Neochlor	2.92 ±0.80*	8.84 ±2.36*	14.8 ±2.55*	25.64 ±5.18*	**49.45 ±3.89***	54.95 ±2.59*	75.09 ±12.2	76.92 ±18.1	95.24 ±2.56	100.73 ±9.1
Chlorantoin	7.92 ±0.80*	15.84 ±1.61*	12.45 ±0.80*	33.94 ±1.61*	39.60 ±7.23*	**47.52 ±4.82***	64.49 ±7.23**	67.89 ±9.64$	76.94 ±8.04	85.99 ±6.43
Chlorinated lime	10.79 ±3.0*	7.19 ±3.95*	10.27 ±3.41*	13.36 ±3.67*	17.47 ±5.88*	47.26 ±6.89*	**52.4 ±7.17***	64.73 ±3.67*	80.11 ±1.24$	95.05 ±2.99
Phan	6.11 ±1.0*	7.33 ±1.20*	9.16 ±0.86*	10.01 ±1.11*	20.51 ±1.79*	20.76 ±1.0*	34.19 ±1.99*	39.07 ±9.51*	46.4 ±5.51*	**59.83 ±7.98****

Note: **$** – $p < 0.05$; ** – $p < 0.01$; * – $p < 0.001$ – relative to the control.

The death of single cells and a slight decrease in marker functions in the culture of HT-29 cells were detected at a concentration of 20 µl/l of chlorination and a clarified solution of perchloric lime.

Thus, the IC_{50} (average inhibitory concentration is the concentration of the drug that suppresses a certain cellular function by 50%) of disinfectants based on the effect on the culture of intestinal origin (HT-29) is Biodez – 60.93 ±9.81 µl/l, Blanidas – 264.30 ±25.12 µl/l, Virkon-S – 283.59 ±31.20 µl/l, Neochlor –593.70 ±33.86 µl/l, Phan – 69.28 ±8.4 µl /l, Chlorination – 289.79 ±30.85 µl/l, Chlorinated lime – 117.35 ±9.44 µl/l. The authors of the scientific works **[41]**, **[42]**, **[43]**, conducted a similar series of experimental studies. However, the effect on the cell line of kidney origin (NEC 293) was: Biodez - 123.65 ±19.56 µl/l, Blanidas – 314.20 ±16.54 µl/l, Virkon-C – 382.48 ±24.87 µl/l, Neochlor – 153.94 ±26.45 µl/l, Fan – 34.13 ±5.7 µl/l, Chlorination – 306.76 ±23.66 µl/l, in our opinion, this is due to non-compliance with the temperature regime during the experiments.

Figure 1 Cells of the NT-29 line, after exposure to disinfectants at a concentration of 2500 µl/l (staining with neutral red).

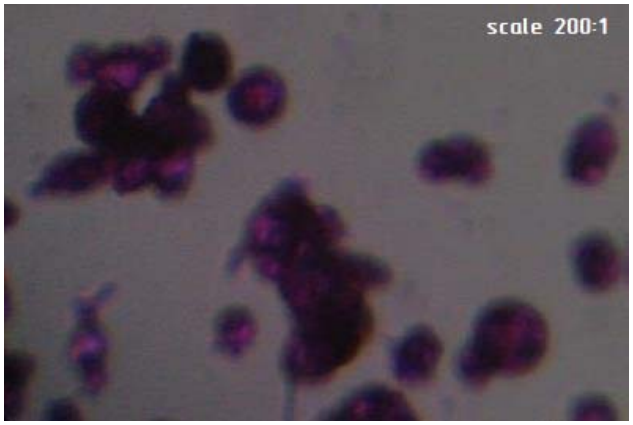

Figure 2 Accumulation of sulforhodamine by HT-29 culture cells (Neochlor – 40 µl/l) (test with SR).

We used the HEK 293 (Human Embryonic Kidney) cell line as a kidney model to determine the organ-specific toxicity of the studied disinfectants (Figure 3).

The obtained results (Table 2) indicate the high cytotoxicity of disinfectants and detergents on the cells of this line. At concentrations of 5,000-10,000 µl/l, all the studied agents cause the detachment of cells from the bottom of the wells and their death. Virkon-S, Blanidas, Neochlor, and Chlorination are the least toxic during the study of in vitro toxicity of the disinfectants and detergents studied.

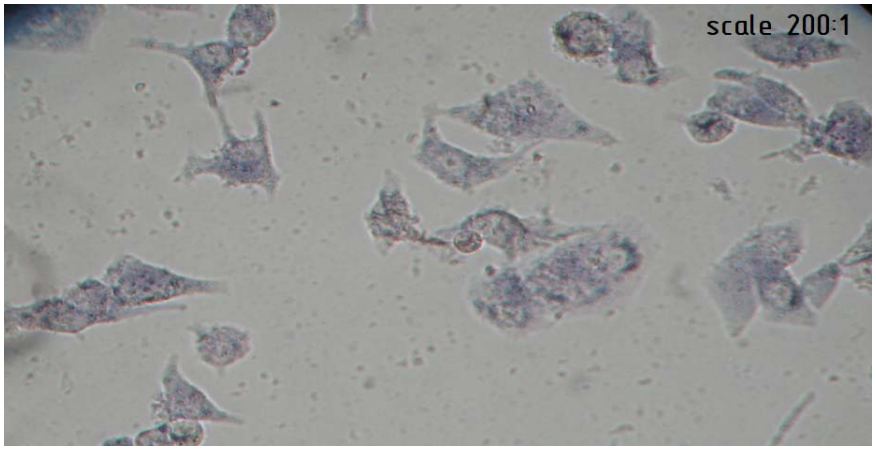

Figure 3 Monolayer of HEK 293 cells (control) ((NRP method)).

Table 2 Cytotoxic effect of disinfectants on cells of the HEK 293-line, M ±m, n = 18 %.

	Cell viability indicators, %									
Concentration, mg/kg	10000	5000	2500	1250	625	312	160	80	40	20
Biodez	0	0	6.90 ±1.48*	14.06 ±4.35*	29.19 ±7.38*	37.15 ±3.69*	**61.03 ±7.38****	79.60 ±33.21	92.87 ±14.76	84.91 ±14.76
Blanidas	0	2.26 ±0.39*	22.60 ±3.91*	44.29 ±3.13*	45.2 ±3.91*	**58.76 ±2.83***	63.28 ±3.91*	70.06 ±1.96**	63.28 ±9.57$	72.32 ±3.91**
Virkon-S	2.36 ±0.18*	11.67 ±0.98*	23.88 ±12.78*	31.84 ±3.21*	42.45 ±5.38*	**53.07 ±4.47***	68.99 ±5.38**	106.14 ±6.34	-	-
Neochlor	0.24 ±0.03*	2.07 ±0.5*	3.62 ±0.98*	16.27 ±2.21*	22.6 ±1.84*	**54.24 ±11.07***	58.76 ±3.69*	63.28 ±3.29*	70.96 ±3.15**	72.77 ±3.52**
Phan	5.42 ±0.68*	14.92 ±1.17*	20.34 ±3.39*	23.73 ±1.69*	27.12 ±6.78*	33.90 ±3.39*	36.61 ±9.02*	43.39 ±2.35*	**54.24 ±6.78***	61.02 ±3.39*
Chlorantoin	7.55 ±0.9*	11.2 ±1.80*	14.59 ±1.85*	18.57 ±3.69*	23.88 ±6.39*	**59.7 ±3.20***	68.19 ±2.58**	75.89 ±3.22$	90.22 ±3.69	92.87 ±3.69

Note: **$** – p <0.05; ** – p <0.01; * – p <0.001 – relative to the control.

Phan, Neochlor, and Blanidas exhibited the most significant cytotoxic effect on the HEK 293 line cells. These disinfectants and detergents at a concentration of 20 µl/l of the growth medium cause about 30-40% cell death.

The IC_{50} of disinfectants based on the effect on the cell line of kidney origin (HEK 293) is Biodez – 173.65 ±19.56 µl/l, Blanidas – 514.20 ±16.54 µl/l, Virkon-S – 402.48 ±24.87 µl/l, Neochlor – 353.94 ±26.45 µl/l, Phan – 44.13 ±5.7 µl/l, Chlorination – 396.76 ±23.66 µl/l (Figure 4). The authors of the scientific works **[44]**, **[45]**, **[46]**, **[47]**, conducted a similar series of experimental studies, but the effect on the cell line of kidney origin (NEC 293) was: origin (HEK 293) is: Biodez – 153.65 ±19.56 µl/l, Blanidas – 414.20 ±16.54 µl/l, Virkon-S – 302.48 ±21.57 µl/l, Neochlor – 323.91 ±22.05 µl/l, Phan – 34.11 ±3.7 µl/l, Chlorination – 396.21 ±20.06 µl/l, in our opinion, such a difference may be related to non-compliance with both the temperature regime during experiments and the non-compliance with time regimes.

Biodez, (the active ingredient is polyhexamethylene guanidine hydrochloride) is more toxic in low concentrations for cells of intestinal origin and somewhat less toxic for cells of renal origin. Blanidas, a chlorine disinfectant, exhibits greater cytotoxicity on cells of the HT-29 line and somewhat less on the HEK 293 cell line. Virkon-S is characterized by high toxicity for the culture of HEK 293, and HT-29 cells. Neochlor (a chlorine-containing disinfectant) is most toxic at low concentrations for cells of kidney origin and somewhat less toxic for cells of intestinal origin. A detergent-disinfectant phan showed high cytotoxicity on HEK 293 and HT-29 cells. Chlorination (chlorinated disinfectant of the third generation) is highly toxic for cells of renal and intestinal origin. The clarified solution of perchloric lime has a significant toxic effect on all the studied cell lines.

The cytotoxic effect of disinfectants was characterized by the phenomena of vacuolization, balloon-like dystrophy, and toxigenic cell lysis (Figure 5). It is worth noting that almost all studied disinfectants and detergents showed high toxicity in vitro on cell cultures of renal and intestinal origin.

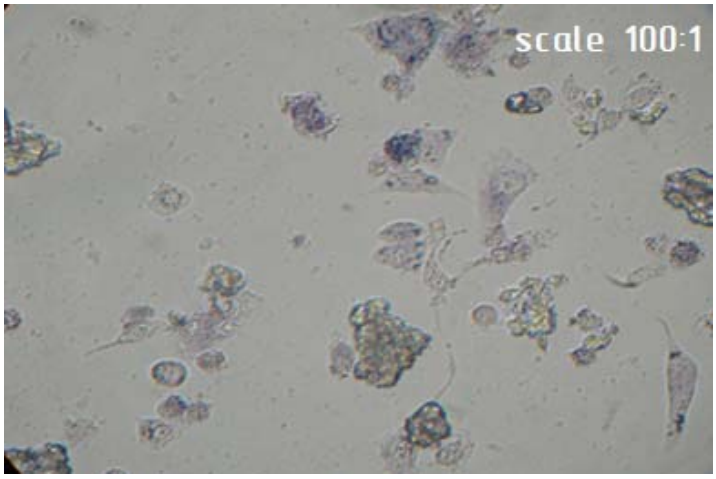

Figure 4 The state of the HEK 293 cell culture monolayer after the introduction of disinfectants into the growth medium at a concentration close to IC50 (MTT method) (×100).

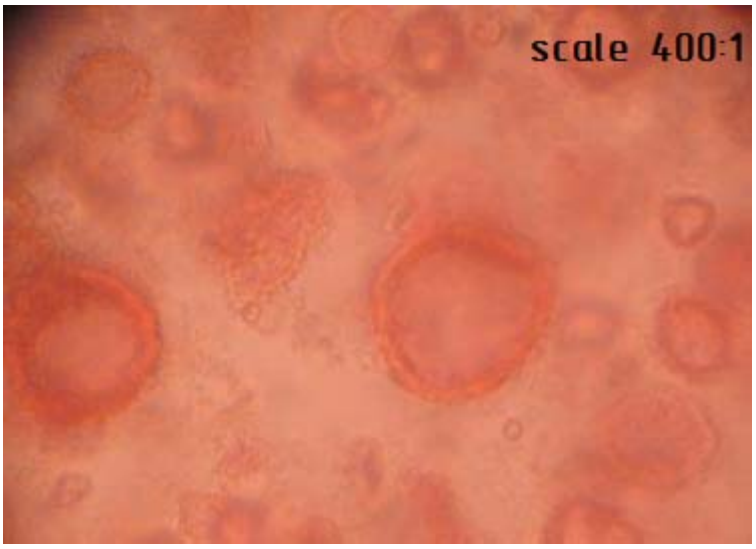

Figure 5 Cytoplasm lysis of HEK 293 cells after the introduction of disinfectants (NRP method).

In further studies, it may be worth evaluating their effect also on other cell cultures, such as those of lung origin and skin.

The average toxic dose (IC50), at which 50% of cells survived and remained attached to the surface, is Biodez – 110.77 ±23.6 µl/l, Blanidas – 594.82 ±36.78 µl/l, Virkon-S – 545.78 ±31.36 µl/l, Neochlor – 603.7 ±49.55 µl/l, Phan – 603.7 ±36.8 µl/l, Chlorination – 393.44 ±54.11 µl/l, clarified solution of perchloric lime – 121.98 ±9.87 µl/l.

It is known that any drug toxicity is determined in model systems, which are both in vivo models using laboratory animals and in vitro based on cell cultures. In both cases, interpreting the results to a greater or lesser extent has the character of possible approximation.

In vitro methods make it possible to explain biological phenomena that, due to the interaction of various factors, are difficult to study in experiments on animals; they contribute to the deepening of understanding by highlighting molecular and cellular mechanisms **[48]**, **[49]**, **[50]**.

To prove the possibility of studying the toxicity of disinfectants at the stage of preliminary toxicological assessment and determining the target organs of toxic effects by in vitro methods, we compared the in vivo toxicity indicators for laboratory animals (according to the literature) and the in vitro cytotoxicity results obtained by us (Table 3). In addition, it shall be emphasized that the determination of cytotoxicity cannot provide complete data on the toxic effect on the entire body.

Based on the comparison of literature data on the toxicity of disinfectants obtained on laboratory animals with our data on cytotoxicity, there can be made a generalization: based on the results of the cytotoxic effect on human cell cultures, preliminary conclusions can be drawn regarding the toxicity of the substance at the stage of screening chemicals for certain purposes, preliminary hygienic regulation, etc. and establish target organs of toxic influence.

Table 3 Toxicity of the studied disinfectants (active substances) in vivo and in vitro.

	Animal	Administration	LD_{50} mg/kg	IC_{50}		$IC_{50\ avg}$
				HEK 293	HT-29	
Biodez	white rats	orally	600 [30]	173.65 ±19.56	60.93 ±9.81	**117.29 ±14.69**
Blanidas	white rats	orally	>2000	514.20 ±16.54	264.30 ±25.12	**389.25 ±20.83**
Virkon-S	white rats white mice	orally	4120 3680	402.48 ±4.87	283.59 ±31.20	**343.04 ±28.04**
Neochlor	rats	orally	2540	353.94 ±26.45	593.70 ±33.86	**473.82 ±30.16**
Phan	white rats	orally	1470	44.13 ±5.7	69.28 ±8.4	**56.71 ±7.05**
Chlorination	Rats (3-colorant in-. 5-dimethyl hydantoin)	orally	542	396.76 ±23.66	289.79 ±30.85	**343.28 ±27.26**
Chlorine lime	Ca $(ClO)_2$. $CaCl_2$ and Ca $(OH)_2$ mixture	orally	-	-	117.35 ±9.44	117.35 ±9.44

Note: * – for a clearer comparison of LD50 and IC50 indicators, the digital values of the latter were expressed as mg/l or mg/dm^3.

However, it shall be noted that in the case of finding the toxicity class of substances, it is impossible to be guided only by the data obtained from the cytotoxic effect. It is necessary, as during toxicological studies on laboratory animals, to take into account the results of other studies, including mutagenic effects, etc.

When comparing the data obtained by us in the experiments and the data of other scientists regarding the toxicity of the studied substances, certain discrepancies can be noted. Thus, the average toxic doses for cell cultures (IC50) of all studied disinfectants are lower than the LD50 obtained in animals.

In addition, data from various scientific sources on the toxicity of disinfectants and their active substances obtained using animals differ significantly depending on the drug's administration method, animal species, duration of exposure, etc. **[51]**, **[52]**. Data on the toxicity of disinfectants for laboratory animals were taken from literature sources.

The results of our research allow us to state that the cytotoxic effect of disinfectants of different chemical natures has a stereotypical character that does not significantly depend on the agent's chemical structure and the cell line used for research (within the class of mammals).

The existing method of determining the residual quantities of disinfectants and detergents on the technological equipment of enterprises for the production, processing, and transportation of food products is based on determining the pH of the surface using universal indicator paper strips with a range of values from 2 to 11. The change in the indicator's colour, detergents, and disinfectants determines the presence of residual disinfectants. But, since a significant part of modern detergents and disinfectants in working concentrations has a pH close to the pH of drinking water (6.0–9.0), this method is not effective enough, and therefore it is impossible to establish the presence of disinfectants and detergents on technological equipment reliably **[53]**.

There are methods for determining disinfectants and detergents in the last portion of washing water. But these methods are specific for each disinfectant, active substance, or chemical class **[54]**, **[55]**.

To assess the risk associated with the use of disinfectants and guarantee the safety of human and animal health, it is required to have sensitive methods for determining their insignificant concentrations in various environments and control the content of residual amounts of toxicants in environmental objects, feed, and food products.

The study's results can be useful during the preliminary testing of disinfectants and the development of a method for determining the residual amounts of disinfectants and detergents in biological objects, particularly milk, and washings from the surface technological equipment, using the determination of cytotoxicity of milk samples.

CONCLUSION

The average toxic dose (IC50), at which 50% of cells survived and remained attached to the surface, is Biodez – 110.77 ±23.6 µl/l, Blanidas – 594.82 ±36.78 µl/l, Virkon-S – 545.78 ±31.36 µl/l, Neochlor – 603.7 ±49.55 µl/l, Phan – 603.7 ±36.8 µl/l, Chlorination – 393.44 ±54.11 µl/l, clarified solution of perchloric lime – 121.98 ±9.87 µl/l, which is two to three times higher than the LD_{50} obtained in animals.

Vacuolization, balloon-like dystrophy and toxigenic cell lysis characterized the cytotoxic effect of disinfectants. It is worth noting that almost all studied disinfectants and detergents showed high toxicity in vitro on cell cultures of renal and intestinal origin.

Studying the cytotoxicity of disinfectants and detergents in vitro using human cell cultures of different histogenesis, which are used during the technological operations of food production, can significantly reduce the number of animals for establishing LD_{50} during the registration procedure of new agents. In particular, based on the results of the cytotoxic effect on human cell cultures, preliminary conclusions can be made regarding the toxicity of the substance at the stage of chemical screening, preliminary hygienic standardization, etc., and there can be identified the target organs of the toxic effect.

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Food taboo and dietary habits among low-income people in Kedah, Malaysia

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ABSTRACT

Food beliefs and taboos about certain foods influence the use and consumption of food in the household. Today, especially in rural areas, some people believe certain foods affect health. This practice has resulted in the non-optimized intake of some food categories. As a result, it is not easy to diversify the types of food for daily diet. This study aims to investigate the beliefs and convictions of rural communities in Kedah regarding certain foods that may influence health. This study also identified the pattern of food intake among residents in rural areas of Kedah State following the belief that some foods can affect health. This study focuses on the rural areas of Kedah State, which include Kubang Pasu, Baling, Pendang, Alor Setar, and Kuala Muda districts. A total of 225 farmers in the rural areas of the selected districts were selected using stratified random sampling. The data were analyzed using SPSS 25 and food intake results. The results of the study show that low-income residents in rural areas of Kedah believe that some foods have an impact on health. A total of 37.11% believe that coffee, carbonated drinks, fresh milk, and low-fat milk cause headaches, stomach aches, heartburn, and nausea, followed by 18.66% who admit that spicy foods such as mutton, beef, and durian cause headaches, high blood pressure and skin problems. The impact of the food taboo has resulted in an overall food consumption rate below 29.9 in households of low-income residents in rural areas in Kedah State. The study's findings suggest that the Malaysian Ministry of Health should develop nutrition and health awareness programs and activities for the rural population. At the same time, there is a need for a comprehensive restructuring of the curriculum and syllabus by addressing the need for healthy eating as early as primary school so that nutrition and health awareness can be embedded in early childhood education.

Keywords: Food taboo, Dietary habits, Low-Income People, Rural Area

INTRODUCTION

Food taboos among communities are inevitable. This is because these food taboos are long-standing. Rural communities are no exception to this practice. This indirectly affects food consumption at the household level. Based on the conceptual framework of UNICEF Food-Care Health, it is explained that cultural norms, taboos, and beliefs contribute to the cause of nutritional deficiencies in households **[1]**. This is because of poor nutrition practices, especially in poor households. Indirectly, this situation has a major impact on household food security status.

Food taboos or beliefs are influenced by domestic eating habits and passed down from generation to generation. Traditional values, customs, and beliefs shape every cultural society and there are consequences if they are ignored or disregarded. A food belief has a long history and is a tradition and norm accepted and embraced by members of society **[2]** food taboos can mean a certain knowledge among family members as well as the obligations and obligations that come with different subjectivities **[3]**. Food beliefs are classified as either permanent or temporary. Permanent food beliefs arise from religious prohibitions, but temporary food beliefs arise from life circumstances **[4]**. This situation tends to reflect a culture where food taboos are imposed in favour of certain groups - the strongest or dominant group, to the detriment of those already vulnerable or marginalized. While food taboos are embedded in community health beliefs, the latter reflects the values associated with a

particular activity or practice. Health beliefs encompass various attitudes, perceptions, and values from different health knowledge sources. Another difference is how health beliefs are formed and maintained in a community. Taboos involve the co-evolution of practices within the framework of social power structures **[3]**. Food taboos are known in almost all human societies as institutionalized rules regulating certain foods' consumption **[5]**. They begin early in an individual's life and may undergo changes throughout life, some to a significant degree, others only when necessary to accommodate external forces **[6]**. Rochow **[7]** defined a food taboo (or ban) as a deliberate avoidance of food for reasons other than a simple aversion due to food preferences. Food taboos can indicate the specific knowledge of certain household members and the responsibilities and roles associated with certain subjectivities. In this way, the awareness and practice of taboos may be most evident within the subgroups most involved in their preservation **[8]**. In addition, people's food taboos are determined by values, attitudes, beliefs, and some environmental and religious circumstances resulting from tradition, culture, and contacts. Food values represent the norms or principles that the individual or group holds about the desirability of food, which (in most cases) is not necessarily related to nutritional value **[6]**. Iradukunda **[9]** explains that food taboos are traditional rules and practices for food selection, preparation, and consumption. The practices are inherited from generation to generation, along with cultural elements through parents, tribal leaders, or influential power holders. At the same time, food taboos are also seen as a way to strengthen the identity and culture of certain communities. At the same time, Oni and Tukur **[10]** stated that there is a relationship between a low level of formal education and adherence to food taboos. Uneducated women are more likely to adhere to food taboos than educated women. Since the proportion of women without formal education in the community is high, indirectly, the practice of taboos in the community is also high. Penafiel et al. **[11]** explained that the belief in taboo eating habits is caused by (i) individuals' mental and physical health status, (ii) lack of knowledge about healthy eating, and (iii) negative attitudes towards different dishes. At the same time, socio-cultural constraints (conflicts in the family, lack of knowledge about nutrition education, competition for food in the family) regarding different diets are also an obstacle to proper food intake. Food taboos also prevent the intake of protein-rich foods such as eggs and meat in the Vihiga district, especially among young children and women of reproductive age **[12]**. In Nigeria, certain species, such as marine and freshwater snails, are strictly protected by some cultures. These foods must not be touched, killed, or eaten **[13]**. Steyn et al. **[14]** found that eating behaviour in African countries is influenced by many factors such as culture, poverty, income, socio-economic level, colonialism, access to goods and services, taboos, and others. This has a direct impact on household eating habits. The study's interesting findings by **[15]** show that the abstinence from eating protein foods practised 20 years ago persists today. This directly harms children under 2 years of age and women of childbearing age who are vulnerable to protein-energy malnutrition. Das **[16]** stated that according to a study in India, ignorance, poverty, and illiteracy are the most important factors explaining false beliefs or misconceptions about food that are directly linked to malnutrition in the population. At the same time, there are also religiously based prohibitions on food. Smith, et al. **[17]** explained that there are also restrictions on food intake in the Christian community. This group avoids certain foods (such as meat and other animal products) during Lent. Similarly, the Catholic community, which fasts on Friday, avoids eating animal products and alcohol on Friday to remember Jesus' crucifixion, but eating fish is allowed on that day.

This situation affects household food habits, especially among low-income people. Nanua, & Mbogoh **[18]** explain food taboos that could negatively affect the dietary behaviour of families. The study by **[4]** shows that meat, eggs, and chicken are avoided in the Karbi tribal area of Assam, India, as it is believed that these foods are spicy and would cause stomach upset and increased bleeding during menstruation. A survey of pregnant women from Maduras conducted by Diana et al. **[19]** found that squid, prawns, pineapple, cabbage, and cold water/ice cream were pregnant women's most common forbidden foods. Squid, prawns, skate, and octopus were taboo for pregnant women of all gestational ages. These types of seafood were considered dangerous during pregnancy and childbirth. Shahar et al. **[2]** found in a study among elderly Malays in Mersing, Johor, that people avoid certain foods because they harm their health. Cucumbers, pumpkin, long beans, aubergine, mustard, leaves, swamp cabbage, coconut shoots, and okra are "cooling foods". In addition, a study by Chakona & Shackleton **[5]** found that 37% of women in the Kat River Valley, South Africa, reported one or more dietary practices that were shaped by local cultural taboos or beliefs. The most commonly avoided foods were meat products, fish, potatoes, fruit, beans, eggs, butternut, and pumpkin. Mohamad et al. **[20]** explained that Ethiopian women living in Addis Ababa avoid eating green chillies, organ meats, and dark green leafy vegetables because they believe these foods are associated with mythical elements.

Food taboo among women pregnancy aims to protect the health of mothers and unborn children **[9]**. Chakrabarti and Chakrabarti **[21]** stated that foods are avoided during pregnancy for the following reasons: (i) miscarriage, (ii) difficulty in delivery, and (iii) fear of abnormalities in the child. Chakrabarti and Chakrabarti **[21]** also found that rural women in West Bengal believed that papaya, parwar (patal), and pineapple would cause miscarriage. Most women admit that "pregnancy is a hot condition" Indirect consumption of hot food during this

period is dangerous for the mother. It can also cause a miscarriage. Tilahun et al. **[22]** also stated that the main reason why pregnant women follow food taboos in the Gedeo zone is due to fear of a large fetus and difficulty in delivery. This causes the birth process to be difficult and prolonged and leads to bleeding.

A study by Mengie et al. [23] found that 67.4% of the female farmers surveyed in eastern Ethiopia adhered to taboo practices in their food intake. This group consumes 67.4% of meat-based foods, followed by chicken eggs (66.2%), carbonated drinks (58.5%), pasta with sauce (56.4%), and milk (36.6%). The main reason this group consumes these foods is the fear of giving birth to a large, heavy child, fear of miscarriage, and difficulties in childbirth. Jones et al. **[24]** also found that women in Madagascar are forbidden to consume eel species during pregnancy, as this can lead to miscarriages or multiple births. At the same time, Middleton et al. **[25]** explain that taboo practices during pregnancy are also associated with childbirth, such as premature births, babies' weight, and children's neurocognitive development. Chakona and Shackleton **[5]** also explain the taboo of eating certain foods among women with the belief and concern that i) that the children will develop bad habits after birth; ii) that they will be born with a disease; iii) that birth will be delayed because certain foods cause women to deliver large babies, and iv) that they cause constant menstruation and infertility. Abu Bakar et al. **[26]** also find that the Mijikenda community is rich in many taboos, some likely influencing food choices. According to the Mijikenda culture, when a mother becomes pregnant with an infant or toddler, "the heat" of the unborn child burns the toddler when the child sleeps with the mother, resulting in severe emaciation. Based on previous studies, many researchers focus more on food taboos in pregnant women and limited discussion of food taboos among low-income people. Therefore, this study aims to identify the food taboo among low-income people and examine the impact on dietary habits in Kedah, Malaysia.

MATERIAL AND METHODOLOGY

Samples

This study focuses on five rural areas in Kedah, Malaysia (Figure 1). A total of 225 low-income people were selected as respondents using stratified random sampling for the studies. The questionnaire was divided into three sections A, B, and C. Section A contains information on the demographic characteristics of the respondents, such as gender, age, marital status, occupation, household size, etc. Section B contains information on the possession of livelihood assets among vulnerable persons. Finally, Section C contains information on food consumption to support respondents' food security based on the Malaysian Food Pyramid.

Figure 1 Study of Location.

The questionnaire contains different questions, including continuous data, a five-point Likert scale, and open-ended questions. The survey was conducted with the help of local research assistants under the guidance of the researchers, and the questionnaire was prepared in the native Malay language (Malay). The survey was conducted

in the form of a face-to-face interview with selected low-income people in selected areas. The researchers visited each respondent to count the number of households so that each household had an equal chance of being selected, and then randomly selected the houses. SPSS Statistic Version 25 was used to examine the data. Food taboos were reported as descriptive data expressed as mean. Summarising the data collection and analysis, the data is presented in Figure 2. The pattern of food consumption was then approximated using the approach of **[27]** in Figure 3.

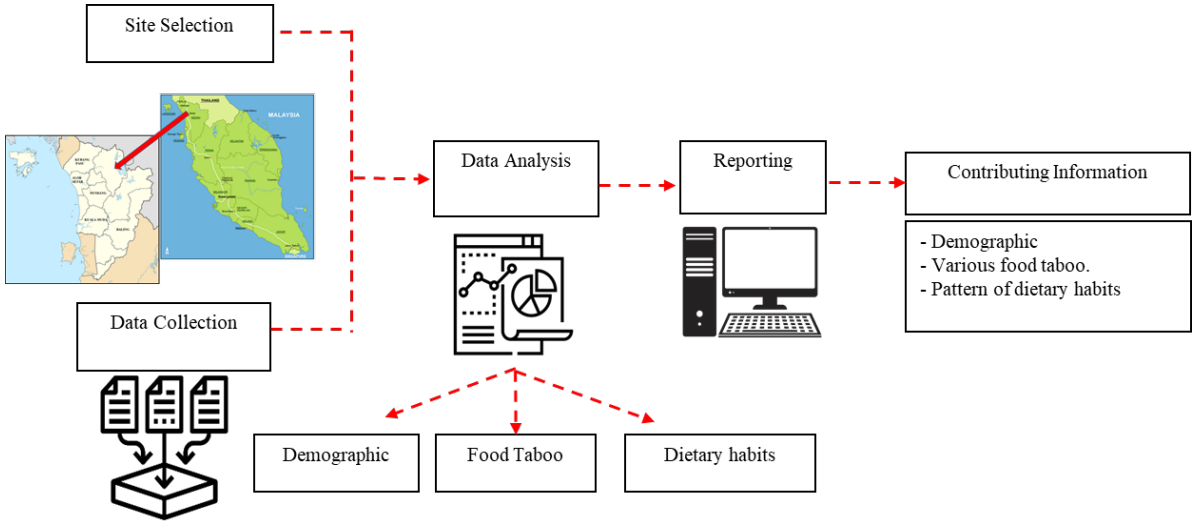

Figure 2 Diagram of data collection and analysis proces.

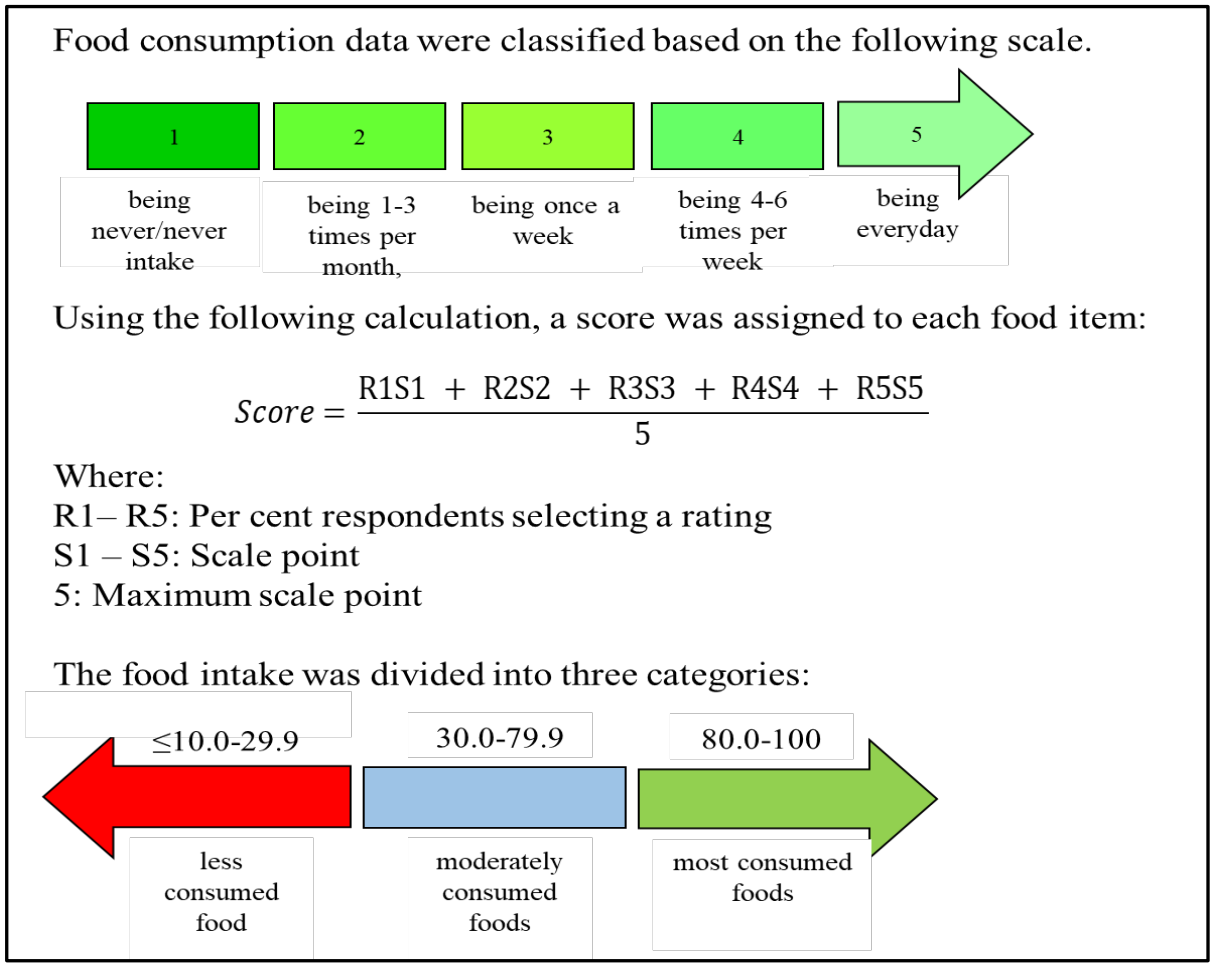

Figure 3 Food consumption calculation.

RESULTS AND DISCUSSION

The study finds out 0.89% of the respondents have no formal education, 8.0% have no formal education (religious school), and 57.3% have only some information from secondary school. 42% of the respondents had

completed primary education and 27.4% had completed primary education. 18.9% of the respondents do not attend school, and only 11.9% receive informal education (religious school). Figure 4 shows that overall the respondents have a low level of education and lack skills and training. The low level of education and lack of skills means that this group has no opportunity to diversify its sources of income. According to Economic Planning Unit (EPU) **[28]**, only 36.4% of household heads in Malaysia had a Sijil Pelajaran Malaysia (SPM) or equivalent educational qualification, while 36% had no qualification and 84.6% had only secondary education. Lack of knowledge makes it difficult to find a better job that improves socioeconomic status and, thus, the quality of life **[29]**. Due to their lack of education,89.9% of household heads work in marginal and semi-skilled jobs **[28]**. This situation has led to limited income, rising expenses, and cost of living being the main cause of the problem of food insecurity in low-income households with limited access to adequate nutrition **[30]**. Besides that, the distribution of respondents by marital status there is 83.11% of the total respondents are married, 0% are single, and 16.89% are widowed.

Respondents aged 43 to 52 years had the highest%age of responses (31.1%), followed by respondents aged 53 to 62 years (30.67%) and respondents aged more than 63 years (24.89%). In comparison, 12.44% of respondents were between 23 and 42 years old, and 0.89% were below 23 years old. The average age of the respondents was 54 years (Figure 5). In terms of land ownership, 48.0% of the respondents own less than 1.0 hectares of land, followed by 1.0 to 3.9 hectares (41.78%), 4.0 to 6.9 hectares (8.44%), and more than 7.0 hectares (1.33%). The average landholding is 1.48 hectares (Figure 6).

The distance from the home to the town is important in measuring the ability of low-income people to access food. The average distance for low income to get food in the city is about 8.56 km (Figure 7). However, food deserts also affect low-income households when the distance is more than 12 km **[31]**. The distance to access food will leave low-income groups exposed to food desserts. Sparks et al. **[32]** define food deserts as areas of high poverty (with a poverty rate of 20% or more) with little access to supermarkets. This is consistent with the USDA **[33]** definition, which states that areas of food dessert arise when numbers of low-income residents have little access to supermarkets or large grocery stores. Cordero et al. **[34]** also agree that food deserts are areas suffering from a lack of physical and social facilities, including a lack of access to food and high nutritional balance. USDA **[33]** also stated that areas of food deserts have little or no access to nutritious food. In short, an insecure food area is where households struggle to obtain sufficient and nutritious food from nearby supermarkets or convenience stores. Communities that have poor access to nutritious and sufficient food have the potential to contract these diseases Coveney & Dwyer **[35]** found that access to nutritious food is poor in rural areas. This is due to high food prices, limited food choices, high rates of diet-related diseases, and poor access to fresh and nutritious food **[36]**. At the same time, Rodriguez and Grahame **[37]** also reported that the transport factor is one of the barriers for low-income residents to get groceries as they do not have access to public and private transport and cannot afford the transport cost. According to the study, 92.44% of those surveyed own a motorcycle. This is followed by 62.67% of respondents who own a car. 16.6% of those surveyed stated that they owned a lorry/van. This shows that the low income in this area has no barrier to accessing food based on transportation.

In the electrical/household appliances category, 100.00% of the respondents owned a television, 88.0% owned a radio, 100.00% owned a gas cooker and 98.66% owned a refrigerator. Communication devices in this study are mobile phones and mobile phones with internet access. Figure 8 shows that 76.44% of the respondents owned a mobile phone.

The classification of standard of living of people in Malaysia is divided into three main categories, viz T20, M40 dan B40 (Table 1). The main purpose of this classification is to facilitate the planning, monitoring, and implementation of the program in a targeted manner according to the needs of each population category. Low-income households in the study were classified as poor because their income was less than MYR2499 compared to RM1941, the actual income of the low-income households in the studies. Meanwhile, the average monthly household expenditure of RM 1169.10 and RM 465.64, or 39.83% was spent on food and beverages. These results related to the finding by Arnawa et. al, **[38]** found in their study that the highest expenditure was on the consumption of cereals, especially rice, which accounted for 38.32% of the farmers' average expenditure on food consumption, followed by expenditure on the consumption of food of animal origin, which reached 26.40%. Expenditure on the purchase of fruits and vegetables ranked third.

Table 1 Poverty Line Income.

Decile Group		Income threshold (RM)
T20 the household earning the highest 20% of the total income of Malaysians	T2	>15,039
	T1	10,960-15,039
M40 households earning 41% to 80% of the total income of Malaysians	M4	8,700-10,959
	M3	7,110-8,699
	M2	5,880-7,099
	M1	4,850-5,879
B40 households with the lowest income of 40%	B4	3,970-4,849
	B3	3,170-3,969
	B2	2,500-3,169
	B1	<2,499

Note: Source: **[39]**, **[40]**.

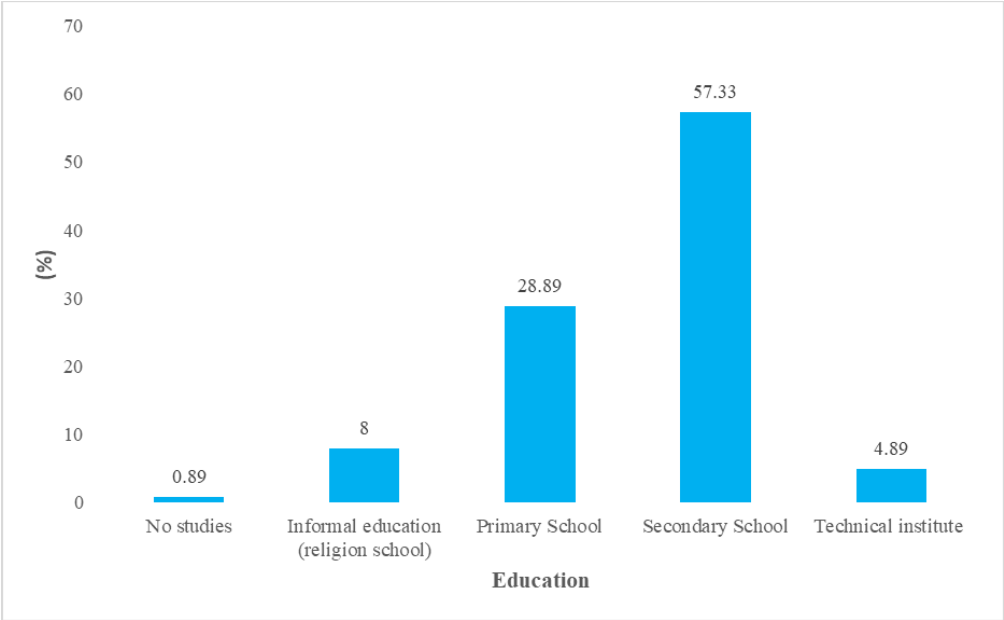

Figure 4 Education level.

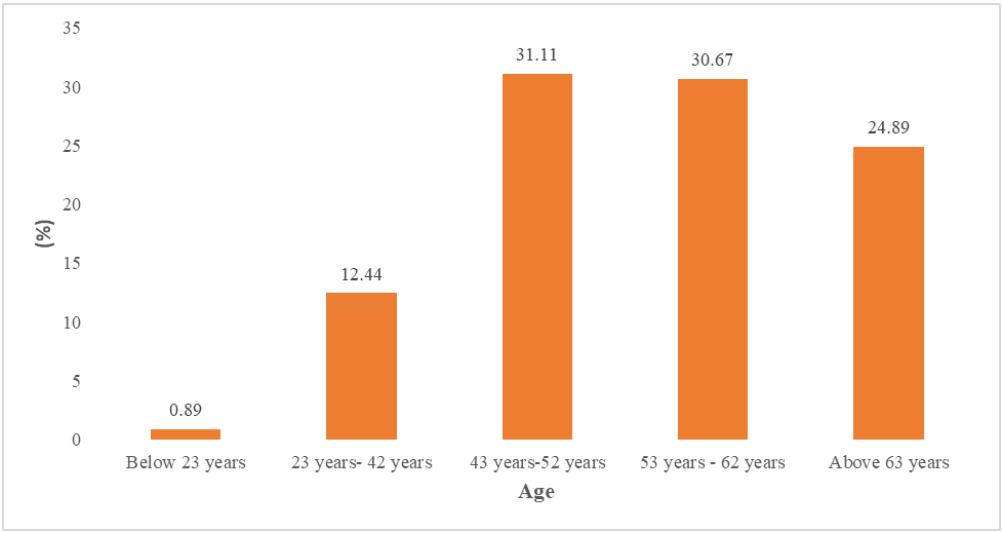

Figure 5 Age.

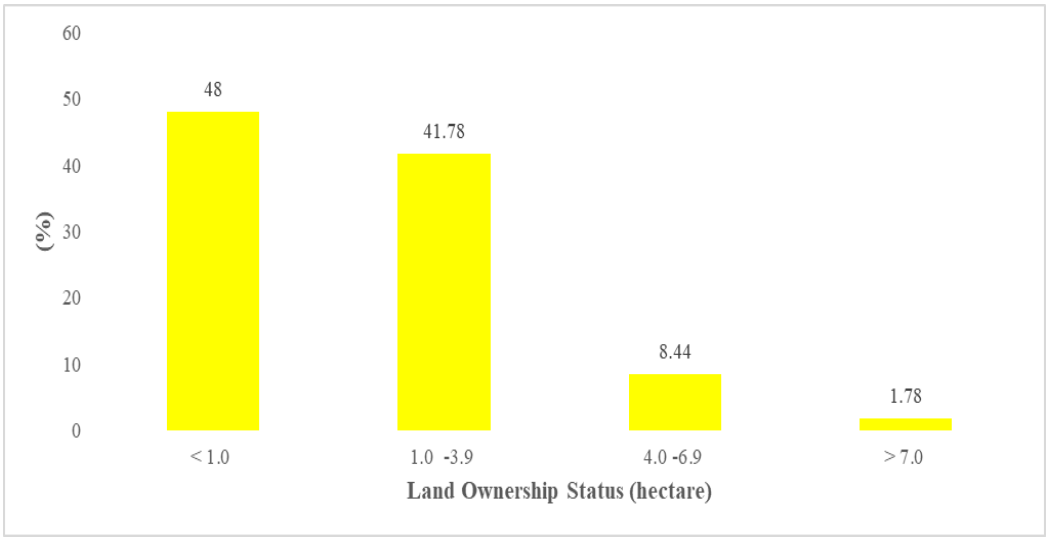

Figure 6 Land ownership status.

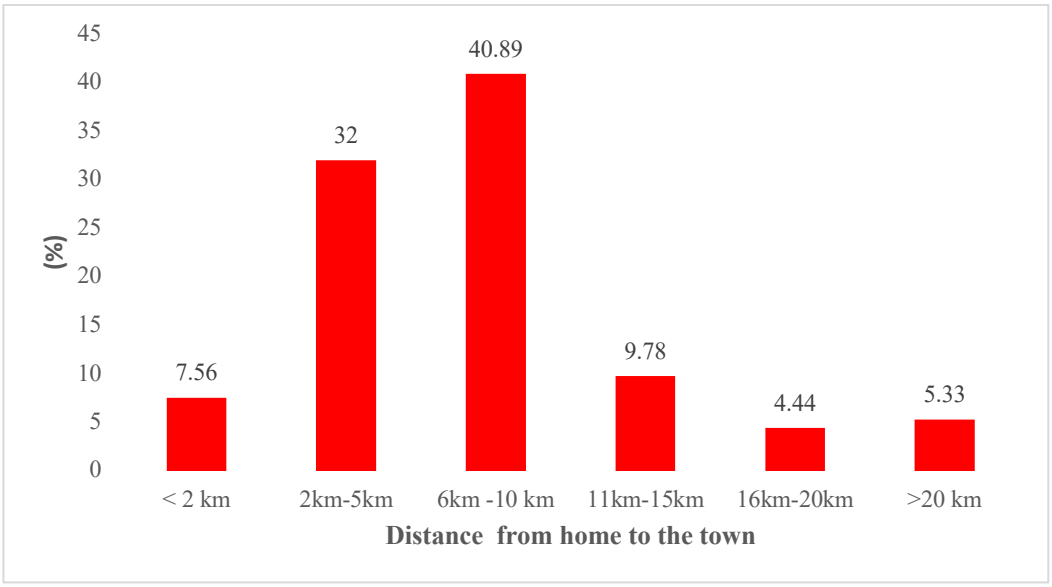

Figure 7 Distance from home to town.

Figure 8 Asset ownership.

Food Taboo Status Among Low-Income Group

This study found that rural communities in Kedah State still believe in taboos on certain foods. Practical and cultural factors are the main factors that lead to this belief still being practised. Adherence to this belief and taboo is difficult to separate in society as it is integrated into the daily life practices of this group. Although there is no study from a health perspective on the effects of eating certain foods on health, this belief is still maintained today. Food consumption also influences behaviour among consumers. Many complex and dynamic factors influence food choices and consumption, as this is closely related to each individual's personality **[41]**. In the context choose food, Arbit et al **[42]** identified five terms related to food, namely sacred, healthy, social, and vinegar. Regarding the sacred, food is closely associated with religious practices and rituals **[43]** Under the moral aspect, food refers to how food choices and eating habits have a positive or negative impact depending on the views of the individual **[44]**. When discussing health, we discuss food choices as the most important prerequisite for a healthy life **[45]**. While in terms of the social aspect of food, which reacts with potential food where it is shared, consumption is accelerated by the community **[46]**. Aesthetics refers to the experience of eating that connects pleasure with the consumption of food **[43]**. However, Gallagher et al. **[47]** also explained that attitudes, social norms, and behavioural control influence the intention to consume food. Attitude strongly influences intention, followed by social norms of behaviour control. This matter is directly related to the practice of taboo food in society. Brown **[48]** explains that social psychologists explain how feelings, thoughts, beliefs, intentions, attitudes, and goals influence behaviour in determining the actions of something. This matter is closely related to the selection and consumption of food in households. At the same time, food neophobia is seen as directly related to attitude and personality. Eertmans et al. **[49]** explain that neophobia can cause some people to avoid certain new foods and reinforce the reasons for different attitudes toward food choices. Neophobia is also a mechanism that protects people from physical harm but is also an obstacle to developing an appreciation for different foods **[50]**.

Table 2 clearly shows the tabooing of food among the low-income groups in the rural areas in Kedah State. The study classified tabooed foods into six main categories: cooling, windy, hot, spicy, itchy, and drinks. The study results show that 37.11% of this group do not drink coffee, carbonated drinks, whole milk, and low-fat milk. They believe these drinks cause headaches, stomach aches, indigestion, and nausea. This practice is also passed on to all their family members. Mutton, durian beef, is also taboo among this group. A total of 18.66% of rural dwellers in Kedah State believe these are spicy foods with health side effects. They believe they will get headaches, high blood pressure, and skin problems (enzymes) if they eat these foods. Similarly, chicken, red meat, seafood, and chicken eggs are classified as itchy foods and cause skin problems (enzymes) when eaten.

Rural communities in the state also believe that some foods, such as cassava shoots, bamboo shoots, yardlong beans, sprouts, groundnuts, and green beans, are classified as windy foods. When eaten, they cause a bloated stomach and nausea. The same goes for eating spicy foods like bamboo shoots, pineapple, and vinegar, which can cause heartburn, stomach pain, and miscarriage. Those with health problems such as numbness/pain in the joints rely on and avoid foods classified as cooling foods such as yardlong bean, star gooseberry, and sprouts. This situation clearly shows that the beliefs and practices of previous communities have been passed down through generations to the present day. Although there are no scientific studies on the effects of food on health, the community still holds on to the belief of the effects of food intake on health. This directly affects the pattern of food consumption in households. In addition, the constrained finances of the low-income group have directly limited food consumption in the household.

Table 2 Food Taboo among low-income people in a rural area, Kedah.

Classification	Food items	Reason for avoidance	age (%)
Cooling foods	yardlong bean, star gooseberry, sprouts	joint numbness/pain	2.66
Windy foods	cassava shoots, bamboo shoot, yardlong bean, sprouts, groundnut, green bean	windy stomach, nausea	5.33
Hot foods	mutton, beef, *durian*	headache, high blood pressure, skin problems (eczema)	18.66
Sharp foods	bamboo shoot, pineapple, vinegar	heartburn, stomach-ache	1.33
Itchy foods	chicken, red meat, seafood (e.g. prawns, prawns paste, mussels), egg chicken	skin problems (eczema)	5.34
Beverages	coffee, carbonated drink, fresh milk, low-fat milk	headache, stomach-ache, heartburn, nausea	37.11

Dietary Habits Among Low Income

Based on the research conducted, dietary habits among low-income groups in rural areas of Kedah State are classified into several major categories as shown in Table 3. The classification according to these categories aims to assess the dietary behaviour of this group. Indirectly, the form and type of food that has priority can be determined. At the same time, the consumption value for each food can be determined more precisely.

Table 3 clearly shows the dietary habits of the low-income groups in the study. For the vegetable category, vegetables that are close to homes, such as bamboo shoots, cassava shoots, and fiddlehead, are also the first choice of this group. However, this vegetable category has a low score of below 29.9. Vegetables available in the market, such as cabbage, choy sum, spinach, and yardlong beans, are also preferred by this group and consumed with a moderate score. Choy Sum has the highest score of 51.64 in the vegetable category compared to other vegetables. Consumption of herbs *(ulam*) is also high, with a score of 41.87. This group believes that taking herbs helps to increase the body's resistance. The results of this study agree with the study by Ballesteros et al. **[51]**, where a study among households in Argentina found that the average intake of fresh fruits and vegetables is very low, well below the levels recommended by the World Health Organization (WHO) and Dietary Guideline for Argentine Residents. The daily consumption of fruit and vegetables is significantly lower among low-income groups due to the lower level of education. However, this study also found that the consumption of fruit and vegetables increases with household income. This shows that the income factor plays an important role in the consumption of fruit and vegetables. The study by Mustafa et al. **[52]** in Bangladesh also stated that low-income groups are more likely to believe in taboo practices of eating vegetables and fruits in their daily diet. Up to 92% of the rural population of Bangladesh forego fruit and vegetable dishes on the daily menu. Most of the rural population is uneducated and does not believe in the importance of fruit and vegetables in their diet. They believe that rice is more important than vegetables

Low-income people in a rural area in Kedah, also consume chicken and chicken eggs as one of the protein sources in their daily diet, with a value between 39.38 and 42.13. This study is related to a study by Narimah et al. **[53]**. Nevertheless, meat consumption is low at 11.91. The reason for this is the high price of meat in the market. This situation led the group to prioritize staple foods for household needs. The study's results also show that this group consumes fruits such as apples, grapes, watermelons, and limes less. The score of 38.04 indicates moderate consumption of this food category. These results related to a study by Diehl et al. **[54]** also found that 50% of low-income residents in Jakarta and India consume chicken eggs, fish, meat, dairy products, and fruit, but only 20% of poor households due to the high prices of these foods.

The same is true for cereal and legume-based foods, where consumption is low at 26.04 to 27.55. The use of food supplements is also low among low-income groups in rural areas, with a score of 28.70. This is because this group is aware of the need for a healthy diet. Those who take herbs can substitute for taking supplements. Seafood such as squid, prawns, and crabs are also an option, but the consumption of these foods is moderate with a value of 35.52. The consumption of chub mackerel is the top choice of this group compared to other fish species, with a value of 78.48, followed by hardtail scad (36.09), slender sprat (34.12), and black skip jack (32.89). For the other categories of saltwater and freshwater fish, consumption is low at 29.9. A study by Djunaidah **[55]** explains that fish consumption is low due to a lack of public understanding and knowledge of the benefits of fish consumption. At the same time, the lack of facilities and infrastructure leads to this problem.

Meanwhile, for beverage categories, tea, coffee, and plain water have a high score (most consumed foods). Cultural factors and long-standing practices have led this group to continue to consider this beverage as their first choice. The study results also show that carbonated, whole milk and low-fat drinks are less popular among this group.

From the research, the average respondent in this study has an income below the prescribed poverty line of RM2449. This group is also classified as poor and vulnerable. This group is also entitled to zakat support from the zakat department under the category of *Asnaf* **[56]**. The low level of education has led this group to adhere to traditional practices and beliefs.

At the same time, the low-income groups in the rural areas of Kedah State also believe that eating certain foods impacts health. Although there is no scientific study on the impact of certain foods on health, this belief has become integrated into the lives of this group. In general, the dietary habits of this group are low, with food intake focused on low prices. This group places more emphasis on quantity than the quality of food. This situation is caused by limited income and ultimately affects the body's nutritional needs. This situation critically affects children's growth and leads to stunting in children **[57]**. In this study, many low-income people still believe in the taboo of eating. It is not easy to change people's eating habits and beliefs. 37.11% believe that coffee, carbonated drinks, and fresh and low-fat milk cause headaches, stomach aches, heartburn, and nausea. This situation influences people's dietary habits regarding these drinks. In addition, people's low education also leads them to

still hold on to the taboo of food. It is very difficult to break the food taboo in communities because this issue is deeply rooted in life, and it is difficult to leave the practice.

Moreover, the rising prices of commodities also affect the food habits of this group. Wardle et al. **[58]** stated a strong relationship exists between knowledge of nutrition and food consumption. The analysis results show that knowledge about nutrition and food intake depends on the education level and occupation type. Indirectly, this statement explains that the level of education plays an important role in determining food intake at the household level.

Table 3 Food consumption score among low-income people.

Food categories	Less consumed food (≤10.0-29.9)		Moderately consumed foods (30.0-79.9)		Most Consumed Foods (80.0-100.0)	
	Food item	**Score**	**Food item**	**Score**	**Food item**	**Score**
Vegetables	Cassava shoots	24.62	Yardlong bean	38.92		
	Water spinach	24.89	Sprouts	42.31		
	Star gooseberry	25.24	Spinach	43.38		
	Fiddlehead	25.24	Cabbage	45.78		
	Bamboo shoot	26.85	Choy Sum	51.64		
			Herbs (ulam)	41.87		
Chicken & Egg			Chicken	42.13		
			Egg Chicken	39.38		
Meat	Beef	25.78				
	Mutton	11.91				
Fruits	Seasonal fruits	29.79	Fruits	38.04		
Beverage	Carbonated drink	21.07			Tea	89.43
	Full cream milk	18.49	Condensed milk	36.63	Coffee	84.18
	Low-fat milk	20.09			Plain water	93.51
Bean/grain	Bean Curd	26.66				
	Groundnut	27.55				
	Green Bean	26.04				
Healthy product	Healthy product	28.70				
Seafood			Seafood (Prawn, Squid, Crab)	35.32		
Saltwater fish	Sardine	28.80	Hardtail Scad	36.09		
	Japanese Threadfin Bream	28.61	Slender Sprat	34.21		
	Yellowtail Scad	24.53	Blackskip Jack	32.89		
	Promfret	18.31	Chub Mackerels	78.48		
	Mangrove Red Snapper	18.23				
	King Mackerel	18.04				
	Barramundi	16.98				
	Snapper	16.18				
	Grouper Fish	15.74				
	Hardtail Scad	36.09				
	Slender Sprat	34.21				
	Blackskip Jack	32.89				
Freshwater fish	Tinfoil Barb	21.15				
	Catfish	22.48				
	Gourami fish	22.84				
	Climbing perch	24.45				
	Channa Striata	24.63				
	Tinfoil Barb	21.15				

CONCLUSION

In general, the food consumption among low-income groups in the rural area of Kedah is less consumed, with a score below 29.9. This is due to poverty, culture, and high prices, which result in some foods not being included in the daily diet. At the same time, food taboos also influence dietary habits among that people.

In reality, many factors influence dietary habits, including climate, availability, religion, emotions, taste, economics, local agricultural practices, and traditions. Therefore, the task of changing food taboos is not easy. Changing dietary habits and beliefs is a complex process that could be achieved through consumer awareness and education. The study's findings have several implications for creating effective nutrition initiatives. The Ministry of Health should continue to promote healthy lifestyles, raise awareness and provide nutrition education. However, basic but effective nutrition techniques must be discovered and implemented carefully. These approaches could also be used to increase nutrition knowledge and awareness and motivate people to change their eating habits. Future nutrition policies should ensure the food consumption of low-income households by providing adequate supplies of staple and micronutrient-rich foods. To this end, policymakers should develop and implement an intervention to raise awareness and prevent malnutrition related to food consumption among children. Knowledge about food consumption should be emphasized more in the curricula, especially in preschool and primary school. In addition, policymakers, health authorities, program planners, and community leaders should work together to plan and implement effective interventions and other intervention programs for Malaysians, especially in low-income households, that include nutrition education and healthy food environments to promote healthy lifestyles.

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

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The study of functional and technological properties of vegetarian ice cream

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ABSTRACT

The use perspective and expediency of plant-based milk, enriched with fiber when combined with organic products, biobased products, and sugar substitute products, has been substantiated in the manufacturing process of vegetarian ice cream. When combined with pumpkin fiber, stevia, bananas, pistachios, coconut oil, and coffee beans with different functional and technological properties, rice milk has a purposeful influence on organoleptic and Physico-chemical properties of food products. Accordingly, the expediency of added rice milk (62%) has been determined to optimize vegetarian ice cream's vitamin and mineral composition. The optimal component ratio has been determined employing experimental studies and multi-criteria optimization: for ice cream "Banana & Pistachio": rice milk – 62%, pumpkin fiber – 2.5%, – 0.5%, banana – 16%, pistachio – 6.8%, coconut oil – 12.2%; “Coffee and chocolate”: rice milk – 62%, pumpkin fiber – 4.8%, stevia – 4%, cocoa powder – 7%, coffee beans – 8%, coconut oil – 14.2%, It has been found that the main physicochemical parameters of the vegetarian ice cream depend on the chemical composition of the ice cream mixture and its freezing conditions. Thus, when the fat content increases, the stability of air bubbles increases, but their sizes decrease. The study results make it clear that the increase in the fat amount is good for the ice cream structure and consistency, while the distance between the fat balls decreases, which, in turn, helps to obtain the product with the smaller ice crystals.

Keywords: vegetarian, functional product, vegetable raw material, ice cream, technology

INTRODUCTION

The primary importance is given to dairy products, considering their biological value, in the organization of a healthy diet. The above is also true of such dairy desserts as the ice cream, the nutritional value of which is due to complete proteins, highly-digestible fats, essential amino acids, calcium, and phosphorus salts, which are vital for the proper functioning of the human body **[1]**, **[2]**. However, the demand for foods of plant origin, having different enhanced biological values, is one of the main trends in the modern world food market **[3]**. Today, the trend to use foods of plant origin instead of animal origin is spreading worldwide. Some nutritionists believe that this is because such foods are better digested and do not have harmful hormones and antibiotics found in foods of animal origin **[4]**, **[5]**. Moreover, the share of people without lactose tolerance and milk allergy, who are forced to use lactose-free products or fully exclude milk proteins from the diet, replacing them with vegetable ones, has increased recently. Such products as rice, soy, almond milk, and others are alternatives to dairy products **[6]**. Considering the increasing demand for vegetarian products, the prospective line of the industry development is the targeted manufacture of vegetarian ice cream with high functional and technological properties **[7]**. There the ice cream is based on flora vegetable milk made of germinated soybean and hemp seeds. Such ice cream is better than traditional milk ice cream in terms of fatty acid composition and vitamin content. However, the development

studies of new types of dry ice cream mixtures made of vegetable raw materials without the components of dairy origin are very limited.

However, there are no conceptual developments in the study direction of the consumer properties of vegetarian ice cream using rice milk enriched with fiber when combined with organic, biobased, and sugar substitute products.

Scientific Hypothesis

Rice milk with pumpkin fiber, stevia, bananas, pistachios, coconut oil, and coffee beans will purposefully affect vegetarian ice cream's functional and technological indicators. Accordingly, improving combined ice cream technology will contribute to expanding the range of ice cream for vegetarians.

MATERIAL AND METHODOLOGY

Samples

The samples of the vegetarian ice cream "Banana & Pistachio" (Figure 1) and “Coffee and chocolate” (Figure 2) were developed due to the study objects. The fruit and berry ice cream manufactured by Alpro “Almond&Salted caramel” with the following components was selected as control 1: drinking water, soluble maize fiber, sugar, soluble oils (rape: shea: coconut), almond-fructose syrup, dextrose, emulsifier of mono- and diglycerides of fatty acids, natural flavour, sea salt, stabilizers (carob bean gum, guar gum, carrageenan), starch, caramelized sugar. The chocolate ice cream “Coconut Milk-Chocolate” manufactured by TM Dalana, with the following recipe composition was selected as control 2: coconut milk, water, sugar, glucose syrup, chocolate (sugar, fat-extracted cocoa powder, cocoa butter), guar fiber, citrus fiber, lemon juice concentrate.

Figure 1 Banana & Pistachio.

Figure 2 Coffee and chocolate.

Chemicals

Sulfuric acid (brand A, chemically pure, Khimlaborreaktiv LLC, Ukraine).

Phenolphthalein solution (NaOH, (Novokhim), Kharkiv, Ukraine).

Sodium hydroxide (NaOH, (Novokhim), Kharkiv, Ukraine).

Animals, Plants and Biological Materials

Rice milk, pumpkin fiber, stevia, bananas, pistachios, coconut oil, cocoa powder, and coffee beans, which satisfy the standard technical documents, were used for the studies.

Instruments

Centrifugal for ice cream test bottles (Nova Safety, Germany).

Water bath Funke-Gerber WB-436 A, Germany).

Ice cream test bottles (butyrometers) Funke-Gerber, Germany).

Ice cream freezer (Spaceman 6225 Berg, Germany).

Laboratory microscope (Daffodil MCX-100 MICROS, Germany).

Laboratory Methods

Organoleptic quality evaluation was carried out according to the five-grade scale. The ice cream overrun was determined with the use of the freezer. A glass with a capacity of 150 cm^3 or more was used. The same glass was alternately weighed empty with the mixture and the ice cream. The glass was filled with the ice cream to the brim **[8]**.

The ice cream overrun (B), % was calculated using the formula (1):

$$B = \frac{M2 - M3}{M3 - M1} \times 100 \quad (1)$$

Where:

M1 is the weight of the empty glass, g; M2 is the weight of the glass filled up with the mixture, g; M3 is the weight of the glass filled with ice cream, g.

The weight fraction of fat in the ice cream was determined by the Gerber method. It was determined with the use of the butyrometer. The summary of the method lies in the dissolution of the ice cream proteins with sulfuric acid, resulting in the fat balls losing their shell and combining into a single fatty layer **[8]**.

Description of the Experiment

Sample preparation: 4 types of the ice cream were used for the studies: developed – "Banana & Pistachio" and "Coffee and chocolate"; control ones – fruit and berry as well as chocolate. The following raw materials were used when manufacturing the vegetarian ice cream: rice milk manufactured by The Bridge (organic); pumpkin fiber manufactured by Golden Kings of Ukraine according to TU U 15.3-24239651-010:2009 **[9]**; stevia according to TU U 15.8-30729147-003-2004 **[10]**; coconut oil (bio) according to DSTU 4562:2006 **[11]**; banana according to DSTU ISO 931:2019 **[12]**; pistachio according to DSTU EEK OOH DDP-10:200 **[13]**; cocoa powder (organic) according to DSTU 5006:2017 **[14]**; coffee beans according to GOST 6805-97 **[15]**.

Number of samples analyzed: During the experimental studies 4 samples of vegetarian ice cream were used, namely: two experimental and 2 control ones. The ice cream "Banana & Pistachio" experimental sample was compared with control 1; "Coffee and chocolate" – with control 2.

Number of repeated analyses: The study was repeated 5 times, while the mathematical statistics methods processed the experimental data.

Number of experiment replication: Each experiment was carried out five times, and the number of samples was three, resulting in fifteen repeated analyses.

Design of the experiment: Production of vegetarian ice cream "Banana & Pistachio" and "Coffee-chocolate" according to the general technological scheme, which consists of the following operations: reception and preparation of raw materials, dosing and mixing of components, filtering (impurity removal), pasteurization, homogenization, cooling, ageing, freezing, hardening and post-hardening of the mixture, packaging, and storage of the finished product.

Statistical Analysis

Statistical processing was performed in Microsoft Excel 2016 in combination with XLSTAT. The accuracy of the obtained experimental data was determined by the Student's test with a confidence probability of ≤ 0.05 for the number of parallel determinations of 5 minimum. The linear programming problems were solved with the use of setting the MS Excel spreadsheet "Solution search" (Excel Solver).

RESULTS AND DISCUSSION

Milk is the main raw material for manufacturing ice cream **[16]**, **[17]**. The absence of milk from the alimentation may cause many nutritional disadvantages, such as calcium, phosphorus and vitamin deficiencies, which could be supplemented by consuming fruits and vegetables **[45]**. Due to the nutrition specifics of vegans, there is a need to use vegetable milk for the ice cream to be manufactured The analysis of scientific and patent information revealed that there are about 35 types of "vegetable milk" today **[19]**, **[20]**. Such types of milk as rice, soybean, almond, sesame and hempseed milk could be used for vegetarian ice cream **[45]**. In the study of **[46]** are presented as raw material hemp milk, and sesame milk are in the formulation of the vegetable ice cream. Sesame milk contains sesamol which causes resistance against oxidation, and lignin, which has antioxidant properties and a considerable quantity of vitamin E. Scientists have proven that soy milk seems to be a good choice for cow milk substitution because is a rich protein source with balance essential amino acids **[47]**. Many benefits of soy milk consumption are related to lowering the risk of cancers, diseases associated with heart and vascular systems, hypercholesterolemia, diabetes, and bone and kidney-related diseases **[45]**. In the study of **[47]**, being easy to be digested, coconut milk is a very rich source of minerals and antioxidants. The prevention of arteriosclerosis and other heart-related illness is given by coconut milk's high oleic and lauric acids **[48]**. However, these types of milk have certain disadvantages during the production of vegetarian ice cream – also a disadvantage, which needs to be controlled, is the stability of the colloidal system.

Accordingly, rice milk was added to the developed vegetarian ice cream to obtain the necessary consistency and texture of the product.

Rice milk reduces the cholesterol level, controls the blood sugar level, provides energy for the body, enhances the performance of the digestive system, supporting healthy intestinal flora **[18]**. Rice milk also has a good influence on skin health due to para-aminobenzoic acid, which is one of the components helping to protect the skin from the sun's negative rays. Rice milk is also an antioxidant and a powerful anti-inflammatory drug for the skin **[41]**, **[42]**. And also improves the consistency and structure of finished products. Inositol alcohol contributes to powerful cell growth, delays the aging process, and normalizes blood circulation **[43]**, **[44]**. The energy content of rice milk is not-too-high compared to other types of vegetables – 47 kcal per 100 g **[19]**, **[22]**.

During the production of vegetarian ice cream, the choice of additional components is no less important. According to research results, **[49]** fruits and vegetables represent the main ingredient for the sorbet, being an important source of tanning substances, ascorbic acid, β-carotene, anthocyans, chlorophylls, pectin, organic acids, proteins and many other bioactive compounds.

So, pumpkin fiber was added to the recipes to remove toxic substances and excess cholesterol, stevia to enhance the performance of the gastrointestinal system, and coconut oil to stabilize the product's shape better **[24]**, **[25]**.

Table 1 Recipe composition of vegetarian ice cream "Banana & Pistachio".

Component name	g/100 g of products
Rice milk	62
Pumpkin fiber	2.5
Stevia	0.5
Banana	16
Pistachio	6.8
Coconut oil	12.2

Table 2 Recipe composition of vegetarian ice cream "Cocoa and chocolate".

Parameter name	g/100 g of products
Rice milk	62
Pumpkin fiber	4.8
Stevia	4
Cocoa powder	7
Coffee beans	8
Coconut oil	14.2

The organoleptic parameters of the food product are one of the first evaluation criteria of the consumer's perception of the finished product **[20-26], [40]**. They depend on the type of raw materials used and the food technology. Accordingly, the organoleptic quality evaluation of the finished product was carried out according to the five-grade scale to choose the best functional food composition in the recipes of the vegetarian ice cream. The results are listed in Table 3.

Table 3 Organoleptic evaluation of vegetarian ice cream according to the five-grade scale.

Parameter	Control 1	"Banana & Pistachio"	Control 2	"Coffee and chocolate"
Appearance	4.5	4.6	4.3	4.7
Taste	4.5	4.7	4.6	5
Smell	4.4	4.7	4.5	4.8
Structure	4.1	4.3	4.5	4.5
Consistency	4.4	4.4	4.4	4.4
Colour	4.7	4.8	4.5	4.9
Overall evaluation	4.4	4.5	4.4	4.7

According to the organoleptic evaluation, the developed samples of the vegetarian ice cream "Banana & Pistachio" and "Coffee and chocolate" got a higher overall score than the control ones due to the improved appearance, color, and taste-aromatic parameters. The vegetarian ice cream has a uniform color which was determined by the addition of banana, pistachio, and coffee beans ("Banana & Pistachio" – slightly green color with brown inclusions, "Coffee and chocolate" – saturated brown color with dark brown inclusions). In the study **[50]**, cocoa and coffee were added as well in these types of frozen desserts, and the result was the acceptance of

the samples by the panellists, the most preferred one is that with a coffee addiction. The substitution of cow milk affected the melting and aeration properties, but this is an expected result.

The appearance, taste, and smell of the ice cream "Banana & Pistachio" are improved due to the addition of banana, which gives the product a sweetish taste and a good smell compared to control one 1, which is too sweet because of high content of sugar and additional sweetening syrups **[27]**, **[28]**. These parameters of the ice cream "Coffee and chocolate" are improved due to the addition of coffee beans, which give the product a saturated brown color and a good coffee taste **[29]**. Similar results were achieved in the research effect of coconut milk, tender coconut and coconut sugar on ice cream's physicochemical and sensory attributes **[51]**.

The profilograms, which allowed us to visualise sensory evaluation results, were built to determine the qualitative differences in the organoleptic evaluation of the developed product (Figure 3 and Figure 4).

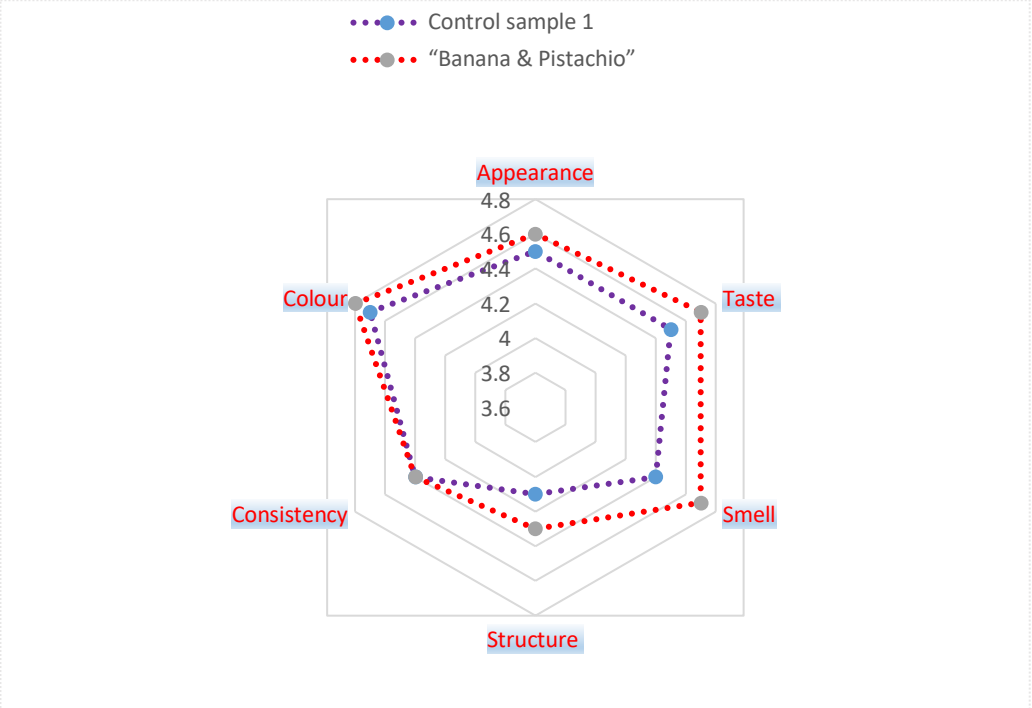

Figure 3 Comparative analysis of control sample 1 ice cream Banana & Pistachio.

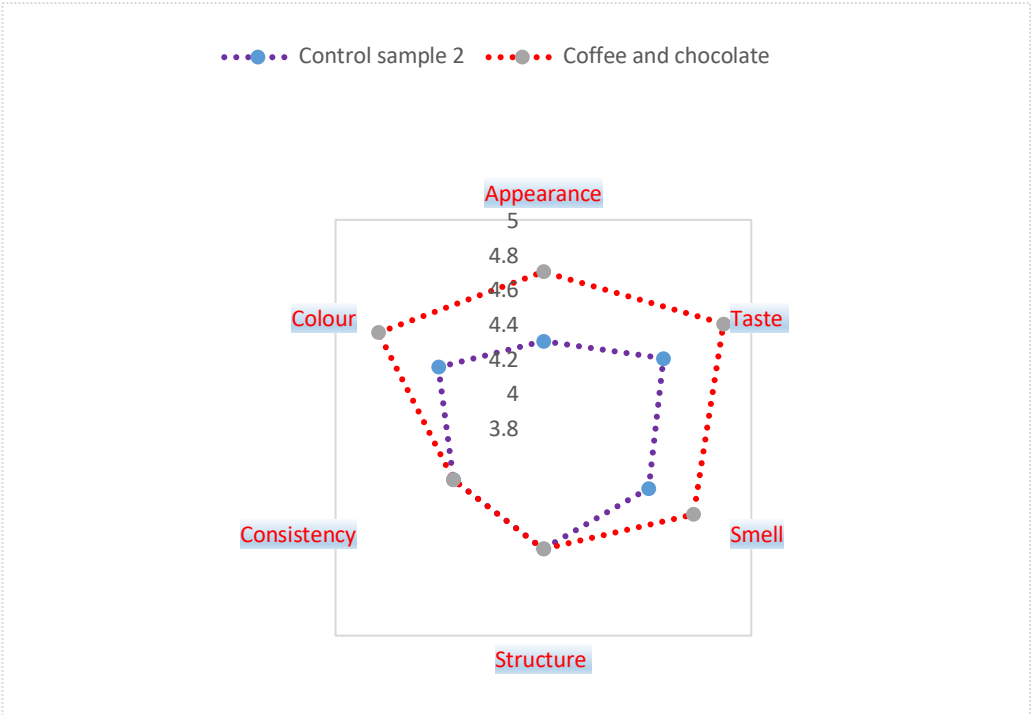

Figure 4 Comparative analysis of control sample 2 with ice cream Coffee and chocolate.

Summing up the results of the comparative evaluation of the organoleptic parameters, one may state that bananas and coffee beans increase the organoleptic parameters **[30]**, **[39]**. All developed recipes had high overall scores compared to the control samples. The most characteristic quality parameter of the ice cream is overrun, that is, its air saturation in the form of small air bubbles.

If the ice cream overrun is low, then the dense consistency of the ice cream is formed, and large ice crystals appear. If the ice cream overrun is too high, then the fragile snow-like structure, as well as the empty taste, is formed, and it can cause the sedimentation of the ice cream volume while storing **[31]**, **[32]**.

The vegetarian ice cream overrun study results are listed in (Figure 5).

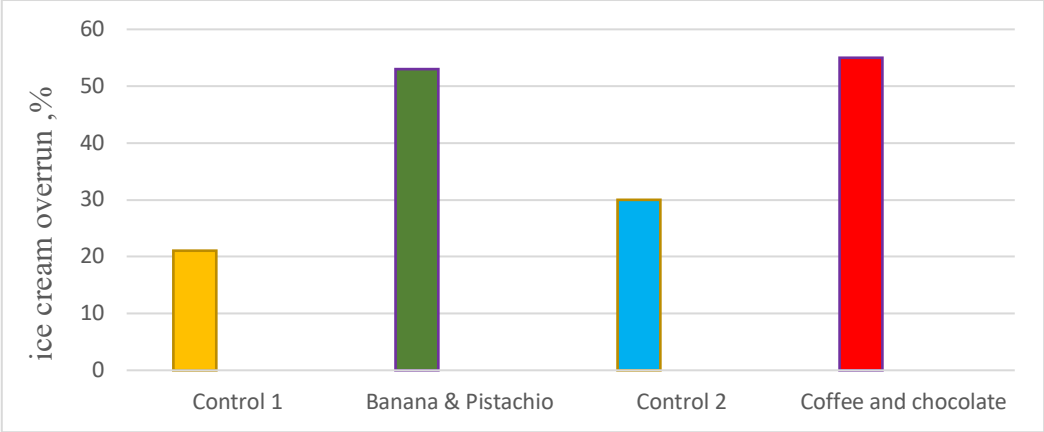

Figure 5 Ice-cream overrun, %.

The air bubbles' stability and sizes depend significantly on the chemical composition of the ice cream mixture and its freezing conditions **[33]**. Thus, when the fat content increases, the stability of air bubbles increases, but their sizes decrease. At the end of freezing, the bubble sizes remain constant upon reaching a certain minimum. The obtained results are listed in (Figures 6-9).

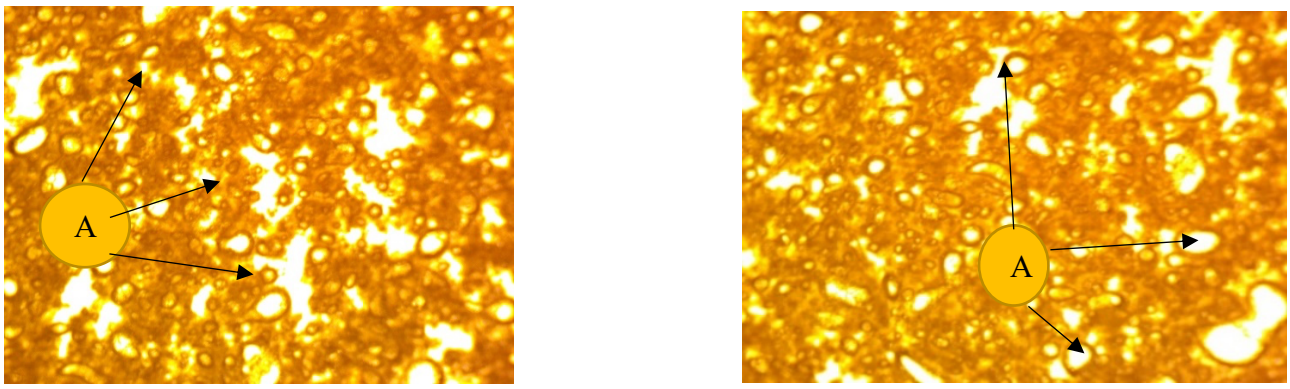

Figure 6 Photomicrographs of the control sample 1 (a.b – air bubbles).

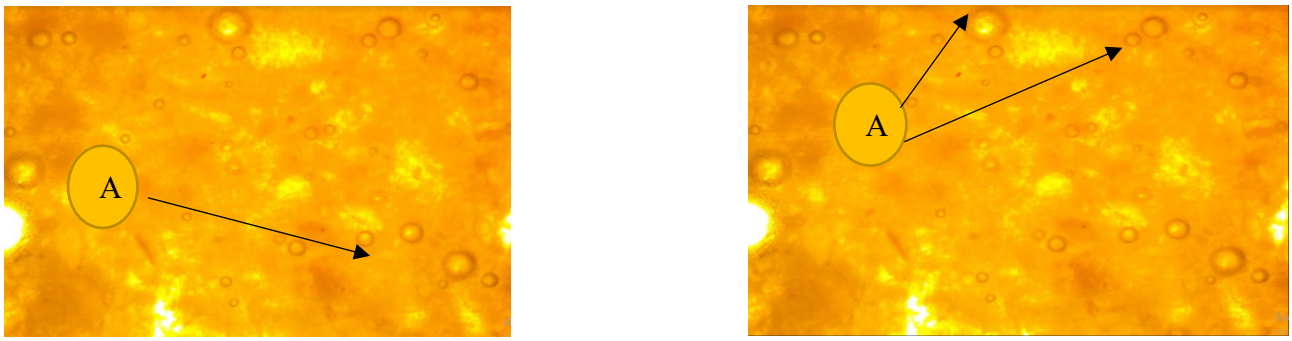

Figure 7 Photomicrographs of vegan ice cream "Banana & Pistachio" (a.b – air bubbles).

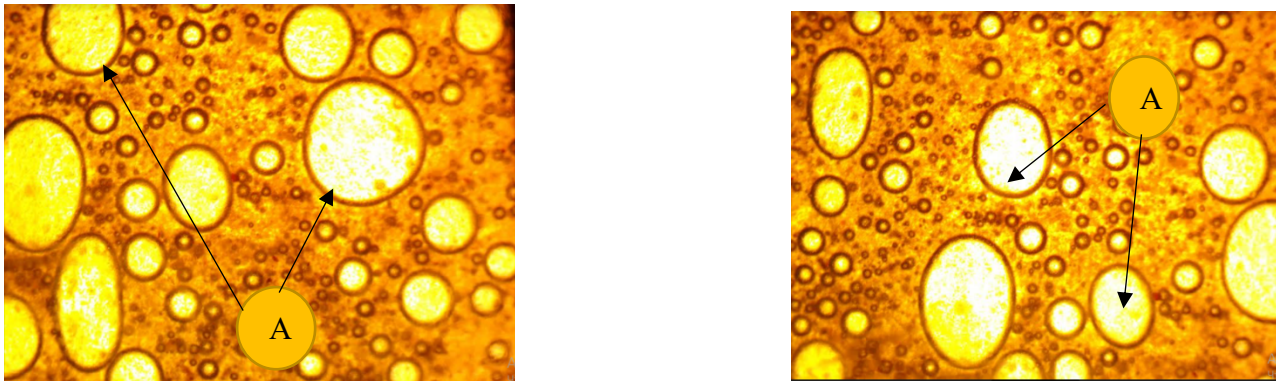

Figure 8 Photomicrographs of the control sample 2 (a.b – air bubbles).

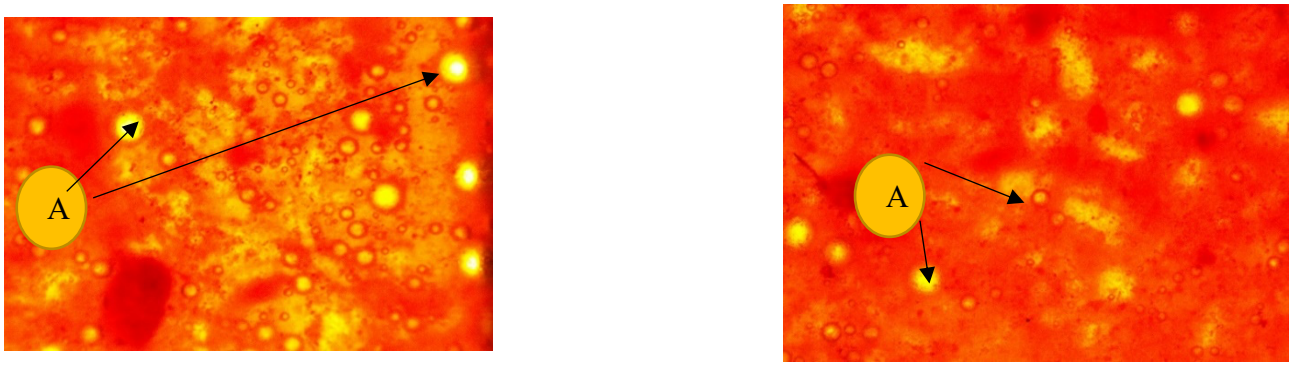

Figure 9 Photomicrographs of vegan ice cream "Coffee-chocolate" (a.b – air bubbles).

Considering the obtained data, one may state that the overrun of the studied samples is higher than that of the control samples **[34]**, **[35]**. The high overrun indicator points at the snow-like consistency, which is caused by the content of solids and fat, the properties of fat, and the freezing efficiency.

The number of fat influences the structure and consistency of the ice cream. The higher the fat content, the smaller the distance between the fat bubbles, which contributes to obtaining the finished product with smaller ice crystals **[36]**. The obtained results are listed in (Figure 10).

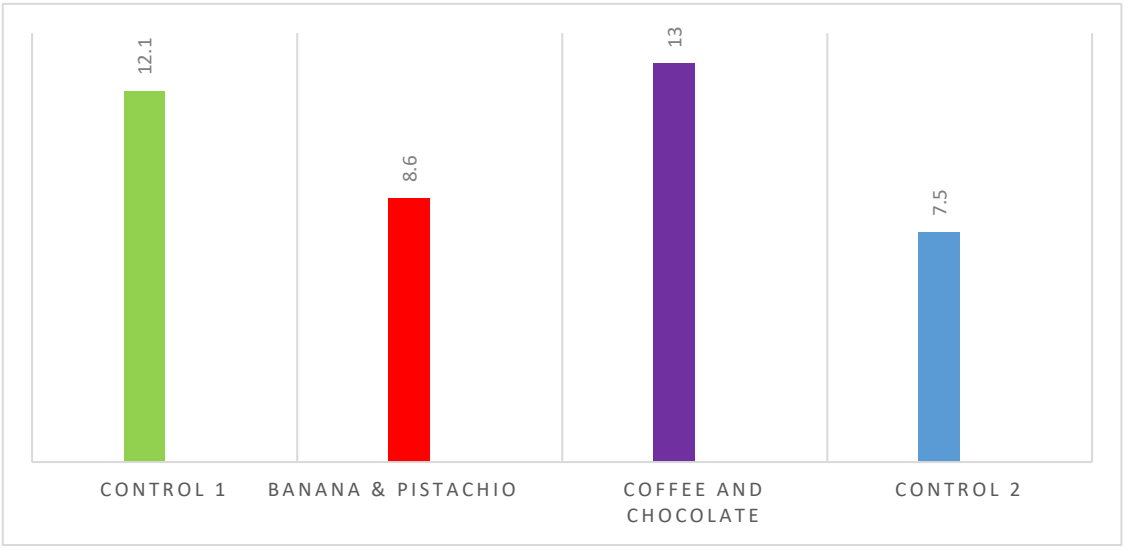

Figure 10 Mass fraction of total fat, %.

Having analyzed the obtained data, one may state that the mass fraction of total fat in the studied samples is lower than in the control samples **[37]**, **[38]**. This is due to the low-fat content in the raw materials used.

CONCLUSION

The expediency and effectiveness of combining rice milk as the main component with pumpkin fiber, stevia, bananas, pistachios, coconut oil and coffee beans in the technology of vegetarian ice cream have been proven, which is confirmed by the positive results of organoleptic and physicochemical tests. Indicators of experimental samples. Through experimental research and multi-criteria optimization, the optimal ratio of components was determined - for "Banana & Pistachio" ice cream: rice milk - 62 %, pumpkin fibre – 2.5 %, stevia – 0.5%, banana – 16%, pistachio – 6.8%, coconut oil – 12.2%; “Coffee and chocolate”: rice milk – 62%, pumpkin fibre –- 4.8%, stevia – 4%, cocoa powder – 7%, coffee beans – 8%, coconut oil – 14.2%. As a result of the conducted research, it was established that the shrinkage of the studied samples is higher than the control samples and ranges between 50-55%. A high whipping index indicates a snow-like consistency, which is determined by the content of solids and fat, the properties of the fat and the efficiency of milling. The mass fraction of total fat in the studied samples is lower than the control ones and amounts to 7.5% and 8.6%, respectively. This is due to the low-fat content in the raw materials used.

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The effect of Kawa Daun (*Coffea canephora*) decoction on blood glucose levels and pancreatic β-cells regeneration in rats with diabetes

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ABSTRACT

Giving coffee leaves *Kawa Daun (Coffea canephora)*, which contains flavonoids and chlorophyll, which are antioxidants, is one of the therapies that may be used to treat diabetes mellitus, which is expected to affect 783 million people worldwide by 2045. This study, therefore, aims to demonstrate *Kawa Daun* decoction's potency in lowering blood glucose levels and restoring pancreatic β-cells in rats with diabetes mellitus. Wistar rats (2-3 months, 200 g, n = 28) were used in this true experimental study, which applied a pre-post-control group design. Regular feeding + no intervention was for the group (K-); *Kawa Daun* was not provided to (K+) DM (alloxan) rats + regular feeding; (P1) DM rats (alloxan) received 3.6 ml/200 g BW/day of *Kawa Daun* decoction along with regular feeding; *Kawa Daun* decoction 7.2 ml/200 g body weight/day in addition to regular feeding was given to (P2) DM rats (alloxan). For 14 days, the intervention was given orally. A spectrophotometer was utilized to detect blood glucose levels, and histological analysis using H&E staining was employed to determine the state of the pancreatic β-cells. In comparison to the (K+) group, the intervention group significantly decreased blood glucose levels ($p = 0.001$), according to the findings. The P2 group's reduction in blood sugar levels (Δ = 139.33 mg/dl 38.45) was more significant than that of the P1 group (Δ = 109.17 mg/dl 35.32). Compared to the (K+) (27.1% damage) group, the intervention group's pancreatic β-cells revealed improvement according to the histopathological examination results. The group's (P2 = 14.9%) damage area was less than the group's (P1 = 22.4%). This study emphasizes how administering *Kawa Daun* decoction can improve blood glucose levels and reconstruct the pancreatic β-cells damage and its protection. Finally, this kind of leaf could be a substitute compound for diabetic herbal therapy.

Keywords: blood glucose, *Kawa Daun*, pancreatic β-cells, flavonoid, antioxidant

INTRODUCTION

Diabetes mellitus is a disease characterized by chronically elevated blood glucose levels caused by impaired glucose metabolism resulting from pancreatic β-cells damage or insulin resistance **[1]**. The International Diabetic Federation reported an increase in the number of people with diabetes mellitus by 537 million in 2021, with an estimated increase to 643 million in 2030 and 783 million in 2045 **[2]**. Indonesia is ranked seventh in the world with a prevalence of diabetes mellitus incidence of 10.7% **[3]**.

Specifically, non-pharmacological treatment is still one of the most popular treatments in Indonesia, primarily since Indonesia is known as a country with a variety of functional foods. One of them is Robusta coffee leaf (*Coffea canephora*). In West Sumatra, coffee leaves, better known as "*Kawa Daun*," are one of the typical drinks of West Sumatra in great demand by the public. *Kawa Daun (Coffea canephora)* has the potential as an antioxidant, anti-diabetic, antibacterial, and anti-inflammatory since its decoction has several bioactive components. The bioactive components contained in *Kawa Daun* are phenolic components, such as caffeine, chlorogenic acid, flavonoids, chlorophyll, and Mangiferin, which can improve the condition of diabetes mellitus pathogenesis **[4]**.

Flavonoids are one of the most abundant secondary metabolite compounds in *Kawa Daun*, with antioxidant, anti-diabetic, and anti-inflammatory properties. The action of flavonoids in improving the condition of diabetes mellitus is by scavenging free radicals of Reactive Oxygen Species (ROS). When ROS activity can be inhibited, oxidation and inflammation processes in the body can be suppressed. It can allow insulin receptors and pancreatic β-cells to regenerate or repair themselves, decreasing blood glucose levels **[5]**.

The results of previous studies explained that giving drinks from Robusta coffee leaves (*Coffea canephora*) can significantly increase insulin levels so that the HOMA-IR index or insulin resistance can be improved. The study was conducted on Wistar rats conditioned to have metabolic syndrome **[6]**. The results of another study also explained that the administration of ethanol extract from Robusta coffee leaves (*Coffea canephora*) could significantly reduce blood glucose levels in Wistar rats fed a high-fat and high-sucrose diet **[7]**.

Based on previous research findings of Anjani (2020) and, the effect of giving *Kawa Daun (Coffea canephora)* decoction on the repair of pancreatic β-cells has not been known. For this reason, this study's significance and objective are to prove the effectiveness of *Kawa Daun* decoction in reducing blood glucose levels and repairing pancreatic β-cells in rats with diabetes mellitus.

Scientific hypothesis

The research hypothesis hinged on the assumption that the *Kawa Daun* decoction *(Coffea canephora)* could positively improve the blood glucose and pancreatic cells' damage of diabetic rats. As an object of the in vivo study in Wistar strain rats, kawa daun (Coffea canephora) in different doses should decrease blood glucose and repair pancreatic disturbance in diabetic rats.

MATERIAL AND METHODOLOGY

This true experimental study used a pre-post-control group design. This research was conducted at the Pharmacy Laboratory and the Laboratory of the Faculty of Health Sciences, Universitas Perintis Indonesia.

Samples

Robusta coffee leaves (*Coffea canephora*) were obtained from the Tanah Datar area of West Sumatra. Sample preparation started with the sample drying process using the drying method in an open container of 27 °C temperature, not exposed to direct sunlight. It aimed to minimize the damage to the bioactive components contained in *Kawa Daun*. The drying process was carried out for two-three days. It was then continued with the manufacture of coarse powder from coffee leaves using a food processor and stored in a dark bottle. The *Kawa Daun* formula was made by boiling 5 g and 10 g of coffee leaves in 200 ml of water until they boiled.

Chemicals

All chemical reagents in the experimental design were of analytical grade quality and were purchased from Sigma-Aldrich, Japan, Leica Biosystem, USA, and Indogen Intertama Supplier, Indonesia.

Experimental Animals

This study used experimental animals obtained from the pharmacy laboratory of Universitas Andalas. The study used white Wistar rats (*Rattus novergicus*) with inclusion criteria of male rats aged 2-3 months weighing 150-200 gr. All groups of rats were given regular feed and drink ad libitum. The given dose was graded, with P1 3.6 ml/200 g BW/day and P2 7.3 ml/200 g BW/day for 14 days. The K- group was a group of healthy mice, only getting regular feed without intervention. Meanwhile, the K+ group was a group of DM rats induced by alloxan, given regular feed, and not given *Kawa Daun*. We involved 28 rats for the experimental groups and separated them in to 4 groups, 7 rats/ group. Before involving these animals, they were acclimatizatized for 7 days with ad-libithum consumption. The Ethics Committee of the Universitas Perintis Indonesia, Padang approved the involvement of these animals following the legislation of the ethical clearance No. 083.1/KEPK.F2/ETIK/2022, KEPK Universitas Perintis Indonesia. We sacrified these animals by dislocating the spinal cord nerve after being anaesthetized with methanol 10%.

Experimental Analysis and Instruments

Proximate and Fiber Analysis: Proximate analysis was conducted on dried *Kawa Daun* powder to determine the protein, fat, carbohydrate, water, and ash content using the 2012 AOAC method **[8]**. Fiber content analysis used the gravimetric method **[9]**.

Chlorophyll Analysis: The chlorophyll content of *Kawa Daun* was obtained through 5 g of powder brewed with 70 °C water for ten minutes, compared to 5 g of powder dissolved in 80% acetone by using Aminot's (2000) standard procedures for chlorophyll determination **[10]**. It was then measured using a 663 nm and 645 nm UV-VIS spectrophotometer of SHIMADZU UV-1280 (Shimadzu Scientific Instruments, Japan). Afterwards, the chlorophyll content was calculated by Arnon's formula **[11]**:

Chlorophyll a = (12.7 × A663) - (2.69 × A645)
Chlorophyll b = (22.9 × A645) - (4.68 × A663)
Total of chlorophyll = (20.2 × A645) + (8.02 × A663)

Total Flavonoid Analysis: A total of 5 grams of *Kawa Daun* powder was homogenized with 200 ml of 96% methanol using a magnetic stirrer for 30 minutes. A total of 60 ml of *Kawa Daun* solution was taken and then centrifuged with Cytology Centrifuge Cytospin^{TM} 4 (Thermo Scientific, USA), taking part of the supernatant. A total of 1 ml of the sample solution was mixed with 9 ml of methanol; 2.8 ml of sample solution, 0.4 ml of 5% $AlCl_3$, and 6.8 ml of 5% acetic acid were pipetted and then incubated for 30 minutes. The measurement of total flavonoids used the $AlCl_3$ calorimetry method of ASTM D240 Oxygen Bomb Calorimeter (Changsha Kaiyuan Instruments Co., Ltd, China), with absorbance calculations utilizing UV-VIS spectrophotometry at a wavelength of 428 nm. The calibration curve employed standard quercetin in methanol (0.005 mg/ml), with concentrations of 0 g/ml, 5 g/ml, 10 g/ml, 20 g/ml, 40 g/ml, respectively and 60 g/ml **[12]**.

Antioxidant Activity Analysis: Antioxidant activity was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. Sample preparation was carried out by weighing 0.1 mg of *Kawa Daun* powder and then putting it into a microtube containing 1 ml of methanol. The homogenized sample was then centrifuged at 4500 rpm for seven minutes. After that, 200 µl of supernatant was stored in a microtube. The blank used was ethanol as much as 200 µl. In addition, the standard solution was prepared by weighing 0.01 g of ascorbic acid, put into a test tube, and adding 10 ml of distilled water and vortex until homogeneous. The dilution was conducted starting from 1000 ppm to 0 ppm, with the formula V1×N1 = V2×N2.

Moreover, antioxidant activity was performed by pipetting 200µl of each solution into a microtube, and 1000 µl of DPPH was added and then mixed using a vortex and incubated for 30 minutes. Observations were then made utilizing UV-VIS Spectrophotometer at a wavelength of 517 nm. The following formula (1) was used for antioxidant activity calculation (1):

(%IC) = (Blank absorbance - Sample absorbance/Blank absorbance) x 100% (1) **[13]**.

Number of samples analyzed: we analyzed 28 pancreatic organs as a histopathological examination sample from different intervention groups.

Number of repeated analyses: 0

Number of experiment replication: 0

Laboratory Methods

Blood Glucose Level Measurement: Enzymatic measurement of blood glucose levels was done twice: after three days of alloxan induction and 14 days after the intervention. Before blood collection, rats fasted for eight-ten hours. Then, 2 ml of blood was taken through the retroorbital plexus, inserted into a microtube, and centrifuged at 4000 rpm for 15 minutes. Blood glucose levels were measured by adding 10 µl of sample and standard with 1000 µl of o-toluidine reagent. For blanks, they were filled with water and added with reagent as much as 1000 µl. Samples, standards, and blanks were then homogenized using a vortex. Then, samples, standards, and blanks were incubated for ten minutes at a temperature of 20-25 °C or five minutes at a temperature of 37 °C. Reading the absorbance utilized spectrophotometry with a wavelength of 500 nm.

Histopathological Examination: Preparing for histopathological examination began with rats killed by spinal cord dislocation. The tissue was washed with physiological NaCl solution, tissue fixation (36 hours) NBF (Neutral Buffer Formaldehyde) 10%. Dehydration was with graded alcohol for 60 minutes, and clearing was with xylol for two hours (2 times). Soaked in liquid paraffin (three hours), the thinly sliced tissue was immersed in a water bath containing water and gelatine at 40 °C, affixing on a slide. Deparaffination of preparations was carried out with xylol I-III (two minutes). Then, it was immersed in the graded alcohol gradually (two minutes). Staining used hematoxylin for ten minutes and eosin for five minutes. Then, it was dipped in graded alcohol (5 times), immersed for five minutes in xylol I, II, and III, and covered with a cover glass. Afterward, a pancreatic necrosis or regeneration feature was observed with Leica MD2000 LED Optilab Microscope (Leica Microsystem, USA) by measuring the injury area with the ImageJ program (Java, National Institute of Health).

Statistical Analysis

Statistical analysis was used to see the effect of giving the *Kawa Daun* decoction in decreasing blood glucose levels and repairing pancreatic β-cells in rats with diabetes mellitus. Paired t-test analysis was employed to measure the effect of giving *Kawa Daun* decoction before and after giving the decoction. In addition, paired t-test analysis was also utilized to determine differences in blood glucose levels between groups. The Post Hoc Bonferroni test followed it if the data were normally distributed, and vice versa. The Kruskal-Wallis test analysis was employed if the data were not normally distributed, followed by the Mann-Whitney test. Statistical analysis was performed using SPSS 26 software, with significant differences in ethical *p*-value >0.05 and 95% CI.

RESULTS AND DISCUSSION

Nutrient Content

Analysis of the nutritional content of *Kawa Daun (Coffea canephora)* used the AOAC 2012 method. The analysis results of the nutritional content of *Kawa Daun* are present in Table 1.

Table 1 Nutritional content of *kawa daun (Coffea canephora)*.

Nutritional Content	(%)
Protein	8.75
Fat	5.21
Carbohydrate	83.86
Water	2.07
Ash	0.11
Coarse fiber	8.95

Note: characteristic of kawa daun (*Coffea canephora*).

Bioactive Components and Antioxidant Activity

Analysis of the bioactive components contained in *Kawa Daun (Coffea canephora)* used the $AlCl_3$ calorimetry method for total flavonoids, UV-VIS Spectrophotometer for chlorophyll, and DPPH scavenging for antioxidant activity. The analysis results of bioactive components and antioxidant activity can be seen in Table 2. From the antioxidant activity test results, *Kawa Daun* is known to have intense antioxidant activity (IC50 = 30.99 ppm) **[14]**. Many factors cause *Kawa Daun* to have high antioxidant activity. Analysis of the intact form of *Kawa Daun* suggests the presence of other bioactive substances, which help increase antioxidant activity, including caffeine, chlorogenic acid, mangiferin, and chlorophyll, and are also known to have anti-diabetic, antioxidant, and anti-inflammatory activities. Aside from bioactive components, nutrients, such as carbohydrates and crude fiber, in *Kawa Daun* can also help increase antioxidant activity through the Maillard reaction caused by heating compounds containing carbohydrate derivatives and amino acids **[15]**.

Table 2 Content of bioactive components and antioxidant activity of *kawa daun (Coffea canephora).*

Sample	Total of Flavonoid (mQE/g)	Chlorophyll (mg/L)	IC50 (ppm)
Kawa Daun	1.3	15.33	30.99

Note: bioactive component of *Kawa Daun* (*Coffea canephora*).

The Kaur et al **[16]** study revealed that the decoction analysis of arabica coffee leaves has a higher flavonoid component than robusta coffee (69.2 mg EQ/g and 47.5 mg EQ/g respectively). However, the *Kawa daun* flavonoid contained in higher enough compound for 1.3 mEQ/g.

The Effect of Giving *Kawa Daun* Decoction on Blood Glucose Levels

Giving *Kawa Daun (Coffea canephora)* decoction was orally carried out for 14 days. Blood glucose levels before and after the intervention can be seen in Table 3. The paired t-test analysis results revealed significant differences in blood glucose levels before and after the intervention in the K+, P1, and P2 groups. A similar statistical test also demonstrated a significant difference in changes in blood glucose levels between the four groups (p <0.001). The Post Hoc Bonferroni test in Table 4 further uncovered a significant difference in changes in blood glucose levels in groups P1 (p = 0.001), and P2 (p = 0.000), compared to group K-, and there was no significant difference in changes in blood glucose levels between treatment groups P1 and P2 (p = 0.119). The intervention results also showed that the decrease in blood glucose levels was the largest in group 2 (139.33 ±38.453). However, if looking at the difference between groups P1 and P2, there was no statistical difference. It indicates that the lowest dose in the intervention could reduce blood glucose levels equivalent to blood glucose levels that received a higher dose intervention.

Table 3 The effect of giving *kawa daun* decoction on blood glucose levels.

Group	Pre-intervention	Post-intervention	(Δ) Pre-Post	*p*-value
K-	87 ±4.336	87.83 ±3.656	-0.83 ±2.927	0.517
K+	479.50 ±91.352	439.50 ±65.127	40 ±37.084	0.046
P1	230.50 ±29.616	121.33 ±13.736	109.17 ±35.324	0.001
P2	240.00 ±33.478	100.67 ±18.907	139.33 ±38.453	0.001

Note: K- = healthy rat + standard feed; K+ = T2DM + standard feed; P1 = T2DM + *Kawa Daun (Coffea canephora)* decoction 3.6 ml/200 g BW/day; P2 = T2DM + *Kawa Daun (Coffea canephora)* decoction 7.2 ml/200 g BW/day.

Table 4 Post hoc test of changes in blood sugar levels before and after the intervention.

Group	Δ Blood Glucose Level (mg/dl)	Value of *p*			
		K-	K+	P1	P2
K-	-0.83 ±2.927	-	0.039*	0.000*	0.000*
K+	40 ±37.084		-	0.001*	0.000*
P1	109.17 ±35.324			-	0.119
P2	139.33 ±38.453				-

Note: $p < 0.05$ = significant.

Functioned as antioxidants, anti-diabetic, anti-inflammatory, phenols, flavonoids, chlorophyll, Mangiferin, and caffeine has been identified as bioactive components in *Kawa Daun*. Flavonoids are known to have antioxidant activity, which is believed to protect the body against damage caused by reactive oxygen species so that they can inhibit the occurrence of degenerative diseases, such as DM. Flavonoids can also lower blood glucose levels with their ability as antioxidants. Moreover, flavonoids protect against cell damage as insulin producers and can restore insulin receptor sensitivity in cells and even increase insulin sensitivity **[17]**. The mechanism of anti-diabetic activity of flavonoids also occurs in the regulation of carbohydrate digestion, regulation of insulin signalling, and insulin secretion resulting from the recovery time of pancreatic β-cells so that insulin production increases and the increase in glucose uptake into the blood is due to repair of insulin receptor cells **[18]**.

The results of this study align with previous studies, which stated that the administration of steeped Robusta coffee (*Coffea canephora*) could reduce blood glucose levels and increase insulin levels so that the HOMA-IR index could be improved, carried out on Wistar rats with metabolic syndrome **[6]**. Another study also showed that the administration of an ethanolic extract from green coffee beans (*Coffea canephora*) provided intense inhibitory activity against the α-glucosidase enzyme to suppress the conversion rate of carbohydrates into glucose and prevent pancreatic β-cells damage **[6]**, **[19]**.

The Effect of Giving Kawa Daun (*Coffea canephora*) Decoction on Pancreatic β-cells Regeneration

Histological pancreatic β-cells can be determined by assessing the percentage of damage to the cells in the pancreas. The comparison of normal cells and damaged cells can be seen in Figure 1, as the comparison of the percentage of damage in groups P1 and P2. In Figure 1. A (K-), pancreatic Langerhans islet (L) had clear boundaries, no edema in the cells, especially in β-cells morphology, clear connective tissue capsule in Langerhans (yellow arrow), and no cell necrosis. Meanwhile, it is inversely proportional to Figure 1. B (K+), where there were signs of pancreatic β-cells damage due to alloxan induction resulting in cell swelling or edema of the Langerhans islet (yellow arrow) and intralobular duct (O), the degranulation of β-cells in Langerhans, the formation of steatoses (S), the destruction of pancreatic acini cells (Ac), and even tissue necrosis was seen. From the microscopic observation results, it was also known that there was extensive damage in the positive control group by 27.1%.

Figure 1. C depicts the changes in pancreatic β-cells after intervention with *Kawa Daun (Coffea canephora)*. It illustrates a repair process in pancreatic β-cells, and a reduction in cell edema and hydrophobic degeneration could also be seen. Pancreatic tissues showed vacuolation and decreased vasculature with cellular infiltration with cells as lymphocytes and mononuclear cells with focal areas of degeneration and necrosis area (LyM). It indicates the recovery process of tissue regeneration and repair. The microscopic feature reveals that the extent of damage ranged from 22.4%, reduced compared to the positive control group.

On the other hand, in Figure 1.D, the damaged area was reduced to 14.9%. These results were not significantly different from the low-dose intervention group. Furthermore, the histopathological feature indicates improvements feature of the Langerhans islet region, and the tissue shows normal pancreatic acini (NA). It is consistent with the absence of a significant difference in blood glucose levels between the low-dose (P1) and high-dose (P2) treatment groups.

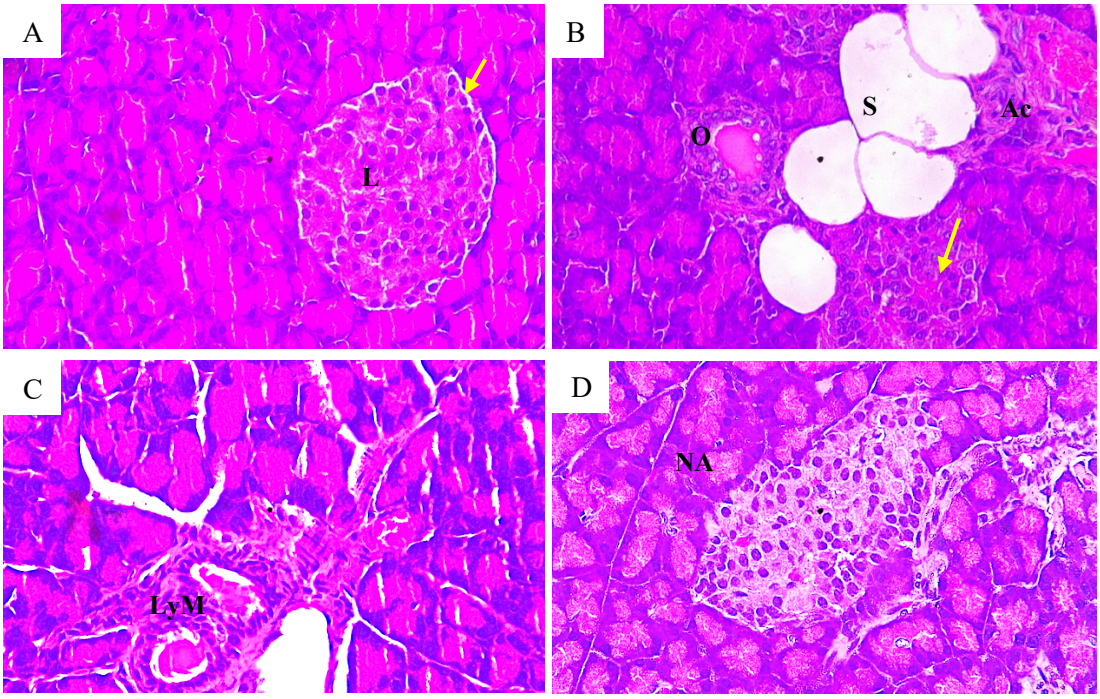

Figure 1 Illustration of pancreatic tissue preparations in x40 magnification: (A) negative control of DM, (B) positive control of DM with alloxan induction, (C) intervention dosage of 3.6 ml/200 g BW/day, (D) intervention dosage of 7.2 ml/200 g BW/day.

Several research results have demonstrated tissue repair after the administration of *Coffea canephora* in some tissues that have undergone degeneration and necrosis. Anti-inflammatory and analgesic properties in *Coffea canephora* reduce the occurrence of edema in tissues previously induced by formalin and modulate pain reduction **[5]**. The results of Handayani **[20]** studies also disclosed an improvement in liver and kidney tissue through an increase in the mean value of ALT (alanine transaminase) and AST (aspartate transaminase) enzymes in liver tissue and an increase in plasma urea levels, thus triggering the kidneys to excrete urea more quickly after *Coffea canephora* intervention, and we believe this effect positively related to the renewal of pancreatic islet cells. Another mechanism thought to improve the condition of pancreatic β-cells is the mechanism of activity of the enzyme α- glucosidase, which is deemed a molecular target in diabetes therapy. This enzyme extracted from *Coffea canephora* has solid inhibitory and high antioxidant activities that suppress insulin resistance, pancreatic cell damage, and impaired tissue glucose utilization. This enzymatic reaction also allows for an improvement in the function of the pancreas of Langerhans, which has been diagnosed with diabetes mellitus **[19]**.

Reduced function of β-cells in diabetes leads to two types of pathogenic conditions caused by immune-mediated diabetes condition (T1DM) and metabolic mechanisms diabetes disorder (T2DM). Some studies analyzed the Langerhans islet morphology and the β-cell gene expression in both T1DM and T2DM, which revealed the genes at the β-cell level and the endoplasmic reticulum stress signal contributes to β-cell failure in T1DM (mostly IRE1 driven) and T2DM (mostly PERK–eIF2α dependent) **[21]**. Another study approved that not only affects pancreatic islet damage but contributes to other vital organ degeneration, such as kidney and hepatic lobes. Primal and Ahriyasna's (2022) findings had approved that the diabetes condition contributed to renal failure and necrosis problems. The study improved the quality of renal nephrons after the high-antioxidant compound from Indonesian traditional leaves extraction. Its high flavonoid level contributes to the regeneration of the nephrotic structure by decreasing the inflammatory effect on the tissue **[22]**. We believe that the internal stress of the β-cell organelle could positively contribute to the cell's normal regulation as an insulin producer. The glomerular infiltration and hypertrophy increase significantly in the diabetic disorder, which persistently generates nephrotic site functional failure. This glomerular disturbance will lead to the expansion of the mesangium and accumulation of the extracellular matrix. Furthermore, it can lead to a loss of podocytes, disruption of the

mesangium (mesangiolysis), and glomerular fibrosis. This renal failure condition proceeded by chronic cardiac hypertension accelerated from the arterial atherosclerosis of the coronary artery **[23]**.

Deliberating the antioxidant composition in *Coffea canephora*, it has been approved that flavonoid compound has a parallel effect on the repairing process of the pancreatic islet. Accordingly, β-cells mass and function are important structures to be primarily affected by diabetes. Growing argumentation evidence supports the efficacy of flavonoids for preventing and attenuating diabetes consequences. In T2DM, the metabolic disruption leads to sustained chronic hyperglycemia, hyperlipidemia, and inflammatory cytokines elevation. These trigger the metabolic pancreatic gland to experience endoplasmic reticulum (ER) stress, lysosomal destabilization, and oxidative stress. The cell injury in the Langerhans islet structure immediately stimulates cell death through apoptosis, autophagy, or necroptosis activities. The disruption of pancreatic islet should be prevented to preserve this organ's physiological function, which could be protected from the flavonoid's antioxidant and anti-imflammatory effects **[21]**, **[24]**. Based on the diabetic rats observed, pancreatic steatosis may be one of the phenotypes of metabolic syndrome, which is characterized by obesity with visceral fat accumulation, diabetic conditions, hyperlipidemia, and hypertension **[25]**. It can be stated that steatosis is induced by exhibiting severe abnormalities in the disposal of hepatic triglycerides (hepatocellular lipids, HCLs) and impaired insulin action in the pancreas.

The pancreatic Langerhans cells death stimuli, such as TNF-α (TNFR-1 receptor) and Fas ligand (Fas-L) in the cell receptors superficies, trigger a series of intracellular signals which activate caspases-3 and -7. These caspases cleavage the poly (ADP-ribose) polymerase, lamin, and XK-related protein 8 substrates, contributing to the apoptotic process in the β-cells system as nuclear condensation, membrane blebbing, even the DNA-fragmentation. TNFR-1 expression will start the necroptosis from the lysosomal membrane permeation and the ROS pathways by promoting the necrosome formation that leads to autophagy. Moreover, the apoptotic pathways of pancreatic β-cells could be reinforced over the ER stress and stimulate the inflammatory and immunity mediators such as nitric oxide (NO), and human leukocyte antigen. In this circumstances, flavonoid's antioxidant function could reduce the pancreatic islet cells' apoptotic and autophagy process. Another research report revealed the protective effects of flavonoid compounds in some tropic plant extraction against diabetes β-cells degeneration. The primary molecular mechanisms by which flavonoids protect β-cells survival are the suppression of oxidative stress and subsequent inhibition of the caspase cascade and DNA damage **[24].**

These β-cells protection pathways, especially from the flavonoid effect, increase the cells' antioxidant capacity, inhibiting the cells' ROS accumulation and lipid peroxidation and protecting the cells' death. Flavonoids are believed to preserve the survival of β-cells by inhibiting pro-apoptotic expressions and downregulating the anti-apoptotic genes. Similarly, oxidative stress also results in insulin resistance in the pancreas, liver, and muscles by increasing Diasyl Glycerol (DAG) and Protein Kinase-C (PKC). It leads to the disruption of phosphorylation in the Insulin Receptor Substrate (IRS-1 and IRS-2), indicating those organs' insulin resistance. Moreover, an increasing amount of fatty acids circulating in vessels is generated due to oxidative stress, which positively will produce large amounts of Nitric Oxide (NO). This overproduction of NO presence may induce endoplasmic reticulum (ER) stress by reducing the level of Ca^{2+} in ER; later on, it is toxic to pancreatic β-cells and finally results in these cells' death **[24]**, **[26]**. Eventually, the flavonoid may interfere with the inflammation signalling cascades and positively prevent NO overproduction and its deleterious consequences in shock and ischemia-reperfusion of tissue injury **[27]**.

In recent reviews, flavonoids stated to have the capacity to enhance glucose-stimulated insulin release and counteract the cytokine-induced dysfunction of pancreatic β-cells. Cytokines are released by the inflammatory cells around β-cells and generate the inducible nitric oxide synthase (iNOS) expression and overproduction of NO, which are leading causes of cell damage **[24]**. We highly approve that the improvement of pancreatic islet repair based on the histopathological features is affected by the previous pathophysiological explanation we stated. The presence of lymphatic and macrophage cells indicates the expression of leukocyte antigen, and the regeneration characteristics indicate the protection of the pancreatic Langerhans islet cells colony. Decreasing neutrophil numbers and preceding autoreactive T-cell accumulation in blood streams are in pre-symptomatic stages of type-1 diabetes and are associated with worsening the pancreatic β-cells function **[27]**, **[28]**.

The histopathological study on Tamarix articulata (FRETA) extract with flavonoid-rich compounds has a potential effect on antidiabetic and antihyperlipidemic circumstances. It has been tested that the experimentally induced diabetes animal had improved the blood lipid profile and histopathological changes in both the liver and pancreas and rectified the Oral Glucose Tolerance Test (OGTT) **[29]**. We acknowldge that the high percentage of antioxidants in flavonoids contributes to the protection of the pancreatic Langerhans islet. It simultaneously repairs the β-cells injury in the induced-diabetic pancreatic organs. By enhancing the expression of some anti-inflammatory proteins and stimulating the presence of leukocyte cells as tissue protection, the antioxidant chains can produce more free radicals, which immediately inhibit excess oxidation leading to tissue damage. Mirmalek

et al. **[30]** indicated that antioxidant contained proteins ameliorate the pancreatic repair of the islet injury. This indicates that the antioxidant as an anti-inflammatory compound has the potential in therapeutic role in pancreatic regeneration resulting in the decreasing percentage of lower intestinal edema, lowering the infiltration of inflammatory cells, and alleviating acinar cell necrosis.

CONCLUSION

Giving *Kawa Daun (Coffea canephora)* decoction could improve diabetes mellitus by lowering blood glucose levels by repairing pancreatic β-cells for 14 days. In addition, there was a significantly improved the blood glucose level in the intervention group with 3.6 mL/200g/day and 7.2 mL/200g/day dose in the positive diabetic group (K+). The histopathological feature reveals that the extent of damaged cells has an effective improvement in both experimental groups, respectively reduced compared to the positive control group. Moreover, the qualitative analysis indicates the improvements in features of the Langerhans islet region and the tissue of the pancreatic acini. Anti-inflammatory and analgesic properties in *Coffea canephora* proved to reduce the occurrence of edema in injured tissues of the pancreatic organ, even for other organs such as hepatic lobes, renal, peripheral innervation, and lymphatic function. Furthermore, flavonoids' high composition in Coffea canephora protects and ameliorates β-cells survival by suppressing oxidative stress and subsequently inhibiting the caspase cascade and DNA damage in diabetic rats. It has a positive consistency without significantly changing blood glucose levels between the low-dose (P1) and high-dose (P2) treatment groups. It suggests that low doses of using *Kawa Daun* have improved the condition of diabetes mellitus equivalent to high doses and can be used for further biochemical analysis, pharmacological developments, and optional nutritional substitution in food and medico-herbal therapy.

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Conflict of Interest:

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

Ethical Statement:

The use of animals in this research was approved by the Ethics Committee of the Universitas Perintis Indonesia, Padang following the legislation of the ethicla clearence No. 083.1/KEPK.F2/ETIK/2022, KEPK Universitas Perintis Indonesia, Indonesia.

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Evaluation of commercial rice grains present in the Amman market

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ABSTRACT

Rice is a staple food that contributes to significant energy intake. Jordan relies on importing to provide the market with the required quantities of rice. Different varieties from different sources with various qualities are available in the market. This study aimed to evaluate the quality of rice available in the markets in Amman city-Jordan. Twenty-five brands (three samples from each brand) were collected. Samples were evaluated regarding chemical composition, dimensions before and after cooking, percentage of different defects, pasting profile (pasting temperature, peak viscosity, peak time, trough, and final viscosity), whiteness, transparency, and milling degree. All rice samples tested comply with the Jordanian standard except for chalky kernels (four brands), heat-damaged kernels (one brand), and insect infestation (two brands). All samples that did not fulfil the Jordanian specifications were from the long-grain rice. Medium-grain rice has higher whiteness, transparency, milling degree, moisture, starch, peak viscosity, trough, and final viscosity than long-grain rice. On the other hand, long-grain rice has a higher protein, pasting temperature, and peak time. There were significant differences in pasting and chemical composition parameters within the two groups of grain sizes. The average elongation ratio for all samples was 1.57 ±0.14, with significant differences between different brands. Due to the higher pasting temperature and peak time, long-grain rice requires more energy during cooking than medium-grain rice.

Keywords: Commercial rice, Amman-Jordan, RVA pasting profile, dimensions, milling degree

INTRODUCTION

Rice is one of the main types of staple cereal food that contribute to about 40-80% of total energy intake **[1]**. Rice is not cultivated in Jordan, so to meet the needs of consumers, Jordan imports different types of rice from different countries, making quality evaluation a vital step. Cooking and eating quality are the main factors affecting consumer acceptability and, in turn, the economic value of rice **[1]**, **[2]**. Eating quality is related to different factors, including starch physicochemical properties, chemical composition, dimensions and elongation upon cooking, defects such as chalky rice content, broken rice, and milling degree (the extent of bran removal) **[1]**, **[3]**. Consumers need rice that conforms to the standards, which evaluate the rice in terms of dimensions and the impact of different factors encountered during harvesting, storage, processing, and distribution on the quality of rice. Cooking and eating quality are usually performed using sensory evaluation of cooked rice. However, this method has several limitations: the texture sensory attributes are not usually clearly defined and standardized **[4]**, the subjective nature of the test, the large sample number and size requirement, and the time-consuming **[2]**. Another approach to determining the rice-eating quality is studying starch, particularly amylose content, as the primary factor affecting quality. However, it is difficult to predict starch behaviour during cooking depending on amylose only **[5]**. Recently, the evaluation of rice eating quality by determining the Rapid Visco Analyzer (RVA) pasting properties has been increasingly adopted by researchers **[2]**, **[4]**, **[6]**, **[7]**, **[8]**, **[9]**. The principle of the RVA pasting profile relies on measuring rice flour suspension viscosity during heating, holding, and cooling cycles, from which valuable information is drawn, such as pasting temperature, peak viscosity, peak time, trough, setback, and final viscosity **[4]**; these parameters were found to be correlated with rice eating quality **[2]**.

To the best of our knowledge, the information on the quality of the commercial rice brands available in the Amman market is scarce. The research aimed to evaluate the available commercial polished rice brands in the Amman market regarding chemical composition, conformance to Jordanian standards, dimensions before and after cooking, and degree of cooking.

Scientific Hypothesis

Several rice brands with different grain lengths are available in Amman markets. There is an expectation of a variation in quality between and within rice brands with different grain lengths. We expect differences in chemical composition (mainly protein and starch), pasting properties, milling degree, and dimensions before and after cooking. We do not expect differences in terms of foreign materials' content.

MATERIAL AND METHODOLOGY

Samples

Twenty-five brands (Table 1) were randomly identified and collected from hypermarkets in Amman/Jordan. Six brands were medium-grain, and 19 were long-grain rice. Three samples (5 kg each) were collected from different batches from each brand. Each sample was divided into three parts: one composed of rice grain for chemical and cooking tests, the second part grounded (0.05 mm screen) intended for the RVA test, and the third part for measuring foreign materials and the degree of milling. Samples were filled in plastic bags and kept refrigerated until tested.

Table 1 Commercial brand names selected from the local market.

Number	Type	Brand name	Origin
1	medium	(M1-USA)	USA
2	medium	(M2-USA)	USA
3	medium	(M3-USA)	USA
4	medium	(M4-USA)	USA
5	medium	(M5-USA)	USA
6	medium	(M6-USA)	USA
7	long	(L1-India)	India
8	long	(L2-Thailand)	Thailand
9	long	(L3-India)	India
10	long	(L4-India)	India
11	long	(L5-India)	India
12	long	(L6-India)	India
13	long	(L7-India)	India
14	long	(L8-India)	India
15	long	(L9-India)	India
16	long	(L10-India)	India
17	long	(L11-India)	India
18	long	(L12-India)	India
19	long	(L13-India)	India
20	long	(L14-India)	India
21	long	(L15-USA)	USA
22	long	(L16-India)	India
23	long	(L17-India)	India
24	long	(L18-India)	India
25	long	(L19-India)	India

Instruments

Near-Infrared Analyzer (NIR, model DA 7250, Perten, Sweden), Rapid Visco Analyzer (RVA model 4500, Perten, Australia), analytical balance (Bel engineering, model M314Ai, Italy), grinding machine equipped with 0.05 mm screen (MF 10 basic, IKA-Werke, Germany), electric rice cooker (Proctor Silex, China), and rice milling meter (Satake, Australia) were used in the study.

Laboratory Methods

Chemical analysis: The moisture, protein, and starch percentage were determined using a Near-Infrared Analyzer using the manufacturer's recommendations.

Foreign materials and defects: One kilogram from each sample was tested manually by visual inspection for broken grains, chalky kernels, damaged kernels, heat-damaged kernels, paddy kernels, rice-based foreign materials, other classes of rice, non-rice-based foreign materials, red kernels, red-streaked kernels, immature kernels, odour, and infestation according to Jordanian standard **[10]**.

Pasting properties: determined using AACC method no. 61-02 **[11]**.

Dimensions before and after cooking: The length (L), width (W), and (T) thickness of ten rice kernels were determined from each sample before and after cooking, and the average was recorded. The following ratios were calculated for each sample:

- Length-thickness ratio (before cooking) = L (before cooking)/T (before cooking)
- Length-width ratio (before cooking) = L (before cooking)/W (before cooking)
- Length-thickness ratio (after cooking) = L (After cooking)/T (After cooking)
- Length-width ratio (after cooking) = L (After cooking)/W (After cooking)
- Elongation ratio = L (cooked) / L (uncooked)

Whiteness, transparency, and degree of milling: milling meter was used to determine whiteness, transparency, and degree of milling using manufacturer recommendations.

Description of the Experiment

Sample preparation: No special sample preparation was performed for testing chemical composition, foreign materials and defects, and whiteness, transparency, and degree of milling. The rice grain was ground with a grinder with a 0.05 mm screen to test the pasting properties. Rice pasting properties were determined using Rapid Visco Analyzer. In a canister, 25 ml of water was added, and after that, 3 g of the milled rice (weight was corrected to 12% moisture content), a paddle was placed in the canister, and the blade was jogged in the sample up and down ten times. The canister with the paddle inserted was placed in the instrument. From the software (TCW), the rice pasting profile test (AACC no. 61-02) **[11]** was selected (Table 2), and the test was begun. Dimensions after cooking were determined by boiling 20g of rice in 500 mL of water using a rice cooker. A pre-experiment was conducted to determine the appropriate cooking time in which rice grains were drawn every 30 sec. and pressed between two small glass plates. Rice grains were considered cooked after the disappearance of the white colour from the centre of the grains.

Table 2: RVA rice pasting profile test

Time	Type	Value
00:00:00	Temp	50 °C
00:00:00	Speed	960 rpm
00:00:10	Speed	160 rpm
00:01:00	Temp	50 °C
00:04:48	Temp	95 °C
00:07:18	Temp	95 °C
00:11:06	Temp	50 °C
Idle temperature: 50 ±1 °C		
End of test: 12 min, 30 sec.		
The time between readings: 4 sec		

Number of samples analyzed: 75 samples were analyzed.

Number of repeated analyses: Measurements were made in duplicate.

Number of experiment replication: Number of replicates was three.

Design of the experiment: Twenty-five different rice brands were randomly collected from hypermarkets in Amman city. From each brand, three different batches were selected. The size of each sample was 5 kg. From each sample, 1 kg was assigned for defects, foreign materials test, and milling degree, 1 kg for rice dimensions tests and RVA-pasting profile analysis, and 1 kg for measuring chemical compositions.

Statistical Analysis

Statistical analysis software: Minitab 19 software (Minitab Inc., State College, PA, USA). Statistical tests performed: A completely randomized design was used to analyze the results using Minitab 19 software (Minitab Inc., State College, PA, USA). Tukey's test was used for means separation based on $p \leq 0.05$. Principal component analysis (PCA) was performed for all data to reduce its dimensionality and visualize different rice sample groups sharing the same characteristics. PCA results were presented as a biplot.

RESULTS AND DISCUSSION

Chemical Analysis

There were significant differences in chemical composition between rice samples (Table 3). The average moisture value was 12.01% ±0.44 for medium-grain rice and 10.19% ±0.60 for long-grain rice. All moisture values conform to the upper limit (15%) specified by the Jordanian standard **[10]**. The average protein value was 7.02% ±0.45 for medium-grain and 8.87% ±0.48 for long-grain rice. The lowest protein values were recorded for medium-grain rice samples (in addition to two samples of long-grain indicated by numbers 8 and 9). The lower protein content was recorded for medium-grain rice, which could be attributed to its higher milling degrees than long-grain rice (Table 7). The protein, fiber, and lipids were located in the outer bran layer, and milling significantly reduced their contents **[3]**. The protein content varies within and among rice **[12]** and is affected by the degree of exposure to solar radiation and fertilization by nitrogen **[13]**. Finally, the average starch value was 91.45% ± 1.60 for medium-grain rice and 90.18% 1.12 for long-grain rice. Medium-grain rice had significantly ($p \leq 0.05$) higher moisture and starch content than long-grain rice; however, long-grain rice had significantly ($p \leq 0.05$) higher protein content than medium-grain rice. It has been reported that the percentage of starch varies from 87 to 91% **[14]**, **[15]**.

Foreign Materials and Defects

Results showed significant differences ($p \leq 0.05$) between rice samples in broken grains, chalky kernels, damaged kernels, heat-damaged kernels, red-streaked grains, and immature kernels (Table 4). There were no significant differences in extraneous organic materials, other rice classes, and red kernels. The Jordanian standards for rice **[10]** stated the upper limits for different defects as follows: 6% for broken grains; 5% for chalky kernels; 3% for black damaged kernels; 2% for heat-damaged kernels; 0.3% for paddy kernels; 0.5% for extraneous organic materials; 1% for other classes of rice; 0.5% for non-organic extraneous materials; 12% for red and red-streaked kernels; and 2% immature kernels. The specifications also stated that rice should be free of visible insects. All rice samples tested comply with the Jordanian standard except for chalky kernels (brand numbers 9, 12, 16, and 17 exceeded specifications), heat-damaged kernels (brand number 21 exceeded specifications), and insect infestation (brand number 7 and 24 exceeded specifications). It was interesting to note that all samples that exceeded specifications were from the long-grain rice. Chalkiness is due to the white colour in the endosperm area, which is undesirable and weakens the rice kernel leading to breaking during rice handling, which reduces head rice recovery **[6]**. It is worth to be mentioned that the foreign materials and defects in rice grains in this research were determined using manual inspection by a trained operator; some researchers suggested using a better method using image processing to avoid the possible errors linked with the first method related to human fatigue while testing a large number of samples **[16]**, **[17]**.

Pasting Properties

Pasting properties in this research were determined using RVA, which has several advantages over other empirical methods represented in well-defined parameters, small sample size, short testing time **[18]**, and correlated with cooked rice sensory properties **[19]**. **[20]** reported that cooked rice acceptability was correlated with high peak viscosity, breakdown viscosity, final viscosity, and hold viscosity. Table (5 A) shows the pasting properties of the different rice samples. Pasting temperature is the temperature at the onset of this rise in viscosity **[1]** when the starch and protein absorb water **[5]**. Pasting temperature is considered an overestimation of gelatinization temperature **[21]**. In this research, the pasting temperature averaged 93.19 ±2.73 °C. Medium-grain rice samples had a lower average pasting temperature (88.58 ±1.43 °C) compared with long-grain rice (94.64 ±0.38 °C). All long-grain rice samples were significantly higher ($p \leq 0.05$) than the medium-grain rice. The pasting temperature indicates the minimum temperature required to cook a sample, directly impacting energy costs. Based on this, and due to its lower values of pasting temperature, medium-sized rice grains are expected to require less energy to be cooked than long-grain rice **[1]**.

Table 3 Percentages[1] of moisture, starch, and protein of rice samples.

Brand	Grain type[2]	Moisture (%)[3]	Protein (%)[4]	Starch (%)[4]
1	M	12.26 ±0.01ab	6.56 ±0.15g	93.55 ±0.18a
2	M	11.57 ±0.28abc	7.19 ±0.02defg	90.14 ±0.51abcd
3	M	12.17 ±0.17ab	7.05 ±0.07efg	89.18 ±0.26cd
4	M	12.1 ±0.42ab	6.92 ±0.34fg	92.65 ±0.69abc
5	M	12.38 ±0.04a	6.84 ±0.19fg	91.29 ±0.24abcd
6	M	11.56 ±0.78abc	7.89 ±0.16cde	91.87 ±0.04abcd
7	L	9.74 ±0.07gh	8.57 ±0.05abc	90.34 ±0.48abcd
8	L	11.28 ±0.03bcd	7.5 ±0.42def	92.69 ±0.04ab
9	L	10.33 ±0.04defg	8 ±0.14bcd	90.07 ±1.32abcd
10	L	9.75 ±0.18fgh	8.85 ±0.13ab	89.74 ±0.52bcd
11	L	10.36 ±0.02defg	9 ±0.14a	89.1 ±0.99d
12	L	10.82 ±0.06cdef	8.78 ±0.10ab	90.4 ±2.83abcd
13	L	10.64 ±0.15cdef	9.15 ±0.07a	90.09 ±1.04abcd
14	L	10.15 ±0.01efgh	8.56 ±0.07abc	89.6 ±1.27bcd
15	L	9.17 ±0.09h	9.11 ±0.01a	88.59 ±0.45d
16	L	10.32 ±0.02defg	9.17 ±0.09a	88.85 ±0.86d
17	L	10.99 ±0.27cde	8.84 ±0.01ab	89.5 ±0.06bcd
18	L	10.05 ±0.50efgh	8.85 ±0.50ab	90 ±0.25abcd
19	L	9.48 ±0.39gh	9.35 ±0.21a	90.095 ±0.36abcd
20	L	10.1 ±0.42efgh	9.23 ±0.39a	90.59 ±1.36abcd
21	L	10.6 ±0.28cdef	8.82 ±0.11ab	90.3 ±0.57abcd
22	L	10.65 ±0.07cdef	9.38 ±0.11a	90.23 ±0.10abcd
23	L	9.31 ±0.15gh	9.28 ±0.03a	90.71 ±0.41abcd
24	L	10.33 ±0.10defg	9.07 ±0.24a	90.93 ±0.11abcd
25	L	9.79 ±0.22fgh	9.04 ±0.43a	90.84 ±0.03abcd
All samples		10.62 ±0.96	8.44 ±0.91	90.48 ±1.39
M-grain		12.01 ±0.44	7.02 ±0.45	91.45 ±1.60
L-grain		10.19 ±0.60	8.87 ±0.48	90.18 ±1.12

Note: [1]Values are expressed as Means ± Standard deviation. According to the Tukey test, the means that do not share the same letter in each column are not significantly different (p >0.05); [2] M: medium-grain rice; L: Long-grain rice; [3] Wet matter bases; [4] Dry matter bases.

There were no significant differences in pasting temperature for samples from long-grain rice; however, there were significant differences ($p \leq 0.05$) in medium-grain rice, and the lowest significant pasting temperature was for samples number 1, 2, 4, and 5. Pasting temperature differences could be related to differences in rice chemical composition. This study founds a strong positive correlation ($R^2 = 0.76$) between rice protein content and pasting temperature (Figure 1). **[5]** attributed the increase in pasting temperature for some types of rice to the resistance of starch granules against swelling, which could be related to the type of starch present. **[22]** reported that amylose content is correlated with high pasting temperature. High amylose content is correlated with the hardness of cooked rice grains, while low amylose content is correlated with stickiness **[23]**, **[24]**.

The highest viscosity during the heating cycle is known as peak viscosity **[25]**, associated with water-holding capacity. The average value of peak viscosity (Table 5 A) was 1093 ±864 cp. Medium-grain rice had significantly higher ($p \leq 0.05$) values (2518 ±443 cp) than long-grain rice (642.4 ±251.2 cp). Sample 6 from medium-grain rice had a significantly lower peak viscosity (1696.5 ±226.980) than the other samples from medium-grain rice. There were significant differences ($p \leq 0.05$) in samples from long-grain. The swelling power and disruption rate are responsible for the variations in peak viscosity **[26]**. It had been reported that rice with high amylose had low peak viscosity, while rice low in amylose had high peak viscosity **[27]**, **[28]**, which suggests that, in our study, long-grain rice had higher amylose content than medium-grain rice. Peak viscosity was reported to be negatively correlated with rice hardness **[29]**. **[19]** reported that consumers preferred rice with high peak viscosity.

Table 4 A Rice foreign materials and defects[1].

Brand number	Broken grains (%)	Chalky kernels (%)	Damaged kernels (black) (%)	Heat-damaged kernels (%)	Paddy kernels (%)	Organic extraneous materials (%)
1	2.8 ±0.64	3.6 ±0.42	0.1 ±0.02	0.1 ±0.02	0	0
2	2.8 ±0.67	1.5 ±0.58	0.1 ±0.09	0.2 ±0.09	0	0
3	3.5 ±0.67	2.5 ±0.91	0.2 ±0.24	0.1 ±0.11	0	0
4	2.2 ±0.08	4.9 ±0.28	0.1 ±0.06	0 ±0.05	0	0
5	3.4 ±0.81	2.8 ±1.02	0.1 ±0.05	0.1 ±0.08	0	0
6	4.3 ±1.02	4 ±0.59	0.9 ±0.11	0.1 ±0.02	0 ±0.05	0
7	0 ±0.03	0	0.3 ±0.1	0.2 ±0.07	0	0
8	1.3 ±0.04	0	0.1 ±0.04	0.6 ±0.22	0	0
9	0	15.4 ±8.81	0.5 ±0.28	0.2 ±0.22	0	0
10	0.4 ±0.05	0.1 ±0.01	0.2 ±0.12	0.2 ±0.00	0	0
11	0.2 ±0.22	0.1 ±0.09	0.1 ±0.14	0.3 ±0.07	0	0
12	0.4 ±0.09	17.8 ±4.05	0.3 ±0.16	0	0	0
13	0.1 ±0.14	0 ±0.06	0.2 ±0.03	0.2 ±0.14	0	0
14	0	0	0 ±0.04	0.2 ±0.05	0	0
15	0.3 ±0.02	0	0.8 ±0.18	0.5 ±0.45	0	0
16	0.9 ±0.66	16.7 ±1.10	0.2 ±0.28	0.3 ±0.01	0	0
17	0.1 ±0.06	13.1 ±2.74	0.1 ±0.18	0.1 ±0.00	0	0
18	0.2 ±0.27	0	0.4 ±0.27	0.1 ±0.03	0	0
19	0.1 ±0.04	0.1 ±0.02	0.5 ±0.15	0	0	0
20	0.1 ±0.03	0.1 ±0.10	0.3 ±0.13	0.1 ±0.08	0	0
21	5.3 ±1.30	0	0.1 ±0.08	2.1 ±0.55	0	0
22	0.1 ±0.19	0	0.1 ±0.08	0.1 ±0.18	0	0
23	0.1 ±0.14	0	0.2 ±0.11	0.1 ±0.12	0	0
24	0.1 ±0.03	0.1 ±0.10	0.3 ±0.15	0 ±0.03	0	0
25	0.5 ±0.47	0	0.3 ±0.05	0.9 ±1.10	0	0

Note: [1]Values are expressed as Means ± Standard deviation.

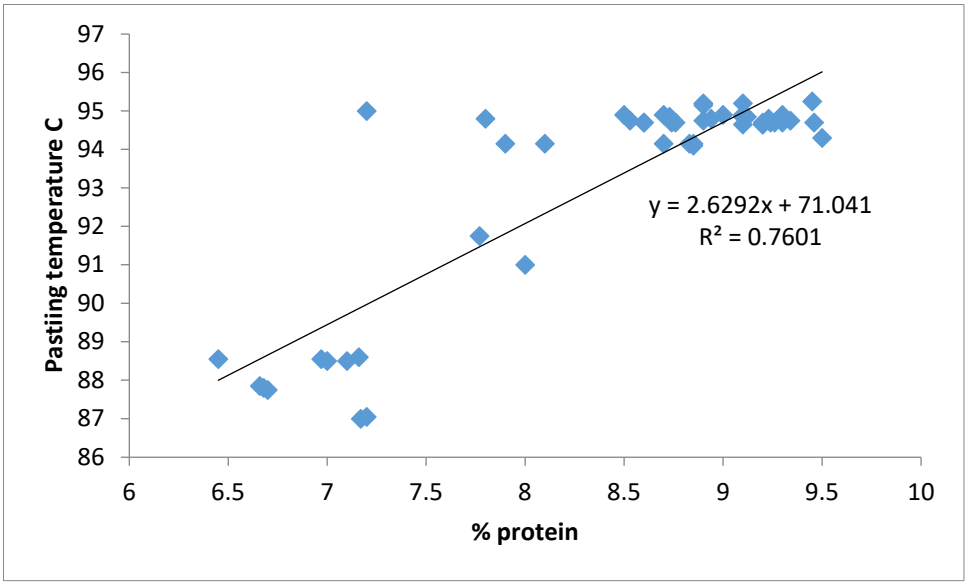

Figure 1 Correlation between % protein in rice samples and the corresponding pasting temperature.

Table 4 B Rice foreign materials and defects[1].

Brand number	Other classes of rice (%)	Inorganic extraneous materials (%)	Red kernels (%)	Red streaked kernels (%)	Immature kernels (%)	Odour[2]	Infestation		
							Free	Infested	Type[3]
1	0	0	0	0	0.5 ±0.12	N	X		
2	0	0	0	0	0.6 ±0.08	N	X		
3	0.1 ±0.17	0	0	0	0.7 ±0.01	N	X		
4	0 ±0.05	0	0	0	1.1 ±0.04	N	X		
5	0 ±0.05	0	0	0	0.8 ±0.36	N	X		
6	0.3 ±0.06	0 ±0.03	0	0.3 ±0.02	0.2 ±0.15	N	X		
7	0	0	0	0.1 ±0.01	0.4 ±0.27	N		X	STW
8	0	0	0	0	0.90 ±0.21	N	X		
9	0	0	0	0.1 ±0.05	1.6 ±0.71	N	X		
10	0	0	0	0.2 ±0.05	0.6 ±0.16	N	X		
11	0	0	0	0.2 ±0.15	1 ±0.35	N	X		
12	0	0	0	0.3 ±0.24	0.8 ±0.39	N	X		
13	0	0	0	0.2 ±0.11	0.7 ±0.15	N	X		
14	0	0	0	0.2 ±0.19	1.2 ±0.37	N	X		
15	0	0	0	0	0.4 ±0.45	N	X		
16	0	0	0	0.3 ±0.20	1 ±0.51	N	X		
17	0	0	0	0.2 ±0.03	0.2 ±0.08	N	X		
18	0	0	0	2.2 ±0.02	0.8 ±0.39	N	X		
19	0	0	0	0.7 ±0.36	0.6 ±0.13	N	X		
20	0	0	0	0.4 ±0.19	0.5 ±0.14	N	X		
21	0	0	0	0.1 ±0.01	1.6 ±0.17	N	X		
22	0	0	0	0.2 ±0.15	1.4 ±0.86	N	X		
23	0	0	0	0.4 ±0.12	0.4 ±0.01	N	X		
24	0	0	0	0.3 ±0.26	0.5 ±0.05	N		X	FB
25	0	0	0.1±0.08	0.4 ±0.05	0.5 ±0.08	N	X		

Note: [1]Values are expressed as Means ± Standard deviation. According to the Tukey test, the means that do not share the same letter in each column are not significantly different (p >0.05); [2] N: normal odour; [3] STW: Sawtoothed grain beetles; FB: Flour beetles.

The time corresponding to peak viscosity is known as peak time and is associated with the time required for rice cooking **[25]**. From Table 5 A, the medium-grain rice had significantly lower values (5.65 ±1.7 min) than long-grain rice (6.99 ±0.1 min). There were no significant differences in peak viscosity time between samples from the same type: medium or long rice grains. The degree of milling is one factor responsible for variation in peak time **[30]**. For energy consumption, rice with a low peak time is preferred **[1]**. It has been reported that the peak time for polished rice ranged between 5.4 to 7 min **[25]**.

There were wide variations in the trough viscosity values (Table 5 B), with the lowest viscosity in the temperature-holding stage **[4]**. The average trough viscosity for all samples was (830.4 ±370 cp). The average value for medium-grain rice was (1348.9 ±194.7 cp), which is higher than the average for long-grain rice (666.6 ±235.8 cp).

Table 5 (A) Rice samples RVA pasting parameters[1] (pasting temperature, peak viscosity, and peak time).

Brand number	Pasting temperature (°C)	Peak viscosity (cp)	Peak time (min)
1	88.2 ±0.95cd	2723 ±144.25a	5.5 ±0.0b
2	87.00 ±1.2d	2992 ±410.12a	5.6 ±0.05b
3	88.5 ±0.0c	2543 ±43.84a	5.7 ±0.04b
4	88.2 ±0.57cd	2589.5 ±60.10a	5.7 ±0.06b
5	88.2 ±0.57cd	2566 ±132.94a	5.7 ±0.04b
6	91 ±0.53b	1696.5 ±226.98b	5.7 ±0.14b
7	94.7 ±0.04a	580.5 ±14.85cde	7 ±0.0a
8	94.9 ±0.34a	192 ±16.97e	7 ±0.0a
9	94.2 ±0.0a	1006.5 ±19.09cd	7 ±0.0a
10	94.8 ±0.07a	696 ±7.07cde	7 ±0.0a
11	95.2 ±0.0a	421.5 ±470.22de	7 ±0.0a
12	94 ±0.0a	1046 ±25.46bcd	7 ±0.0a
13	94.7 ±0.0a	610 ±4.24cde	7 ±0.0a
14	94.8 ±0.14a	718 ±42.43cde	7 ±0.0a
15	94.9 ±0.04a	672 ±.3460cde	7 ±0.0a
16	94.8 ±0.04a	658.5±2.12cde	7 ±0.05a
17	94.1 ±0.08a	1081.5 ±119.50bc	7 ±0.0a
18	94.8 ±0.18a	686.5 ±159.1cde	7 ±0.0a
19	94.7 ±0.0a	583 ±144.25cde	7 ±0.0a
20	95.08 ±0.32a	450 ±14.14cde	7 ±0.0a
21	94.93 ±0.53a	241.5 ±50.21e	7 ±0.0a
22	94.7 ±0.0a	727.5 ±13.44cde	7 ±0.0a
23	94.8 ±0.14a	673 ±57.98cde	7 ±0.0a
24	94.8 ±0.0a	577 ±189.51cde	7 ±0.0a
25	94.9 ±0.0a	584 ±241.83cde	7 ±0.0a
All samples	93.19 ±2.73	1093 ±864	6.68 ±0.58
M-grain	88.58 ±1.43	2518 ±443	5.65 ±1.7
L-grain	94.64 ±0.38	642.4 ±251.2	6.99 ±0.01

Note: [1]Values are expressed as Means ± Standard deviation. According to the Tukey test, the means that do not share the same letter in each column are not significantly different ($p > 0.05$).

A significant difference ($p \leq 0.05$) between medium and long-grain rice was observed in terms of breakdown viscosity (Table 5 B). The breakdown viscosity is the difference between peak viscosity and trough viscosity, 1169 ±450 cp for medium rice grain and -3.16 ±11.87 cp for long rice grain. High breakdown viscosity is correlated with improved cooked rice palatability **[25]**.

The final viscosity is the viscosity reached at the end of the cooling stage **[5]**. All rice samples' average final viscosity value was 1840 ±847cp (Table 5 B). Medium-grain rice had significantly higher ($p \leq 0.05$) final viscosity values (2887 ±365 cp) than long-grain rice (1510 ±666 cp). The setback viscosity is the difference between peak and final viscosity **[4]**. The average setback value for all rice samples was 732.4 ±552 cp. Medium-grain rice had significantly lower ($p \leq 0.05$) values (371 ±712 cp) than long-grain rice (846.6 ±443.7 cp). The setback viscosity indicates starch's ability to retrograde **[31]**, whereas a lower setback viscosity indicates a lower tendency of starch to retrograde **[4]**.

Table 5 (B) Rice samples RVA pasting parameters[1](trough viscosity, final viscosity and set back).

Brand number	Trough viscosity (cp)	Breakdown viscosity (cp)	Final viscosity (cp)	Set back (cp)
1	1447.5 ±154.86ab	1275.5 ±10.6a	3104 ±8.49ab	381 ±135.79ghijk
2	1545 ±370.52a	1447.0 ±39.6a	2835 ±282.54abc	-147 ±141.42k
3	1267 ±76.37ab	1276.0 ±32.5a	2731 ±98.99abc	188 ±55.15hijk
4	1158 ±28.28abcd	1431.5 ±31.8a	2547 ±48.08bcd	-42.5 ±108.19jk
5	1210.5 ±9.19abc	1355.5 ±123.7a	2591 ±.6930bc	25 ±63.64ijk
6	1465.5 ±27.58ab	231.0 ±199b	3516 ±151.32a	1819.5 ±378.30a
7	587 ±14.14fghi	-6.5 ±0.71c	1122 ±.6930ghi	541.5 ±54.45efghi
8	194.5 ±17.68i	-2.5 ±0.71c	284 ±32.53j	92 ±15.56ijk
9	1007 ±.198bcdef	-0.50 ±0.71c	2829 ±1.41abc	1840.5 ±45.96a
10	703 ±8.49defgh	-7.0 ±1.41c	1433 ±57.98fgh	737 ±50.911efgh
11	791 ±45.26cdefg	31.0 ±50.9bc	2128.5 ±45.97cdef	1342 ±0abcd
12	1054 ±24.04bcdef	-8.0 ±1.41c	2411 ±70.71bcde	1365 ±45.26abc
13	615 ±2.83efghi	-5.0 ±1.41c	1094 ±24.04ghi	484 ±28.28fghij
14	723.5± 41.72defg	-5.5 ±0.71c	1630.5 ±71.42efgh	701.5 ±14.85cdefg
15	676 ±.3960efgh	-4.0 ±0.0c	1373.5 ±54.45fgh	701.5 ±14.85efgh
16	664 ±1.41efghi	-5.5 ±0.71c	1638.5 ±75.66iefgh	980 ±73.54bcdef
17	108 9±117.38abcde	-7.5 ±2.12c	2594 ±.28150bc	1489.5 ±194.45ab
18	690 ±159.81defgh	-3.5 ±0.71c	1610 ±373.35fgh	901 ±246.07cdefg
19	590 ±144.25fghi	-7.0 ±0.0c	1401 ±367.67fgh	818 ±223.45cdefg
20	454.5 ±12.02ghi	-4.5 ±2.12c	947.5 ±12.02hij	497.5 ±26.16efghij
21	244 ±50.91hi	-2.5 ±0.71	404.5 ±82.73ij	163 ±32.53hijk
22	734 ±13.43defg	-7.0 ±0.0c	1792 ±36.77defg	1064 ±23.33bcde
23	678.5 ±57.28efgh	-5.5 ±0.71c	1492 ±114.55fgh	819 ±56.57cdefg
24	582 ±189.51fghi	-5.0 ±0.0c	1360 ±.47730fgh	783.5 ±287.79cdefg
25	588 ±243.25fghi	-4.0 ±1.41	1139 ±439.82ghi	554 ±199.40efghi
All	830.4 ±370	278.3 ±549.0	1840 ±847	732.4 ±552
M-grain	1348.9 ±194.7	1169 ±450	2887 ±365	371 ±712
L-grain	666.6 ±235.8	-3.16 ±11.87	1510 ±666	846.6 ±443.7

Note: [1]Values are expressed as Means ± Standard deviation. According to the Tukey test, the means that do not share the same letter in each column are not significantly different ($p > 0.05$).

Dimensions Before and After Cooking

The length values of uncooked medium-rice grains (5.58 ±0.08 mm) with no significant differences between them (Table 6 A). However, the length values of uncooked long-rice samples (8.01 ±0.51 mm) varied considerably with significant differences. The average width value of uncooked medium-grain rice samples was 2.56±0.09 mm, significantly higher than long-grain rice (1.86 ±0.12 mm). There were significant differences in uncooked grain width within medium and long-grain rice groups. It has been reported that cooking quality is correlated with rice gain width **[31]**. The average thickness value of the medium-grain rice samples was 1.83 ± 0.03 mm, significantly higher than long-grain rice (1.56 ±0.07 mm). There were no significant differences in thickness values within medium and long-grain rice samples.

After cooking, the average length value of medium-rice grains was 8.95 ±0.34mm, significantly lower than long-grain rice (12.63 ±1.41mm). There were no significant differences within medium-grain rice samples; however, there were significant differences within long-grain samples. The average width value of cooked medium-grain rice was 3.39 ±0.23, significantly higher than long-grain rice (2.5 ±0.17). There were no significant differences between medium-grain rice samples and significant differences between long-grain samples.

The uncooked medium-grain rice L/T ratio (Table 6 B) was 3.05 ±0.06, significantly lower than long-grain rice (5.14 ±0.07). There were no significant differences in the L/T ratio between medium-grain rice and significant differences between long-grain samples. Uncooked medium-grain rice had an average L/W ratio of 2.21 ±0.12, significantly lower than long-grain rice (4.33 ±0.37). Similarly, there were no significant differences in the L/W

ratio between uncooked medium-grain rice and significant differences between uncooked long-grain samples. The cooked medium-grain L/T ratio was 3.42 ±0.49, significantly lower than long-grain rice (6.73 ±.00). For cooked rice L/W ratio, medium-grain rice had an average value of 2.72 ±0.23, significantly lower than long-grain rice. For both cooked rice ratios (L/T and L/W), there were no significant differences between medium-grain rice and significant differences between long-grain rice. The L/W ratio determines the shape of rice grain: ratios >3 are considered slender shaped, while ratios ≤3 are considered bold, according to the International Rice Research Institute **[32]**.

One of the most important ratios is the elongation ratio **[33]**, which indicates rice cooking quality. The average value of the elongation ratio for all rice samples was 1.57 ±0.14. There were significant differences between rice samples. Elongation in one direction (length) is preferred to elongation in both length and width **[1]**. It was reported that storage conditions (time and temperature) affected the physicochemical properties of rice grains **[34]**.

Table 6 (A) Dimensions[1] of rice grains before and after cooking.

Sample number	Dimensions of rice grain					
	Before cooking			After cooking		
	L [2] (mm)	W (mm)	T (mm)	L (mm)	W (mm)	T (mm)
1	5.6 ±0.02	2.6 ±0.08	1.8 ±0.04	8.7 ±0.02	3.2 ±0.11	2.4 ±0.18
2	5.6 ±0.0	2.5 ±0.04	1.8 ±0.04	8.7 ±0.46	3.3 ±0.10	2.5 ±0.14
3	5.6 ±0.03	2.6 ±0.02	1.9 ±0.02	8.8 ±0.28	3.4 ±0.28	3.5 ±1.36
4	5.6 ±0.15	2.7 ±0.01	1.9 ±0.01	9 ±0.07	3.4 ±0.32	2.5 ±0.16
5	5.6 ±0.03	2.5 ±0.10	1.8 ±0.01	9 ±0.16	3.5 ±0.05	2.5 ±0.10
6	5.4 ±0.04	2.4 ±0.05	1.8 ±0.01	9.5 ±0.01	3.6 ±0.36	2.7 ±0.03
7	8 ±0.18	1.8 ±0.01	1.6 ±0.05	12.4 ±0.71	2.5 ±0.12	1.9 ±0.06
8	7.6 ±0.09	2.1 ±0.08	1.8 ±0.01	10.4 ±0.11	2.8 ±0.03	2.1 ±0.10
9	8.3 ±0.01	2 ±0.01	1.6 ±0.01	15.7 ±0.92	2.6 ±0.16	1.8 ±0.33
10	7.8 ±0.23	1.7 ±0.0	1.6 ±0.04	11.4 ±0.42	2.3 ±0.08	1.9 ±0.06
11	8.4 ±0.13	1.9 ±0.01	1.5 ±0.05	12.3 ±0.05	2.4 ±0.09	1.8 ±0.01
12	7.4 ±0.49	1.8 ±0.28	1.5 ±0.04	13.2 ±0.62	2.5 ±0.07	1.8 ±0.0
13	7.4 ±0.20	1.7 ±0.04	1.5 ±0.03	11.6 ±0.69	2.4 ±0.13	1.8 ±0.16
14	8 ±0.01	1.9 ±0.01	1.6 ±0.03	13.1 ±0.42	2.4 ±0.05	1.8 ±0.03
15	7.8 ±0.16	1.8 ±0.06	1.6 ±0.01	11.8 ±0.29	2.4 ±0.05	1.9 ±0.09
16	8.5 ±0.27	2 ±0.01	1.6 ±0.06	14.6 ±0.19	2.5 ±0.14	1.9 ±0.01
17	7.7 ±0.05	1.8 ±0.01	1.5 ±0.04	13 ±0.7	2.5 ±0.02	1.9 ±0.04
18	8.6 ±0.09	1.8 ±0.0	1.6 ±0.04	13.5 ±0.31	2.7 ±0.05	2 ±0.04
19	8.5 ±0.0	1.8 ±0.12	1.6 ±0.07	13.1 ±0.20	2.6 ±0.0	1.9 ±0.11
20	8.6 ±0.14	1.8 ±0.0	1.5 ±0.01	13 ±0.07	2.5 ±0.13	2 ±0.05
21	7 ±0.19	2 ±0.01	1.6 ±0.06	9.6 ±0.26	2.9 ±0.08	2.2 ±0.22
22	8.5 ±0.08	1.8 ±0.04	1.5 ±0.03	13 ±0.24	2.6 ±0.07	1.9 ±0.09
23	8.4 ±0.18	1.8 ±0.06	1.5 ±0.05	13 ±0.03	2.3 ±0.18	1.9 ±0.04
24	8.4 ±0.17	1.9 ±0.06	1.6 ±0.03	13.7 ±0.13	2.4 ±0.03	2 ±0.07
25	7.6 ±0.35	1.8 ±0.04	1.6 ±0.01	11.6 ±0.03	2.3 ±0.15	1.9 ±0.04
All samples	7.34 ±1.14	2.02 ±0.32	1.63 ±0.13	11.75 ±2.01	2.71 ±0.42	2.09 ±0.44
M-grain	5.58 ±0.08	2.56 ±0.09	1.83 ±0.03	8.95 ±0.34	3.39 ±0.23	2.68 ±0.56
L-grain	8.01 ±0.51	1.86 ±0.12	1.56 ±0.07	12.63 ±1.41	2.5 ±0.17	1.90 ±0.13

Note: [1]Values are expressed as Means ± Standard deviation; [2]L: The length, W: width, and T: thickness.

Table 6 (B) Dimensions[1] of rice grains (ratio) before and after cooking.

Sample number	Dimensions of rice grain				
	Ratios				Elongation
	Uncooked		Cooked		
	L^2/T	L/W	L/T	L/W	
1	3.1 ±0.06	2.1 ±0.06	3.6 ±0.26	2.8 ±0.09	1.5 ±0.0
2	3.1 ±0.11	2.2 ±0.04	3.4 ±0.37	2.6 ±0.06	1.3 ±0.24
3	3 ±0.05	2.3 ±0.25	2.8 ±1.17	3.1 ±0.37	1.6 ±0.04
4	3 ±0.06	2.1 ±0.00	3.6 ±0.19	2.7 ±0.23	1.6 ±0.04
5	3.1 ±0.04	2.2 ±0.10	3.6 ±0.08	2.6 ±0.08	1.6 ±0.04
6	3 ±0.04	2.2 ±0.06	3.5 ±0.04	2.7 ±0.28	1.7 ±0.01
7	5.2 ±0.04	4.5 ±0.07	6.4 ±0.58	4.9 ±0.52	1.5 ±0.57
8	4.2 ±0.16	3.6 ±0.17	5 ±0.28	3.7 ±0.0	1.4 ±0.04
9	5.2 ±0.04	4.2 ±0.02	8.9 ±1.14	6.1 ±0.01	1.9 ±0.11
10	5 ±0.01	4.2 ±0.13	6 ±0.41	4.9 ±0.34	1.5 ±0.10
11	5.7 ±0.11	4.5 ±0.05	7 ±0.19	5.3 ±0.31	1.5 ±0.05
12	4.8 ±0.42	4.2 ±0.35	7.3 ±0.35	5.4 ±0.10	1.8 ±0.21
13	4.8 ±0.04	4.3 ±0.21	7.3 ±0.02	4.9 ±0.56	1.6 ±0.05
14	5.1 ±0.11	4.2 ±0.05	7.4 ±0.12	5.4 ±0.28	1.6 ±0.06
15	5 ±0.06	4.3 ±0.05	6.3 ±0.18	4.9 ±0.01	1.5 ±0.06
16	5.5 ±0.37	4.3 ±0.16	7.9 ±0.04	5.9 ±0.42	1.7 ±0.04
17	5 ±0.11	4.3 ±0.01	6.8 ±0.95	5.2 ±0.32	1.7 ±0.08
18	5.5 ±0.18	4.7 ±0.05	6.9 ±0.28	5 ±0.02	1.6 ±0.06
19	5.5 ±0.25	4.6 ±0.30	6.9 ±0.49	5.1 ±0.08	1.5 ±0.03
20	5.7 ±0.12	4.9 ±0.08	6.7 ±0.21	5.2 ±0.29	1.5 ±0.02
21	4.4 ±0.04	3.4 ±0.09	4.4 ±0.33	3.3 ±0.19	1.4 ±0.0
22	5.5 ±0.18	4.6 ±0.13	7 ±0.21	5.1 ±0.04	1.5 ±0.01
23	5.4 ±0.06	4.6 ±0.05	7 ±0.14	5.6 ±0.43	1.6 ±0.03
24	5.4 ±0.01	4.4 ±0.23	7 ±0.18	5.7 ±0.01	1.6 ±0.05
25	4.8 ±0.18	4.2 ±0.27	6 ±0.12	4.9 ±0.30	1.5 ±0.07
All samples	4.64 ±0.98	3.82 ±0.92	5.94 ±1.69	4.51 ±1.18	1.57 ±0.14
M-grain	3.05 ±0.06	2.21 ±0.12	3.42 ±0.49	2.72 ±0.23	1.56 ±0.15
L-grain	5.14 ±0.07	4.33 ±0.37	6.73 ±1.00	5.08 ±0.68	1.57 ±0.14

Note: [1]Values are expressed as Means ± Standard deviation; [2]L: The length, W: width, and T: thickness.

Whiteness, Transparency, and Degree of Milling

Rice is usually consumed in the milled form **[33]**, where the bran layers are removed. Because proteins and lipids are located in the bran, milling increases starch content and decreases lipids and protein content **[36]**. Therefore, the composition and pasting properties are changed, making reporting the degree of milling essential when evaluating pasting properties **[3]**. Satake degree of milling (SDM) is the commonly used method to determine the degree of milling **[3]**. Measurement is based on measuring whiteness and transparency and calculating SDM using a special algorithm. SDM express results in a value between 0 to 199, where 0 represents brown rice, and 199 represents white wholly milled rice **[35]**. Table (7) shows the whiteness, transparency, and degree of milling for different rice samples. The average whiteness value of medium-grain rice was 40.84 ±1.62, significantly higher ($p \leq 0.05$) than long-grain rice (32.09 ±4.28). There were no significant differences between medium-grain rice samples, while there was a significant difference between long-grain rice samples. Medium-grain rice samples' transparency average was 3.03 ±0.44, significantly higher ($p \leq 0.05$) than long-grain rice (2.49 ±0.41). There were significant differences ($p \leq 0.05$) between and within medium and long-grain rice samples. The average milling degree for medium grain was 100.83 ±6.39, significantly higher than long grain rice, 57.92 ±19.1. There were no significant differences between medium-grain rice, but there are between long-grain rice.

Table 7 Whiteness, transparency, and milling degrees[1] of rice samples.

Brand number	Whiteness	Transparency	Milling degree
1	42.30 ± .28a	3.04 ±0.09abcd	107.5± 0.71a
2	38.75 ±0.48ab	3.44 ±0.04a	94.5 ±6.36ab
3	39.90 ±2.55a	3.16 ±0.34ab	97.5 ±9.19ab
4	41.95 ±0.07a	3.13 ±0.07abc	106.5 ±0.71a
5	40.75 ±1.48a	3.22 ±0.08ab	101.5 ±6.36ab
6	41.40 ±0.14a	2.18 ±0.21fghij	97.5 ±2.12ab
7	30.55 ±0.5de	2.87 ±0.01abcdef	52.5 ±2.12de
8	33 ±0.71de	2.96 ±0.01abcde	65 ±2.83cde
9	38 ±1.56abc	1.81 ±0.08ij	82 ±7.07bc
10	30.10 ±0.14e	2.64 ±0.08bcdefg	49.5 ±0.71de
11	31.15 ±0.5de	2.79 ±0.26abcdef	55.5 ±4.95de
12	38.15 ±0.07abc	1.96 ±0.13ghij	81.5 ±0.71bc
13	32.3 ±0.14de	2.80 ±0.02abcdef	60.5 ±0.71cde
14	33.90 ±0.42cde	2.82 ±0.04abcdef	68.5 ±2.12cd
15	29.55 ±0.5e	2.46 ±0.04cdefghi	46.5 ±2.12de
16	35.10 ±0.71bcd	1.58 ±0.08j	67.5 ±3.54cde
17	41.35 ±0.07a	2.42 ±0.08defghi	98.5 ±0.71ab
18	31.55 ±0.35de	2.73 ±0.28bcdef	56.5 ±3.54de
19	31.20 ±0.85de	2.71 ±0.26 bcdef	55 ±5.66de
20	30.20 ±0.28e	2.69 ±0.14bcdef	50.5 ±0.71de
21	20.95 ±0.92f	1.91 ±0.00hij	4.5 ±3.54f
22	31.15 ±0.64de	2.85 ±0.01abcdef	56 ±2.83de
23	30.05 ±0.07e	2.85 ±0.18bcdefgh	49 ±1.41de
24	31.70 ±3.82de	2.6 ±0.3bcdefgh	56 ±19.1de
25	29.55 ±1.06e	2.33 ±0.38efghi	45 ±7.07e
All samples	34.19 ±5.35	2.62 ±0.47	68 ±25.05
M-grain	40.84 ±1.62	3.03 ±0.44	100.83 ±6.39
L-grain	32.09 ±4.28	2.49 ±0.41	57.92 ±19.1

Note: [1]Values are expressed as Means ± Standard deviation. According to the Tukey test, the means that do not share the same letter in each column are not significantly different ($p > 0.05$).

PCA biplot

Figure 2 shows the PCA biplot for different rice samples using the first two principal components responsible for 99.97% of the variation between samples. The first two principal components separated the rice sample into two distinct groups: one for medium-grain rice and the other for long-grain rice. Medium-grain rice samples are closer to each other than long-grain rice, indicating similar quality. Medium grain rice correlates with high milling degree values, peak viscosity, starch, trough, width, and thickness after cooking. On the other hand, long-grain rice is correlated with high values of pasting temperature, peak time, protein content, setback, uncooked and cooked L/T, and L/W ratios. A previous study reported correlations between pasting properties, cooking, and appearance quality **[37]**.

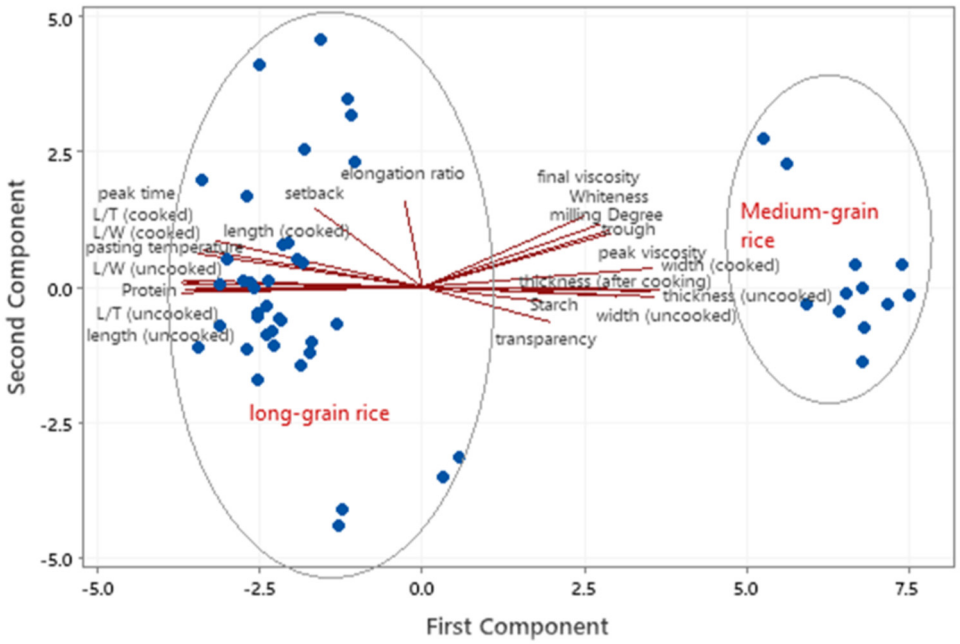

Figure 2 PCA biplot of different parameters tested.

CONCLUSION

All rice samples tested comply with the Jordanian standard except for chalky kernels (four brands), heat-damaged kernels (one brand), and insect infestation (two brands). All samples that exceeded specifications were from the long-grain rice. Medium-grain rice has higher whiteness (40.84), transparency (3.03), milling degree (100.83), moisture (12.01%), starch (91.45%), peak viscosity (2518 cp), trough (1348.9 cp), and final viscosity (2887 cp) than long-grain rice (32.09, 2.49, 57.92, 10.19%, 90.18%, 642.4 cp, 666.6 cp, and 1510 cp, respectively). On the other hand, long-grain rice has a higher protein (8.87%), pasting temperature (94.64 °C), and peak time (6.99 min) than medium-grain rice (7.02%, 88.58 °C, 5.69 min, respectively). There were significant differences in pasting and chemical composition parameters within the two groups of grain sizes. The average elongation ratio for all samples was 1.57 ±0.14, with significant differences between different brands. Due to the higher pasting temperature and peak time, long-grain rice requires more energy during cooking than medium-grain rice. The limitations of this research are not measuring the cooked rice by instrumental texture and sensory evaluation and not measuring the amylose content. Further studies are recommended to measure cooked rice by instrumental texture and sensory evaluation and to measure amylose content to correlate with what had been found in this study.

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The authors declare no conflict of interest.

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

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Development of active and biodegradable film of ternary-based for food application

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ABSTRACT

The effectiveness of plastic packaging in protecting food is quite appreciable, but its non-biodegradable characteristic raises concerns about environmental impacts. This has drawn attention to the development of alternative materials for food packaging from bio-based polymers. Chitosan, a polysaccharide with biodegradable, biocompatible, and non-toxic properties, is widely used in the formulation of food films. The objective of this work was to create a biodegradable and sustainable chitosan-based film whose active and intelligent action is obtained from red cabbage anthocyanins and the addition of propolis. The edible film's thickness and total polyphenol content were 61.0 ±0.1µm and 20.08 ±0.5 mgAG g^{-1}, respectively. The content of phenolic compounds and the biodegradation showed significant results (p <0.05), besides the good thermal stability to 200 °C and transparency. The proposed formulation developed an edible, biodegradable, and active (antioxidant) film with interesting heat-sealing resistance, moisture barrier and gas transfer, which contributes to increasing food shelf life.

Keywords: anthocyanin, biodegradable, biopolymer, chitosan, packaging, propolis

INTRODUCTION

Bio-sourced packaging materials are an interesting alternative to conventional polymers **[1]**, **[2]**, **[3]**. Bio-based polyesters are economically competing with conventional ones due to their biodegradability, availability, compostable nature, barrier properties and good mechanical **[4]**, **[5]**. Chitosan is a polysaccharide obtained by partial deacetylation of chitin found in insect exoskeletons, fungi, and crustacean shells **[6]**, and is the second most abundant natural polysaccharide after cellulose **[7]**. The excellent properties, biodegradability, biocompatibility and non-toxicity of chitosan allow its potential application in the food industry as packaging or coatings that improve the preservation of these products, drawing on its antimicrobial property against a wide range of bacteria, moulds and yeasts **[8]**, **[9]**, **[10]**. Chitosan can interact with the bacterial cell membrane and destabilize it, which makes chitosan films powerful vehicles for antimicrobial compounds that have difficulty reaching their cellular target due to low solubility or permeability **[11]**. Among other advantages is observed high film-forming ability **[12]**, antioxidant activity **[11]**, **[13]**, gas barrier properties **[13]**, **[14]**, and moisture barrier **[15]**, which make it a promising compound for different food products. Propolis is a complex mixture of resinous, gummy and balsamic substances of various consistency, texture and colour. It comprises 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other substances, including organic residues. It presents several antioxidant substances (caffeic acid, feluric acid and caffeic phenyl ester acid) besides flavonoids in its ethanolic extracts **[16]**. Anthocyanins, members of the phenolic family, are responsible for different nuclei in flowers, vegetables and fruits **[17]**. These have been used as active compounds in film formulations for food coatings due to their strong antioxidant power and antimicrobial potential **[18]**, **[19]**. In

addition, anthocyanins can change their chemical structures and colours at different pH values, which is suitable for use in pH-sensing smart food packaging **[20]**, **[21]**. Different anthocyanins have been applied in the development of smart food packaging, such as anthocyanins extracted from red cabbage **[22]**, **[23]**, **[24]**. Thus, it is observed that both compounds used in this study (chitosan and anthocyanins), present important properties in forming material for active and intelligent packaging, with the main objective of increasing the shelf life of foods **[21]**.

Scientific hypothesis

Food film, based on chitosan, propolis and red cabbage, with active and biodegradable properties, can increase the shelf life of food and replace non-biodegradable plastic materials.

MATERIAL AND METHODOLOGY

Samples

The edible film is made from biopolymer materials such as chitosan, propolis and red cabbage.

Chemicals

All reagents used in the execution of this research were of analytical grade and obtained from the Food Preservation & Innovation Laboratory on Campus 2 of the University of Blumenau, Brazil. The acid acetic p.a. was bought from Dinamica Quimica Contemporânea Ltda, and the chitosan (low molecular weight) with a deacetylation degree ≥75% was purchased from Sigma-Aldrich.

Animals, Plants and Biological Materials

It was used extract from red cabbage (*Brassica oleracea*), and propolis produced in the Vale do Itajaí region, South Brazil.

Instruments

A sphere spectrophotometer (SP60, Lovibond) was used for colour testing. Scanning electron microscopy (SEM) was performed with a VEGA 3 SEM scanning electron microscope, Tescan. A HERMLE universal centrifuge, Z300K and UV-1800 spectrophotometer, SHIMADZU were used for total polyphenol content analyses. Thermogravimetric (TGA) analysis was performed in a simultaneous DTA-TG and DTG-60 analyzer, SHIMADZU. An analytical balance (Ohaus Corp. Pine Brook, N. J. USA) was used and for thickness measurements a digital micrometre (0.1 µm resolution) (Mi-tutoyo Co., Kawasaki-Shi, Japan).

Laboratory Methods

The project was carried out in the Biochemical Engineering, Chemical Analysis, and Food Preservation & Innovation laboratories at the University of Blumenau, in southern Brazil.

Film colour was determined **[21]** with a CIE-Lab colour scale applied at 3 different points on each sample to measure L* (clarity), a* (reddish-green) and b* (yellowish-green). Illuminant D65 and an observation angle of 10° were applied for the measurements. Scanning electron microscopy (SEM) of the surface and the morphology of the cross sections of the films were obtained according to **[21]**. In this procedure, the samples were coated with a thin gold layer prior to observation (Q150R ES, Quorum), and an accelerating voltage of 10 kV was applied. Images of the films' top surface and cross section were taken at magnifications of 5000x and 350x.

Total polyphenol content was determined by the Folin Ciocalteau method, with results expressed as mg gallic acid, 100 g^{-1} fresh mass (FM). Briefly, samples (10 g) were macerated and mixed with 20 mL of 96% ethanol. The solution was centrifuged at 4 °C and 120 rpm for 10 min. The supernatant was collected and submitted to the Folin Ciocalteu reagent and sodium carbonate 20% (w/v). The absorbance reading of the samples was performed at a wavelength of 756nm and gallic acid was used as a standard solution.

The biodegradability test was adapted **[25]**, using film samples (C) cut into squares (11cm^2), which were kept inserted 1cm below the soil for 14 days. The soil material used was collected from the University of Blumenau (Latitude: -26.9333 Longitude: -49.0500).

Thermogravimetric analysis (TGA) of the samples was performed according to **[21]**, and the heating rate was set to 10 °C min^{-1} in the range of 30 °C to 600 °C.

Description of the Experiment

Sample preparation: The anthocyanins were extracted from a solution containing 100 g of red cabbage, previously crushed, and 200 mL of distilled water with 1% glacial acetic acid. The solution was stored under refrigeration (4 °C) for 24 hours and then vacuum filtered with Whatman strip filter paper nº 1.

The films were produced following the casting methodology. For this, 4g of chitosan were dissolved in 3 different solutions: (1) 150 mL of a 1% acetic acid solution (control, C), (2) 150 mL of a 1% acetic acid and 1.5 mL propolis extract solution (CP) and (3) 150 mL of red cabbage extract and 1.5 mL propolis extract solution (CPC).

These solutions were kept under stirring (100 rpm) for 5 hours and then, after adding 1.2 mL of glycerol, remained under stirring for another 1 hour. The filmogenic solutions developed were poured into 30 cm x 10 cm refractories and kept at 20 °C, without light, for 7 days. After this period, the films were removed from the refractories. Thickness measurements were taken at 6 different locations of each film sample using a digital micrometre (resolution of 0.1 μm) (Mitutoyo Co., Kawasaki-Shi, Japan).

Number of samples analyzed: The number of samples analyzed was 3.

Number of repeated analyses: Three repeated analyzed were performed for each treatment factor. The total sample analyzed was 9 samples.

Number of experiment replication: The number of experiment replication was 3.

Design of the experiment: The three film samples, developed and analyzed, consisted of the following formulations: chitosan-based (C), which represents the control sample, chitosan and propolis (CP), and chitosan, propolis and red cabbage (CPC).

Statistical Analysis

Data were analyzed using Statistica software (version 7.0, StatSoft Inc., Tulsa, USA). All measurements were performed in triplicate and reported as the mean ± standard deviation (SD). Significance among mean values was determined at 5% level ($p \leq 0.05$) of significance by one-way analysis of variance (ANOVA) with following post-hoc Tukey's test.

RESULTS AND DISCUSSION

Analysis of colour parameters

Different types of anthocyanins are observed in nature, of which six are the most widespread (cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin). Being polar in nature, anthocyanins are soluble in polar solvents such as methanol, ethanol and water. This is the reason why it was extracted with solutinic acetic acid. These solvents are being acidified to stabilize the anthocyanins in the flavylium cation **[17]**. Table 1 shows the results obtained for the colour parameters. Chitosan is a light and soft polymer, so the C sample had a lighter and translucent colour. On the other hand, the CP sample, because it contains propolis, has a more orange tone **[26]**, and the CPC film showed a more intense colouration due to the presence of anthocyanins from red cabbage **[27]**, whose initially red tone, over time, due to the acid medium, becomes orange **[21]**.

Table 1 Colour parameters values for chitosan (C), chitosan and propolis (CP), and chitosan, propolis and red cabbage (CPC) films according to the CIE-Lab scale.

Samples	L*	a*	b*	CIE-lab
C	93.5 ±2.1^{a}	-3.1 ±0.9^{b}	9.6 ±0.8^{b}	
CP	84.7 ±2.6^{a}	-3.5 ±0.6^{b}	40.5 ±1.4^{a}	
CPC	55.7 ±1.5^{b}	25.5 ±2.7^{a}	46.9 ±1.5^{a}	

Note: Different lowercase letters denote significant difference among the films samples ($p \leq 0.05$). L* (clarity), a* (reddish-green) and b* (yellowish-green).

Figure 1 shows the films developed (chitosan = C, CP = chitosan + propolis) and CPC = chitosan + propolis + red cabbage), demonstrating the different colouration and opacity.

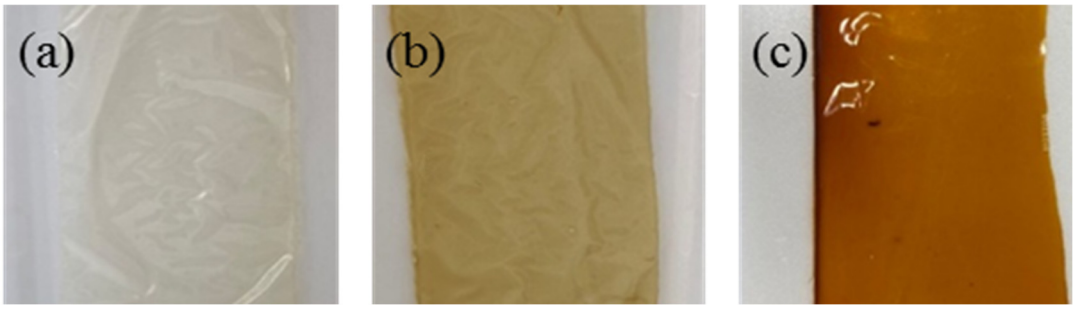

Figure 1 Films developed, (a) chitosan (C), (b) chitosan and propolis (CP), and (c) chitosan, propolis and red cabbage (CPC).

Studies have shown the variation in the colouration of materials composed of anthocyanins **[23]** when submitted to different pH environments, highlighting the potential of these materials for application in intelligent packaging **[27]**. Translucent films are desirable for food application, as it makes it possible to better visualization of the internal contents of the package. More opaque packaging, in turn, reduces the incidence of light, which is interesting for specific products by protecting them against deterioration caused by photooxidative reactions **[28]**.

Studies demonstrate the antimicrobial action of films developed and applied to food, using propolis **[29]**, antioxidant action **[30]** and intelligent function **[31]** when using extracts composed of anthocyanins, resulting in a smart material **[32]**.

Scanning electron microscope (SEM)

Morphological images of the surface and cross-section of the produced biodegradable films are shown in Figure 2.

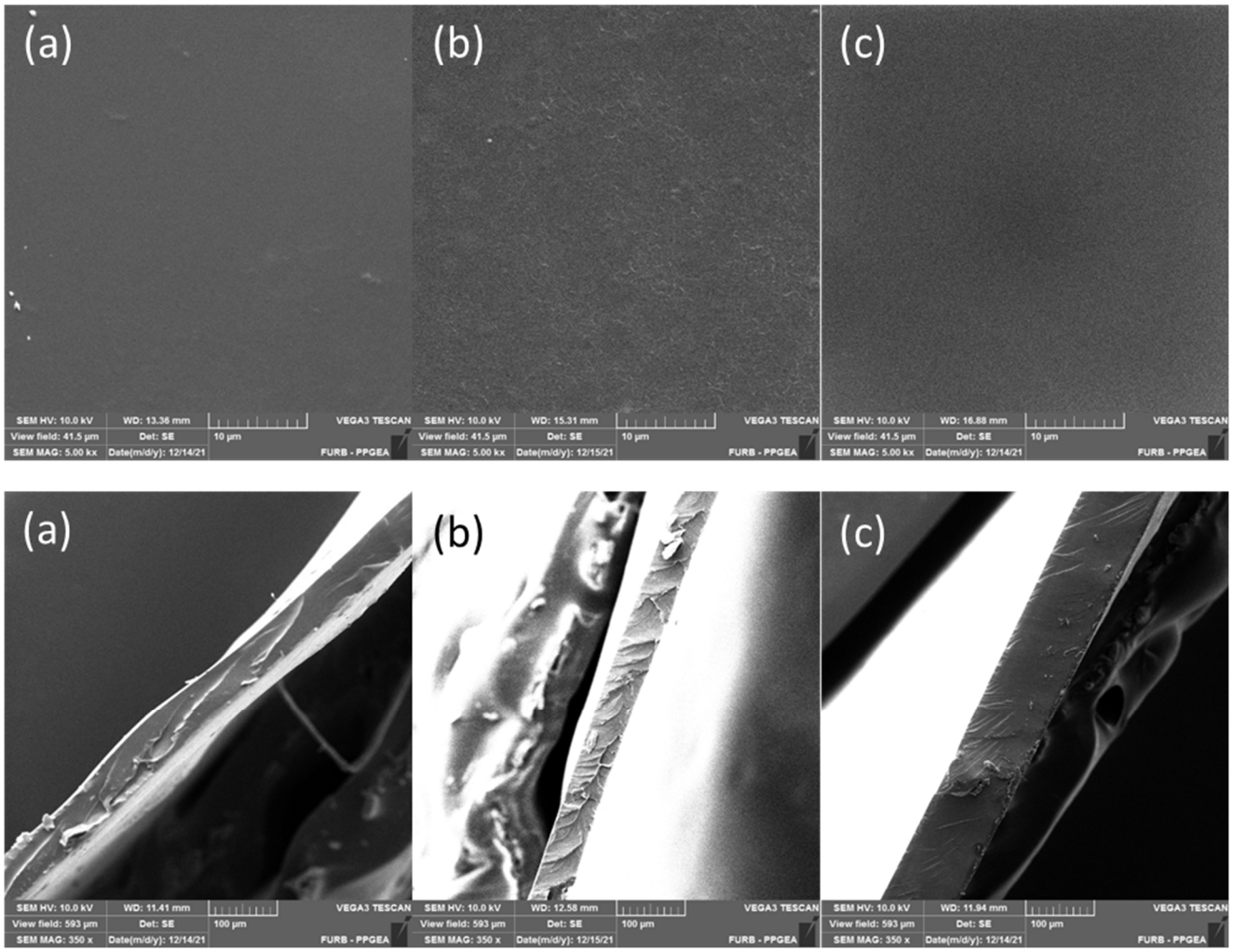

Figure 2 SEM of the surface area (upper image) and cross-section (bottom image) of chitosan film (a), chitosan and propolis (b) and chitosan, propolis and red cabbage (c).

Figure 2 shows a compact and homogeneous structure in all films, indicating good compatibility between all components of the formulations. The addition of propolis makes the structure less uniform, suggesting that there is interaction between the components of chitosan and propolis. However, purple cabbage extract results in a continuous and uniform surface **[33]**, identical to the addition of propolis **[34]**.

The film samples had a thickness of 59.0 to 62.0 ±0.1 μm, similar to that verified by **[33]**. The physical characteristics of the films are very important for marketing and food quality, including when associated with other conservation methods **[35]**, **[36]**. The biopolymer-based films' thickness, colour, and opacity properties are parameters of considerable influence in food packaging and coating applications since they can positively or negatively affect both the quality of the applied food and consumer decisions **[26]**.

Total polyphenol content

The total polyphenolic content quantified in films C, CP and CPC are presented in Table 2. As cited in other studies, propolis **[37]** and red cabbage **[38]** have active agents, such as flavonoids and phenolic compounds.

Table 2 Total polyphenol content in chitosan (C), chitosan and propolis (CP), and chitosan, propolis and red cabbage (CPC) films.

Samples	Total polyphenol content mgAG g^{-1}
C	00.0 ± 0.0^{c}
CP	12.4 ± 0.5^{b}
CPC	20.1 ± 0.5^{a}

Note: Different lowercase letters denote significant difference among the films samples ($p \leq 0.05$). All values are expressed as the mean ±SD (standard deviation).

The results demonstrate that propolis and red cabbage extract present interesting amounts of phenolic compounds **[39]**, responsible for the antioxidant activity of the films **[40]**. The antioxidant property of the films contributes to the prevention of food oxidation and, therefore, extends the shelf life of food products **[41]**. Studies show increased antioxidant activity with the addition of propolis **[42]**. The total phenolic content and DPPH in chitosan films increased significantly ($p < 0.05$) when propolis was added, demonstrating the increase proportional to the concentration of propolis **[43]**. Chitosan has also been reported to have intrinsic antimicrobial activities **[44]** against Gram-negative and Gram-positive bacteria **[45]**.

Biodegradability test

It was observed that after 2 weeks, the C film inside the soil degraded. Figure 3 shows the film's images on day 0 (a) and after 14 days submerged in soil (b). The film has a high potential for degradability **[46]**, which can be explained by its natural composition (biopolymers and chitosan), which facilitates its degradation in a moist, nutrient-rich environment such as soil **[47]**. The films developed with chitosan in this study are non-toxic, are biodegradable and mechanically stable. Thus, they could be used as food packaging material capable of protecting the food content and the environment **[48]**. The presence of propolis in the films did not alter the degradation properties of the soil. Thus, the chitosan-propolis composite films can be considered rapidly degrading materials when discarded in nature **[26]**.

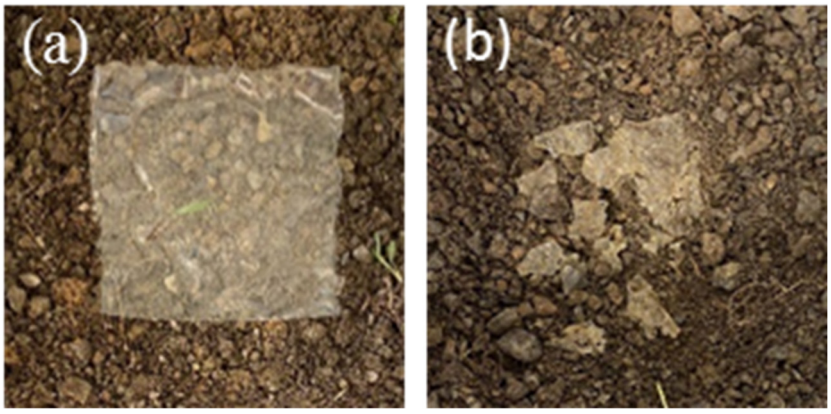

Figure 3 Biodegradability test of C-film (chitosan), (a) day 0 and (b) after 14 days submerged in soil.

Thermogravimetric analysis (TGA)

The thermal property of film reflects its ability to resist decomposition at high temperatures. TGA is frequently used to investigate the thermal stability of film **[49]**. The thermal degradation results of the C, CP and CPC films are shown in Figure 4.

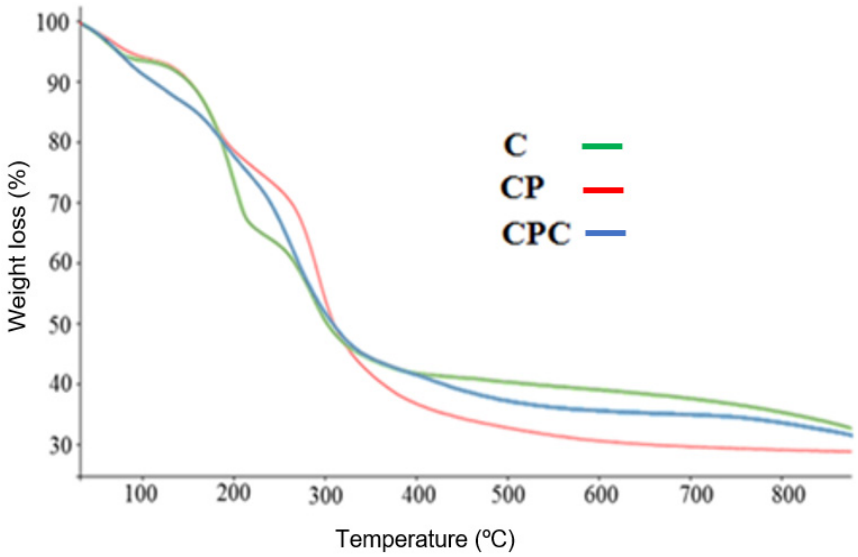

Figure 4 Thermogravimetric analysis of chitosan (C), chitosan and propolis (CP), and chitosan, propolis and red cabbage (CPC) films.

All films showed mass loss due to thermal degradation up to 400 °C. The C film showed a gradual mass reduction of 32%, the CP film of 37% and the CPC of 38% until reaching the temperature of 400 °C **[50]**. This is due to the loss of moisture from the film and from the glycerol. The three films obtained good thermal stability up to 200 °C, which can be attributed to the more compact structure formed by the components, making it difficult for the samples to lose moisture **[51]**. The results were similar to those **[26]** that used chitosan and bio-waste enriched with propolis extract, which showed the first mass loss between 30 and 100 °C, due to the evaporation of water and ethanol in their structures, and the second mass loss (46.0% and 66.9%), between 100 and 700 °C, due to the decomposition of their structure. Studies proved that increased natural extracts such as banana peels extract **[52]**, and purple-fleshed sweet potato extract **[53]**, can reduce chitosan film's weight loss. Films with heat-sealing properties show resistance to thermal degradation and potential application for food packaging **[54]**.

CONCLUSION

The colouration of the films showed a significant difference (p <0.05), demonstrating the influence of propolis and red cabbage extract (anthocyanins) on the colouration and transparency of the material. It is observed that the interaction of the formulation components contributed to obtaining a film with uniform texture and homogeneous colouration, becoming stable at temperatures close to 200 °C, which also indicates the possibility of being heat-sealed. The three compounds used in the development of the film have excellent properties (active, intelligent, edible and biodegradable) that contribute to increasing the shelf life of foods. In addition, the film produced showed good degradability in soil (within 14 days), demonstrating potential as a substitute for petroleum-based plastics.

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Development of sour cream with vegetable oils using a food emulsion stabilised by an emulsifying complex

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ABSTRACT

This scientific work describes the research that aims to study the use of a finely dispersed, aggregately stable food emulsion with a mass fraction of blended oil of 50% and xanthan gum in the composition of sour cream with vegetable oils as an analogue of traditional sour cream. The samples of fat-containing fermented-milk bases as a component of sour cream with vegetable oils with a fat content of 10-20% were obtained using two methods. The first method consists in normalising the fat content of the fermented-milk base obtained by fermentation of skimmed cow's milk with a food emulsion, and the second one – is in the fermentation of a normalised mixture consisting of a food emulsion and skimmed cow's milk. When comparing the duration of fermentation of skimmed cow's milk and normalised mixtures with a fat content of 10 to 20%, it was established that in order to achieve the minimum value of the titrated acidity of the clot of 60 °T, the duration of fermentation of skimmed cow's milk is 6 hours, of a normalised mixture with a fat content of 10% – 8 hours, 15% – 12 hours, 20% – 16 hours. According to the organoleptic quality indicators, the samples of fat-containing fermented-milk bases with a fat content of 20%, obtained by two methods, had an indiscrete but unsuitable thick consistency, which was adjusted using xanthan gum. According to the organoleptic quality indicators, it was established that in order to obtain a sour cream with vegetable oils with an indiscrete and thick consistency, 0.15% of xanthan gum should be added to the fat-containing base obtained by the first method, and 0.20% – to the fat-containing base obtained by the second method. The study of determining the content of polyunsaturated fatty acids in sour cream with vegetable oils with a fat content of 20% shows an increased content of omega-3 and omega-6 fatty acids – 2.13% and 10.88%, respectively, compared to sour cream obtained by the traditional technology.

Keywords: food emulsion, fermented milk product, sour cream, polyunsaturated fatty acids, xanthan gum

INTRODUCTION

To date, the issue of resource conservation in the milk processing industry is relevant due to the decrease in the production of cow's milk as a raw material **[1]**. Instead, there is a tendency to increase the production of milk-containing products, in the content of which fats of vegetable origin replace milk fat. Accordingly, considering the acceptable price and comparing it with milk products, the demand for such products also increased **[2]**.

Thus, the technology of milk-containing products is rapidly developing to provide consumers of all social groups with high-grade food products.

It should be noted that one of the ways to resolve the bacterial equilibrium problem in the human body is to include fermented milk products in the diet, as they contain probiotics that have a positive effect on the gut

microflora **[3]**. Sour cream is a fermented milk product **[4]**. In the production technologies of sour cream and sour creamy consistency products, fats of non-dairy origin are usually used, such as palm and coconut fats and milk fat substitutes **[5]**. However, such fatty components may not satisfy the human body's needs, particularly polyunsaturated fatty acids in vegetable oils **[6]**. The constant analysis of the structure of the population's nutrition indicates a deficiency of polyunsaturated fatty acids, especially omega-3, in the presence of consuming an excess amount of saturated fatty acids **[7]**. Usually, in the technologies of sour creamy consistency products, fat components are added to skimmed milk, and the normalised mixture total volume dispersion is carried out by stirring, which is a rather energy-consuming process. In such cases, the obtained fat products may have unsatisfactory quality during storage because fat globules without stabilisation by emulsifiers may be unevenly distributed in normalised mixtures and have an average size of more than 2 μm. The fat phase dispersion with obtaining fat globules with an average size of not more than 2 μm increases the nutritional value, improves organoleptic and improves the finished product's physical and chemical quality indicators **[8]**. Therefore, using vegetable or blended oils precisely in dispersed form is relevant to obtain new types of sour creamy consistency products. There is the technology of a food emulsion with a mass fraction of fat of 50%. The food emulsion contains blended oil and is an emulsion-type fat concentrate with an average size of fat globules of not more than 2 μm and a stability index of 100%. Such parameters of the food emulsion are achieved due to the use of an emulsifying complex (sodium caseinate + a mixture of polyglycerol and higher fatty acids) and established homogenisation modes **[9]**. Also, since vegetable oils, compared to milk fat **[10]**, cannot structure food systems, stabilisers or thickeners should be used **[11]**. Using stabilisers and thickeners makes obtaining a sour creamy consistency product with high consumer properties possible.

Scientific Hypothesis

The research work hypothesis was based on the assumption that using a food emulsion in the technology of a fermented-milk sour creamy consistency product would avoid dispersing the total volume of the milk-and-vegetable mixture. Accordingly, such a solution would ensure a reduction of the technological process and increase the content of polyunsaturated fatty acids. Using stabilisers and thickeners would allow for obtaining a product with high organoleptic quality indicators.

MATERIAL AND METHODOLOGY

Samples

The research work was carried out with samples, the composition of which is given in Table 1.

Table 1 Formulations of test samples and control samples.

Components	Fat Mass Fraction, %			
	0.0	**10.0**	**15.0**	**20.0**
Control Sample*				
Cream obtained from cow's milk (with a fat mass fraction of 20%)	-	-	-	100.0
Total	-	-	-	100.0
Fat-Containing Fermented-Milk Base No. 1*				
Skimmed cow's milk	100.0	80.0	70.0	60.0
Food emulsion (with a fat mass fraction of 50%)	-	20.0	30.0	40.0
Total	100.0	100.0	100.0	100.0
Fat-Containing Fermented-Milk Base No. 2				
Fermented skimmed cow's milk	-	80	70	60
Food emulsion (with a fat mass fraction of 50%)	-	20	30	40
Total	-	100.0	100.0	100.0

Note: Direct application bacterial preparation. It is not indicated in the formulated composition.

Chemicals

Distilled water, H_2O (TOV Novokhim, Ukraine).
Phenolphthalein alcoholic solution, $C_{20}H_{14}O$, 1.0% (Shostka Chemical Reagents Plant, Ukraine).
Sodium hydroxide, NaOH, 0.1 N (TOV Khimlaborreaktiv, Ukraine).
Cobalt sulphate solution, $CoSO_4$, 2.5% (TOV Khimlaborreaktiv, Ukraine).
Sodium methylate, CH_3ONa (ATK Ukraine, Ukraine).
Sodium sulphate, Na_2SO_4 (AT ZPD, Denmark).
MRS-agar (Conda, Ukraine).

Animals, Plants and Biological Materials

Iprovit SSK bacterial preparation (Institute of Food Resources NAAS of Ukraine, Ukraine) containing *Lactococcus lactis* ssp. *lactis; Lactococcus lactis* ssp. *cremoris; Lactococcus lactis* ssp. *diacetilactis; Streptococcus salivarius* ssp. *thermophilus*.

Instruments

Laboratory thermometer, (TOV Standard-Lab).
Mohr pipettes, (TOV SkyLab).
Bunsen beaker, (TOV SkyLab).
Conical flask, (TOV SkyLab).
Glass rods, (TOV SkyLab).
Titration assembly, (TOV Labour-Technik).
Thermostat TSO-80, (TOV Ukragrotest).
Gas chromatograph (GE LifeSciences BPG 100/500, Germany).
Petri dish (TOV Termolab).
Counter of colonies of microorganisms JL-1C (TOV Spectrolab).
Microscope XS-5520 LED (TOV Micromed).

Laboratory Methods

The titrimetric method determined the titrated acidity, which is based on the neutralisation of acids contained in the investigational product with a sodium hydroxide solution in the presence of an indicator, according to GOST 3624 **[12]**. The fatty acid content was determined by chromatographic according to DSTU ISO 15885/IDF **[13]**. Organoleptic quality indicators were assessed by tasting and compared with standard indicators according to DSTU 4418 **[14]**. The number of viable lactic acid bacteria was determined by the method of sowing serial dilutions in agar nutrient media according to GOST 10444.11 **[15]**.

Description of the Experiment

Sample preparation: Food emulsion with a mass fraction of blended oil (sunflower oil and linseed oil (85:15)) of 50% was obtained by homogenisation of a coarsely dispersed mixture, which consists of a previously prepared fat component and the obtained aqueous solution of sodium caseinate **[16]**. The fat-containing fermented-milk bases and the control sample were obtained by the fermentation of a normalised mixture (the fat-containing fermented milk base No. 1), cream, and by the normalisation of fermented skimmed cow's milk with a food emulsion (the fat-containing fermented milk base No. 2), according to the formulations given in Table 1. Xanthan gum was added to fat-containing fermented-milk bases with a fat content of 20% No.1 and No. 2 with mass fractions from 0.1 to 0.3% at 20 °C and stirred for 5 minutes.

Number of samples analysed: During experimental investigations, 9 samples were used, and the titrated acidity and organoleptic quality indicators were determined in 7 samples, of which and the fatty acid composition was determined in 2 samples.

Number of repeated analyses: All measurements were performed 3 times.

Number of experiment replication: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: Normalised mixtures and skimmed cow's milk were fermented in a thermostat at a temperature of 30 °C for 16 hours. Every two hours of the fermentation process, the titrated acidity was determined in normalised mixtures and skimmed cow's milk according to the method **[12]**. The normalisation of fermented skimmed cow's milk with a food emulsion was carried out by stirring at a temperature of 20 °C for 5 minutes. A sour cream with vegetable oils was obtained by adding xanthan gum to fat-containing fermented-milk bases No. 1 and No. 2 (with a fat content of 20%) at a temperature of 20 °C and stirring for 5 minutes. Determination of the number of viable lactic acid bacteria in fat-containing fermented-milk bases No. 1 and 2 was carried out according to the method **[15]**. The organoleptic quality indicators of fat-containing fermented-milk bases No. 1 and No. 2 were assessed by tasting and compared with standard indicators in the regulatory documentation **[14]** for sour cream as traditional one. The chromatography method determined the content of omega-3 and omega-6 polyunsaturated fatty acids in the sour cream with vegetable oils and the control sample **[13]**.

Statistical Analysis

The STATISTICA Microsoft Excel editor combined with XLSTAT processed experimental data using mathematical statistics methods. The accuracy of the obtained experimental data was determined using the Student's t-test with a confidence coefficient ≤0.05 with many parallel definitions of at least 5 (confidence probability $p = 0.95$).

RESULTS AND DISCUSSION

The methods of obtaining a fat-containing fermented-milk base for sour cream with vegetable oils, consisting of skimmed cow's milk and a food emulsion, were substantiated at the first stage of the research investigation. Figure 1 shows the value of the titrated acidity of samples of normalised mixtures and skimmed cow's milk during fermentation at 30 °C.

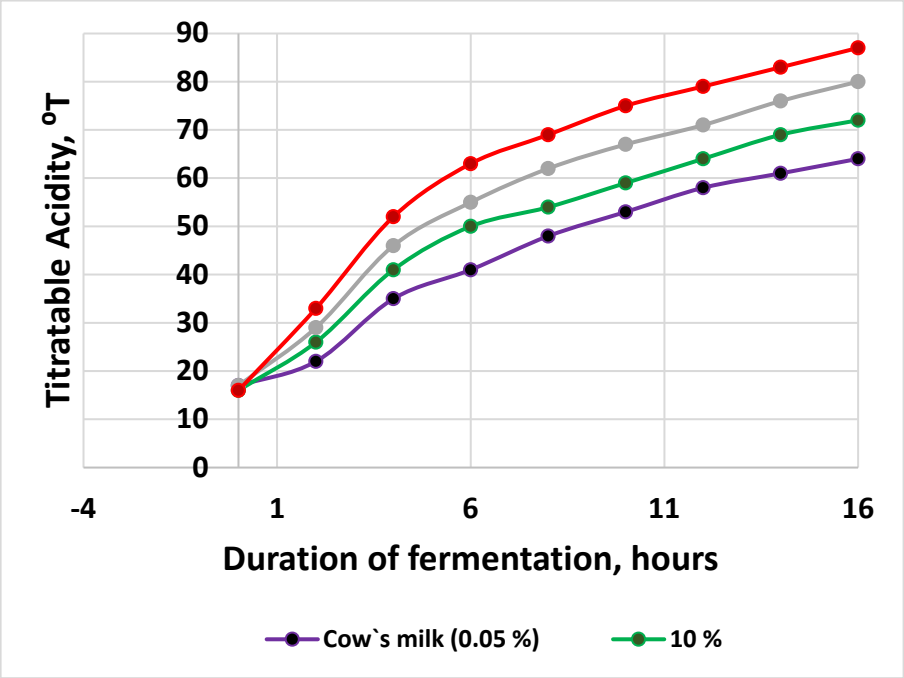

Figure 1 Values of the titrated acidity of samples of normalised mixtures with a fat content of 10, 15, 20% and skimmed cow's milk during the fermentation process.

According to Figure 1, it is clear that during the fermentation, a fermented-milk coagulum with a titrated acidity of 60 °T was obtained from all test samples, which meets the regulatory requirements according to DSTU 4418 **[14]**.

However, the fermentation time of the samples to reach the specified acidity value was different. Thus, to achieve the minimum value of the titrated acidity of the coagulum (60 °T), the duration of fermentation of full-fat cow's milk is 6 hours, and of a normalised mixture with a mass fraction of blended oil of 10% is 8 hours, of 15% – 12 hours, of 20% – 16 hours. Therefore, the components of a food emulsion inhibit the development of lactic acid microflora compared to the duration of the skimmed cow's milk fermentation.

Sour cream, according to the traditional technology, is obtained by the fermentation of cream with bacterial cultures **[17]**, and sour creamy consistency products are usually obtained from a mixture consisting of skimmed milk and vegetable oils (fats), and bacterial cultures **[18]**, **[19]**.

It should, however, be noted that the fermentation process of cream containing milk fat (of 10-40%) and used in the production of sour cream according to the traditional technology is longer compared to the fermentation process of cow's milk with a fat content of 0.05%-2.5% due to deteriorating the fermentation microflora **[20]**. Also, not only milk proteins but also the fat phase are involved in the formation of the fermented-milk coagulum and its quality indicators **[21]**.

With this in mind, it is possible to obtain a fat-containing fermented-milk base by fermenting a mixture normalised by the content of blended oil (skimmed cow's milk + a food emulsion) or by mixing a previously obtained fermented milk base (skimmed cow's milk fermented with a bacterial preparation) with a food emulsion, that should be tested experimentally.

The Iprovit SSK bacterial preparation specification states two fermentation temperature regimes 30-34 °C and 36-38 °C **[22]**. Linseed oil in a food emulsion blended as a fat phase is vulnerable to the oxidative deterioration **[23]**, **[24]**. According to the regulatory requirements for blended oil **[25]** contained in a food emulsion, its storage temperature is up to 30 °C. Therefore, the fermentation of the normalised mixture was carried out at a temperature of 30 °C to avoid oxidative deterioration of linseed oil.

Also, it should be noted that the lactose content, which ensures the process of lactic-acid fermentation **[26]**, **[27]** in normalised mixtures, is sufficient to obtain fermented milk coagulum with a titrated acidity of 60 °T.

The number of viable lactic acid bacteria in fat-containing fermented-milk bases and their organoleptic quality indicators are shown in Table 2 and Table 3.

Table 2 Number of viable lactic acid bacteria in fat-containing fermented-milk bases.

Sample		Number of viable lactic acid bacteria, CFU/g
The norm, no less than [14]		1.0×10^7
Fat-Containing Fermented-Milk Base No. 1	fat content 10 %	10^8
	fat content 15 %	
	fat content 20 %	
Fat-Containing Fermented-Milk Base No. 2	fat content 10 %	
	fat content15 %	
	fat content 20 %	

Table 2 shows that all samples of fat-containing fermented-milk bases after fermentation have a standard amount of viable lactic acid bacteria as for classic sour cream.

According to the research results given in Table 3, it was established that the deterioration in consistency is observed with the increase in the mass fraction of blended oil in the fat-containing fermented milk base No. 1. Thus, the fat-containing fermented milk base with a mass fraction of fat of 20% acquired an unsuitable thick consistency. All three samples of the fat-containing fermented milk base No. 2 acquired an unsuitable thick consistency. This consistency characteristic is explained by the fact that blended oil is not a fat phase capable of forming and texturing this food system, compared with milk fat **[28]**, **[29]**.

Table 3 Organoleptic quality indicators of fat-containing fermented-milk bases.

Indicator	Blended Oil Mass Fraction, %		
	10	**15**	**20**
	Fat-Containing Fermented-Milk Base No. 1		
Appearance and consistency	Homogeneous mass with a glossy surface, thick		Homogeneous mass with a glossy surface and unsuitable thick consistency
Taste and smell	Pleasant, fermented-milk, slightly nutty		
Colour	White with a cream shade, equal throughout the mass		
	Fat-Containing Fermented-Milk Base No. 2		
Appearance and consistency	Homogeneous mass with a glossy surface and unsuitable thick consistency		
Taste and smell	Pleasant, fermented-milk, slightly nutty		
Colour	White with a cream shade, equal throughout the mass		

Therefore, there is a need to improve the consistency of samples with mass fractions of blended oil of 20%, obtained by various methods using a thickener and structure stabiliser, which will allow obtaining finished products with the maximum possible content of polyunsaturated fatty acids. The use of thickeners and/or stabilisers is widely used to improve the technology of sour cream **[30]**, which contributes to obtaining higher quality indicators, particularly the consistency of finished products.

At the research second stage, the composition of sour cream with vegetable oils using xanthan gum as a natural thickener and structure stabiliser was substantiated **[31]**, **[32]**.

Xanthan gum forms a substance that is similar to a gel **[33]**, but its solution has great mobility - under mechanical action, the viscosity decreases. After stopping the action, the viscosity returns to the previous one **[34]**, **[35]**, which will positively affect a sour cream with vegetable oils.

Organoleptic quality indicators of samples of sour cream with vegetable oils with a mass fraction of blended oil of 20% based on the fat-containing fermented-milk bases No. 1 and 2 with different xanthan gum content are given in Table 4.

As can be seen from Table 4, to obtain an homogeneous, thick consistency, 0.15% of xanthan gum should be added to the fat-containing base No. 1, and 0.20% – to the fat-containing base No. 2. The different content of the used xanthan gum in the composition of fat-containing fermented-milk bases is explained by the fact that the fat-containing base No. 2 has a more unstructured consistency due to adding a food emulsion separately to the fermented-milk base made from skimmed cow's milk. At the same time, during the fermentation process, the normalised mixture No. 1 formed a more structured fermented milk coagulum involving fat globules with partially coagulated protein **[36]**, **[37]**.

Table 4 Organoleptic quality indicators of samples of sour cream with vegetable oils with a mass fraction of blended oil of 20% with different contents of xanthan gum.

Indicator	Xanthan Gum Content, %				
	0.10	**0.15**	**0.20**	**0.25**	**0.30**
Fat-Containing Fermented-Milk Base No. 1					
Appearance and consistency	Homogeneous mass with a glossy surface and unsuitable thick consistency	Homogeneous mass with a glossy surface, thick	Homogeneous mass with a glossy surface, viscous		Homogeneous mass, gel-like
Taste and smell	Pleasant, fermented-milk, slightly nutty				
Colour	White with a cream shade, equal throughout the mass				
Fat-Containing Fermented-Milk Base No. 2					
Appearance and consistency	Homogeneous mass with a glossy surface and unsuitable thick consistency	Homogeneous mass with a glossy surface and unsuitable thick consistency	Homogeneous mass with a glossy surface, thick	Homogeneous mass, viscous	
Taste and smell	Pleasant, fermented-milk, slightly nutty				
Colour	White with a cream shade, equal throughout the mass				

The fat content of sour cream with vegetable oils (20%) corresponds to the average fat content of sour cream, which is a positive point since there is an adverse reaction to a low-fat diet with the recognition that the consumption of foods, which are high in carbohydrates, is probably harmful **[38]**.

The content of omega-3 and omega-6 polyunsaturated fatty acids in sour cream with vegetable oils is shown in Figure 2.

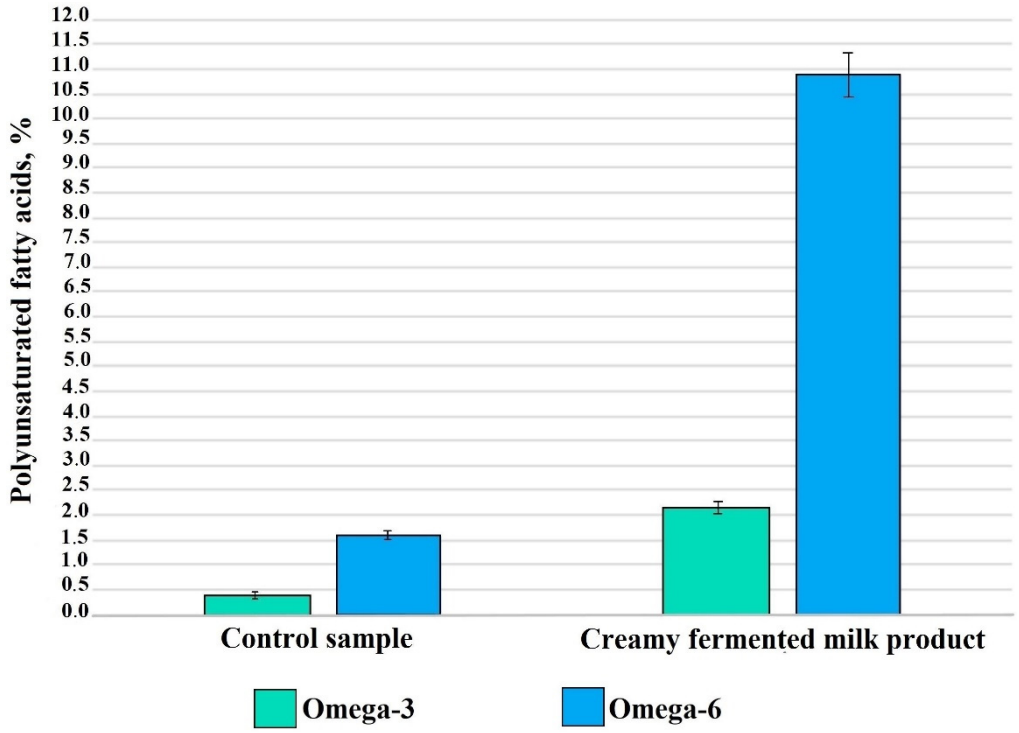

Figure 2 The content of omega-3 and 6 polyunsaturated fatty acids in sour cream with vegetable oils compared to the control one.

As can be seen from Figure 2, the content of omega-3 and omega-6 polyunsaturated fatty acids in sour cream with vegetable oils is higher compared to sour cream obtained by traditional technology and is 2.13% and 10.88%, respectively.

As is known **[39]**, polyunsaturated fatty acids, especially omega-3 and omega-6, are not synthesised by the human body and must be obtained with food. The important role of polyunsaturated fatty acids in preventing and treating many diseases has been proven **[40]**. According to the content of omega-3 and omega-6, their ratio in sour cream with vegetable oils is 1:5, due to the use of blended oil **[41]**, which corresponds to the recommendations of the World Health Organisation regarding this ratio (1: (5-15)) for widely consumed food products **[42]**.

CONCLUSION

The research was conducted on the possibility of using a food emulsion with a mass fraction of blended oil of 50%, with an average size of fat globules of not more than 2 μm and xanthan gum, and as a natural thickener and stabiliser in the composition of sour cream with vegetable oils as an analogue of traditional sour cream. The first method of obtaining a fat-containing fermented-milk base for sour cream with vegetable oils, consisting of a food emulsion and fermented skimmed cow's milk, and the second method of obtaining a fat-containing fermented-milk base, consisting of a normalised mixture, which contains a food emulsion and skimmed cow's milk, are substantiated. The duration of skimmed cow's milk fermentation is 6 hours, of normalised mixture with a fat content of 10% – 8 hours, 15% – 12 hours, 20% – 16 hours. A deterioration in consistency is observed with an increase in the mass fraction of blended oil in the fat-containing fermented-milk base from 10 to 20%. Thus, the fat-containing fermented-milk base obtained by the first method with a fat content of 20% and the fat-containing fermented-milk bases with a fat content of 10-20% obtained by the second method acquired an unsuitable thick consistency. To obtain a sour cream with vegetable oils with an indiscrete and thick consistency, 0.15% of xanthan gum should be added to the fat-containing base with a fat content of 20%, obtained by the first method, and 0.20% – to the fat-containing base with a fat content of 20%, obtained by the second method. It was found that the content of omega-3 and omega-6 polyunsaturated fats is 2.13% and 10.88%, respectively, which is higher compared to sour cream with a fat content of 20% obtained by the traditional technology, in a sour cream with vegetable oils, which includes fat-containing bases obtained by two methods. Thus, the possibility of using food emulsion and xanthan gum as part of sour cream with vegetable oils with a fat content of 20% has been proven. Under the industrial production conditions of the developed sour cream with vegetable oils, the product label will indicate that it is a product made from raw dairy materials with a complete replacement of milk fat with vegetable oils. The developed sour cream with vegetable oils makes it possible to expand the range of milk-containing fermented-milk products with increased polyunsaturated fatty acids. Also, it is recommended to be used both as a finished product and as a semi-finished product for obtaining food-fermented products with the content of plant raw materials by including vegetable, fruit, and berry heterogeneous or homogeneous fillers.

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Ethical Statement:

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

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The variability of acrylamide content in potato French fries depending on the oil used and deep-frying conditions

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ABSTRACT

The research aimed to investigate the variability of the acrylamide content in French potato fries depending on the type of oil and the length and conditions of deep-frying. Deep-frozen pre-fried potato French fries primarily intended for catering establishments were deep-fried parallel in two oils (multi-component oil and rapeseed oil) at the same conditions (175 °C/4 min and 200 °C/3 min) until the limit for total polar compounds (TPCs) content (24%) was reached. The samples were analysed immediately after removal from the package, after the first frying and when the TPCs was exceeded. High-performance liquid chromatography/electrospray ionization tandem mass spectrometry (HPLC/ESI-MS/MS) was used to determine acrylamide. Mathematical and statistical evaluation of the results was according to the indicators of descriptive characteristics, i.e., arithmetic mean, standard deviation (SD), and coefficient of variation (%). Analysis of variance (ANOVA) was used to compare groups, i.e., the assumption of agreement of variance was verified by the F test (F). All pairwise differences in means were tested using Tukey's HSD test (Honest Significantly Different) and Scheffe´s test. The critical value of α, compared to the standardized difference between the means, was established using our chosen risk of 5%. The highest acrylamide values were measured in samples deep-fried in rapeseed oil at 200 °C/3 min in sample 2b (451.13 µg/kg when deep-fried immediately) and in sample 2d (383.24 µg/kg after exceeding TPCs). The lowest values of acrylamide were found in samples deep-fried in multi-component oil at a temperature of 200 °C/3 min in sample 1d (183.35 µg/kg after exceeding TPCs) and at a temperature of 175 °C/4 min in sample 1c (240.75 µg/kg after exceeding TPCs). The decreased tendency of acrylamide in both types of oils and variants of temperature after exceeding TPCs compared to the state immediately after frying is confirmed for all samples. Potato-based products are a significant source of acrylamide production and subsequent consumption. Monitoring its presence in food is, therefore, an important legislative requirement.

Keywords: French fries, Oil, Acrylamide, Deep-frying, Liquid chromatography

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) rank as the fifth highest-produced commodity for human consumption. In 2019, 371 million metric tons of potatoes were produced worldwide, with China, India and Ukraine accounting for 45% of global production **[1]**. Potato tubers need to be processed before consumption. Due to their culinary versatility, different cooking practices are applied, with the most widespread being boiling, microwaving, toasting, roasting and frying **[2]**. Frying is widely used in industrial and domestic sectors because it can create unique sensory properties in fried food **[3]**. Several chemical reactions occur in the oil during frying and deep-frying, such as oxidation, hydrolysis, oxidative and thermal polymerization, which may cause the formation of various process contaminants **[4]**. The benefits of the frying are its speed and operational simplicity. Even though deep-frying is an old and popular process, it is still poorly understood **[5]**. French fries are a semi-finished product and a finished product that is sold frozen in a wide variety of assortments, usually in the form of strips of various thicknesses, but also in the form of slices, crescents, and quarters, so with or without the skin, or even as whole

small tubers. Traditional fries are the most popular variant, defined as fried potato pieces in the shape of strips with a cross-section of 10 x 10 mm and a length exceeding 6-7 cm. When pre-fried, they contain about 4% fat, while ready-to-eat French fries can contain about 7% fat **[6]**. Repeated frying and deep-frying of food in the same vegetable oil leads to the formation of new compounds resulting from hydrolytic, thermal, and oxidative reactions **[7]**, **[8]**. Interactions of oils with food matrices lead to further changes affecting food quality and digestibility. These are changes in fatty acid composition, decreased nutritional value considering vitamins and antioxidant compounds, and increased accumulation of dimer and polymerized molecules and other chemical compounds with toxic potential for human health **[9]**, **[10]**.

Short-chain fatty acids, aldehydes, ketones, alcohol, and nonvolatile products generated by lipid oxidation have higher polarity than triglycerides of fresh edible oil. These are considered total polar materials /compounds (TPM/TPCs), and fried food with TPM has been found to exhibit various ill-health effects **[11]**. Total polar compounds (TPCs) the content in deep-fried oils is a reliable indicator of oxidative degradation of frying oils. For public health concerns, the content of TPCs in frying oil is regulated at not more than 25%, in Taiwan **[12]**. Several authors **[13-16]** have determined TPM in a wide range of food. Temperature and frying time affect the kinetics of a wide spectrum of oxidation products and the formation of acrylamide **[17]**. Acrylamide (CH2=CH-CONH, 2-propenamide) is a white, odourless, crystalline solid with a relative molecular weight of 71.08 g x mol^{-1} **[18]**. Acrylamide is produced during the heat treatment of food. Due to its simple structure, it can be formed by various mechanisms, which include the reactions of carbohydrates, proteins, amino acids, lipids, and probably other minor food components. However, it primarily reacts with the amino acid asparagine and glucose **[19]**. Asparagine content is relatively higher in potato-based products than the reduced sugar content. Reducing sugar level influences the acrylamide formation of the products **[20]**, **[21]**. Acrylamide is formed in food at a temperature of 120-210 °C, but most of it is formed in the temperature range of 120-170 °C. However, its formation is also possible at a temperature of 100 °C, when an N-glycoside is formed, cleaved between the bonds of C-N atoms, and an intermediate product is formed from which acrylamide can be formed **[22]**. The primary formation mechanism of acrylamide in food is the reaction of the free amino acid, asparagine, and reducing sugars at temperatures above 120 °C through the Maillard reaction **[23]**. Acrylamide is produced in foods rich in carbohydrates, which are processed at high temperatures by frying, baking, and roasting. These are most often potato crisps, French fries, and baked potatoes. A slightly lower content is found in bread, breakfast cereals (roasted), coffee, coffee substitutes, gingerbread, crackers, wafers, and biscuits **[24]**.

Acrylamide is a chemical processing contaminant that is generated during the thermal treatment of heat-processed foodstuffs such as fried potato, cereal-based products, and roasted coffee. This compound presents a public health concern and a priority for the European Food Safety Authority (EFSA). In 2017, the European Commission published a regulation to control acrylamide levels within the primary sources of exposure **[25]**. In 2019, a new recommendation for monitoring the presence of acrylamide in other foods was also introduced **[26]**. The US Food and Drug Administration (FDA) has also provided guidance to the industry regarding monitoring and controlling acrylamide in food products, although it has not established maximum recommended levels **[27]**. French fries are one of the main contributors to the dietary intake of acrylamide **[28]**, **[29]**, although estimated levels of acrylamide intake often fail to take into account consumer behaviour during the preparation of this food **[30]**. Fried potatoes (French fries, crisps, and hash browns) are prone to acrylamide formation due to the high content of precursors in fresh tubers and the intensity of the thermal treatment applied during frying **[31]**, **[32]**. Acrylamide in food potentially increases the risk of developing cancer for consumers in all age groups. Since acrylamide is present in a wide range of everyday foods, this concern applies to all consumers, but children are the most exposed age group on a body weight basis **[33]**. Acrylamide exhibits several toxic effects at once, increasing its overall toxicity. In addition to carcinogenic, neurotoxic, mutagenic, teratogenic, and genotoxic properties, its influence on reproduction has also been proven **[22]**. The potato industry is continuously perfecting acrylamide mitigation strategies, including selecting suitable potato varieties, controlling transport and storage conditions, monitoring and adjusting process conditions, and using alternative technologies. An essential strategy is to provide end users with information about suitable food preparation methods **[34**, **35, 36, 37, 38, 39]**.

The research aimed to investigate the variability of the acrylamide content (µg/kg) in French potato fries depending on the type of oil and the length and conditions of deep-frying.

Scientific Hypothesis

H1: We expect that acrylamide in French fries will rise at higher frying temperatures.

H2: After the first frying of French fries, the acrylamide content will be lower than after reaching the TPCs limit value, which means wear/burn-through oil.

H3: We expect differences in acrylamide content in French fries fried in rapeseed and multi-component oil.

MATERIAL AND METHODOLOGY

Samples

Deep-frozen pre-fried potato French fries primarily intended for catering establishments were deep-fried parallel in two oils (multi-component oil and single-component oil) (Figure 1). Composition of pre-fried and deep-frozen potato French fries: potatoes, sunflower oil. Nutritional information in 100 g of deep-frozen product: fats 4.5 g, saturated fatty acids 0.5 g, carbohydrates 24 g, sugars 0.5 g, proteins 3 g, salt 0.1 g.

For deep-frying French fries, edible multi-component and rapeseed oils have a wide spectrum of long-term and short-term thermal food preparation (cooking, steaming, frying, baking) and a cold kitchen (salads, marinades, sauces etc.).

Figure 1 Deep-frozen pre-fried potato French fries preparation.

Multi-component oil (1): sunflower oil, sunflower oil HO, rapeseed oil, anti-foam agent E 900. 100 g of the product contains 9.2 g of saturated fatty acids, 56.4 g of monounsaturated fatty acids, 34.4 polyunsaturated fatty acids and 0 g of carbohydrates.

Single-component (rapeseed) oil (2): 100% rapeseed oil suitable for frying. 100 g of the product contains 91.5 g of fat, of which 8.6 g are saturated fatty acids, 0 g are carbohydrates.

Chemicals

Acetic acid HPLC grade (Fischer Scientific, Loughborough, UK), d3-acrylamide (2,3,3-D_3-AA) (98%) (Cambridge Isotope Laboratories, Androver, USA), ethyl acetate 99%, (Centralchem, Bratislava, Slovak Republic), $K_4[Fe(CN)_6.3H_2O$ p.a. (Centralchem, Bratislava, Slovak Republic), $ZnSO_4.7\ H_2O$ p.a. (Centralchem, Bratislava, Slovak Republic), methanol gradient grade ≥99.9% (Sigma-Aldrich Laborchemikalien, Germany).

Instruments

Deep-fat fryers Bartscher SNACK III A162810E with smart thermostat (Bartscher, Germany).

Oil tester Testo 270 (Testo SE, Germany).

Vacuum rotatory evaporator Heidolph WB 2000 (Heidolph Instruments, Schwabach, Germany)

HPLC system 1200 series (Agilent Technologies, Santa Clara, California, USA) coupled to an Agilent 6460 Triple Quad detector equipped with an ESI interface.

Centrifuge Sigma 2-16KC (Sigma, Osterode am Harz, Germany).

Nylon syringe filter (Q-Max RR Syringe Filters, Frisenette ApS, Knebel, Denmark).

Laboratory Methods

The method for determining acrylamide was according to Ciesarová et al. **[40]**. Samples were extracted to acetic acid (0.2 mM) water solution and pre-extracted to ethyl acetate to avoid the negative impact of salts in the chromatography system. The sample preparation was as follows: 2.0000 g of a homogenized sample was weighed into a 10 mL centrifuge tube with a cap, then 100 µL of the internal standard solution (0.0020 g of d3-acrylamide in 100 mL of water) and 18 mL of acetic acid (0.2 mM) were added. After shaking by a vortex mixer for 30 s, the mixture was sonicated for 5 min. Then, 1 mL of Carrez solution I (15 g of $(K_4[Fe(CN)_6].3H_2O$ in 100 mL of water) and 1 mL of Carrez solution II (30 g of $(ZnSO_4.7\ H_2O)$ in 100 mL of water) were added and mixed for

1 min. After that, the mixture was centrifuged in Sigma 2-16KC at 8720 x g for 10 min at 5 °C. A volume of 5 mL of the clear supernatant was transferred to a separator funnel; 5 mL of ethyl acetate was added and mixed well. The ethyl acetate layer was removed, and the extraction step was repeated twice with 5 mL of ethyl acetate. 3 x 5 mL of ethyl acetate layers were collected and evaporated in a vacuum rotatory evaporator Heidolph WB 2000 at 35 °C to dryness. The residue was dissolved in 1 mL of acetic acid solution (0.2 mM) and filtered through a 0.45 µm pore size nylon syringe filter. LC-MS/MS analysis was performed with an HPLC system 1200 series coupled to an Agilent 6460 Triple Quad detector equipped with an ESI interface. The analytical separation was performed on Atlantis dC18 (100 mm, 3 µm) column (Waters, Milford, MA, USA) using an isocratic mixture of 5 mL of methanol, 1 mL of acetic acid and 500 mL of deionized water at a flow rate of 0.4 mL/min at 25 °C. The ESI mass spectrometry detection was performed in a positive ESI+ mode with drying gas (N_2) flow 8 L/min and 350 °C temperature, nebulizer pressure 50 psi, capillary voltage 2.5 kV. Data acquisition was performed using multiple reaction monitoring (MRM) with the transition for acrylamide: 72 → 55 and d3-acrylamide: 75 → 58. The quantification of acrylamide was calculated with a calibration curve of the standard compound in the range from 10 to 4000 ng/10 mL. The analysis time was 11 min; the retention time of acrylamide and d3-acrylamide was 2.0 min. The LOD of the applied procedure was 10 µg/L; LOQ was 15 µg/L.

Description of the Experiment

Number of samples analyzed: 9.

Number of repeated analyses: 3.

Number of experiment replication: All experiments were done at least in triplicates.

Design of the experiment: Deep-frozen pre-fried potato French fries were deep-fried parallel in two oils (multi-component oil and single-component oil) at the same conditions (175 °C/4 min and 200 °C/3 min) until the limit for TPCs content (24%) was reached. The samples were analysed immediately after removal from the package, after the first deep-frying and when the TPCs were exceeded. Four commercial deep-fat fryers of capacity 8 L were used for frying French fries samples. Fresh oils were loaded into the fryer separately and heated to 175 and 200 °C. The same batch of French fries (100 g – one frying cycle) was deep-fried, and the same frying conditions (175 °C/4 min and 200 °C/3 min) were applied. Afterwards, the French fries were placed in a plate, and extra oil was sucked using tissue paper. The frying procedure was held constantly for several days (6 h per day according to reached 24% TPCs content). At the end of each frying day, the deep fryer was shut off, and the oil was cooled down. French fries sampling intervals for the determination of acrylamide content are listed above.

Labelling of samples:

- Control – pre-fried and deep-frozen potato French fries.
- 1a – French fries deep-fried in multi-component oil, 175 °C/4 min, sampling immediately after the first deep-frying.
- 1b – French fries deep-fried in multi-component oil, 200 °C/3 min, sampling immediately after the first deep-frying.
- 1c – French fries deep-fried in multi-component oil, 175 °C/4 min, sampling after exceeding TPCs.
- 1d – French fries deep-fried in multi-component oil, 200 °C/3 min, sampling after exceeding TPCs.

- 2a – French fries deep-fried in single-component oil, 175 °C/4 min, sample immediately after the first deep-frying.
- 2b – French fries deep-fried in single-component oil, 200 °C/3 min, sampling immediately after the first deep-frying.
- 2c – French fries deep-fried in single-component oil, 175 °C/4 min, sampling after exceeding TPCs.
- 2d – French fries deep-fried in single-component oil, 200 °C/3 min, sampling after exceeding TPCs.

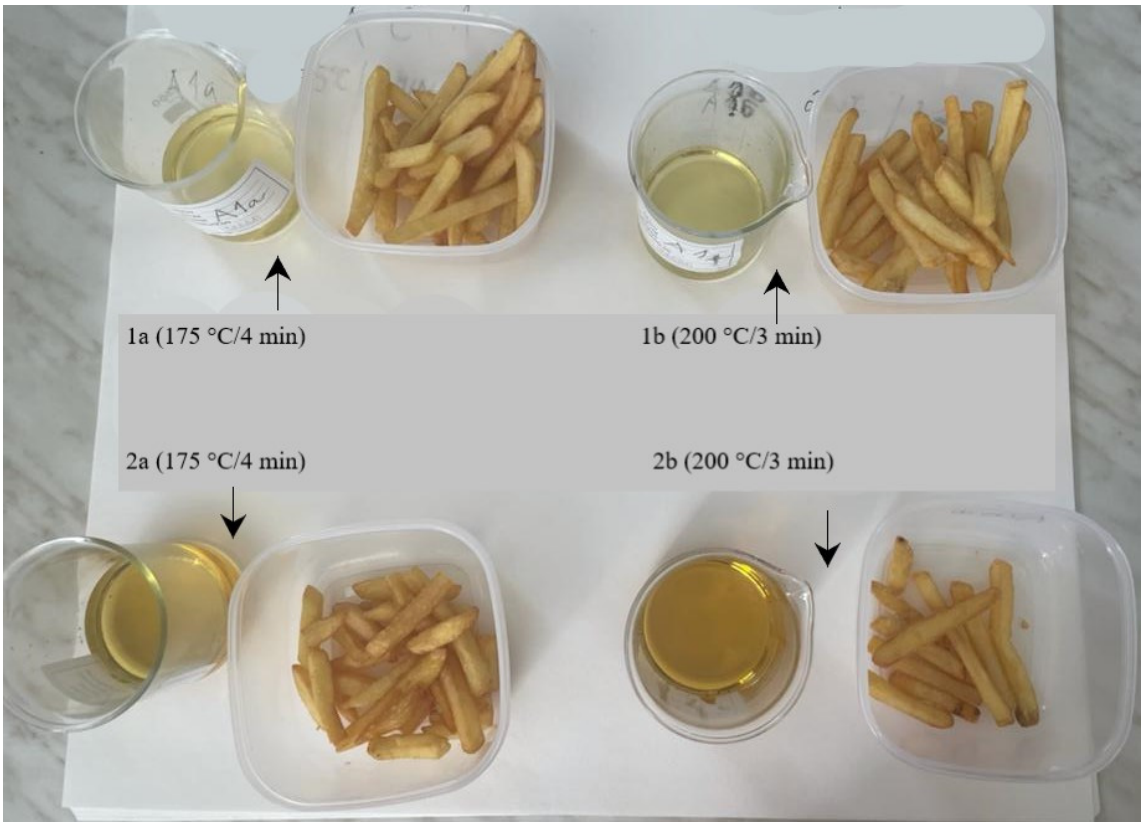

Figure 2 Fried potato chips fried in parallel in two oils (multi-component oil and single-component oil) under the same conditions (175 °C/4 min and 200 °C/3 min) up to the limit for TPC content.

Measurement of TPCs content was performed by oil tester Testo 270. The TPCs content enables a statement on the deterioration of deep-frying oils because of heat. TPCs estimation was based on constant dielectric changes directly measured on hot oil with deep frying oil tester Testo 270.

Table 1 Evaluation of the oil quality based on measurement of TPCs content by Testo 270.

Display indication	Classification
>1% and <14% polar compounds	Fresh oil
>14% and <18% polar compounds	Slightly used
>18% and <22% polar compounds	Used, but still OK
>22% and <24% polar compounds	Much used, it is recommended to exchange
>= 24 % polar compounds	Oil deterioration

LC-MS/MS was used to determine acrylamide. The results were evaluated using the software MassHunter Workstation version B.04.00 and Agilent MassHunter Workstation version B. 04.01.

Statistical Analysis

The SAS Enterprise Guide Version, 1.5 system program, performed the mathematical and statistical evaluation of the results. The obtained data were statistically evaluated according to the indicators of descriptive characteristics, i.e. $\bar{X}$ – arithmetic mean, SD – standard deviation and coefficient of variation (%). Analysis of variance (Kruskal-Wallis test, ANOVA) was used to compare groups, i.e., the assumption of agreement of variance was verified by the F test (F). All pairwise differences in means were tested using Tukey's HSD test (Honest Significantly Different) and Scheffe´s test. The critical value of α, compared to the standardized difference between the means, was established using our chosen risk of 5%.

RESULTS AND DISCUSSION

Several companies operating in the Slovak market today offer a wide range of potato products. French fries are delivered to catering establishments as food for ordinary consumers as 1st and 2nd level convenience, i.e., peeled sliced potatoes in the shape of fries or pre-fried frozen potato French fries intended for deep-frying (semi-finished product). As there is experience from gastronomic practice, their quality is different, not to mention the wrong choice of oil for deep-frying or unsuitable conditions for deep-frying. French fries can be prepared at home or by professional food services from fresh or deep-frozen par-fried potato, with the most extended heat preparing methods being baking and frying.

Setting goals and implementing the experiment was based on Slovak legislation requirements **[41]**. The legislation states that only fats designed for heat preparation and the given purpose can be used for continuous frying and deep-frying of prepared dishes. These fats and oils can be used for a maximum of 24 hours, while their operating temperature must not exceed 180 °C unless otherwise specified by the manufacturer. Food and catering establishments must also have regularly calibrated temperature measuring devices. However, practice shows that operators of fast-food restaurants often violate these requirements and use deteriorated oils for frying. Concerning the above study, this paper will report on the effect of various oil types and deep-frying conditions on the acrylamide content in French fries. The first prerequisite for evaluating the results was the creation of a calibration curve. Calibration was performed by diluting the acrylamide stock solution (5 mg in 100 mL of water) in the range of 10-4000 ng/10 mL with 50 µL of the internal standard (d3-acrylamide).

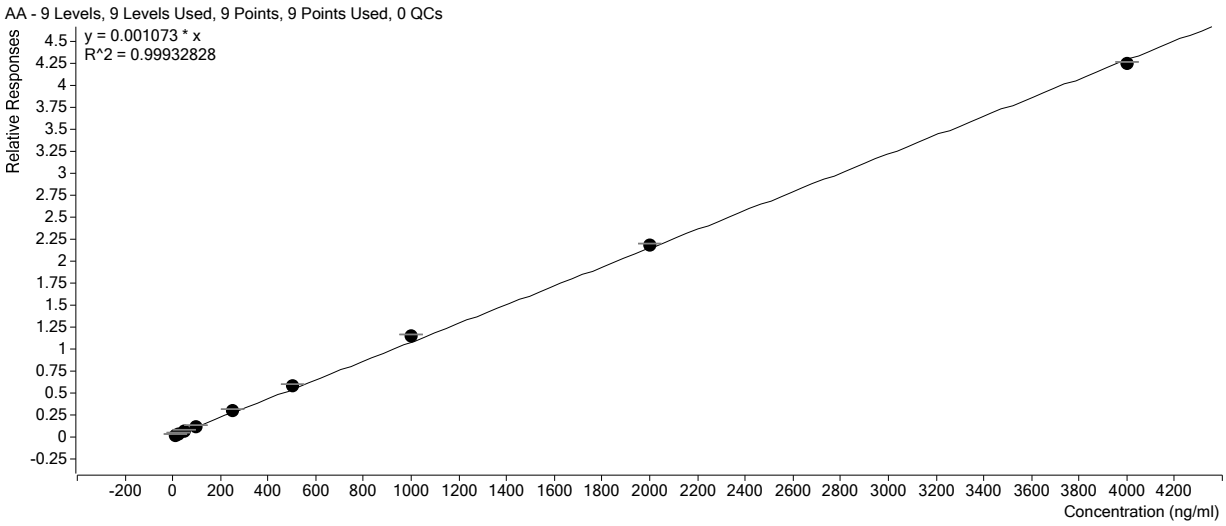

Figure 3 Calibration curve for acrylamide content (µg/kg) determination.

A supervised learning algorithm's inference of a linear relationship allows prediction a variable based on another variable using simple linear regression. The regression plot of standardized residuals versus standardized predicted values indicates that the points are randomly and evenly dispersed around the line of mean throughout the plot, as shown in Figure 4. No significant trend was observed from the scatter plot. The residual plot suggests no violation of Linearity.

Since frying oil degradation is greatly accelerated by foods, the frying condition should be controlled carefully. The decisive parameter for determining the content of acrylamide in French fries was the monitoring of the TPCs value, which is related to the quality and deterioration of the oil. As already mentioned, deep-frozen pre-fried potato French fries primarily intended for catering establishments were deep-fried parallel in two oils (multi-component oil and single-component oil) at the same conditions (175 °C/4 min and 200 °C/3 min) until the limit for TPCs content (24%) was reached. The samples were analysed immediately after removal from the package, after the first frying and when the TPCs were exceeded.

A lower TPCs content (3.5) was found in fresh rapeseed oil (samples 2) compared to fresh multi-component oil (4.5). Exceeding the TPCs values and thus the oil deterioration occurred after several hours of continuous deep-frying of French fries. The results are shown in Table 2.

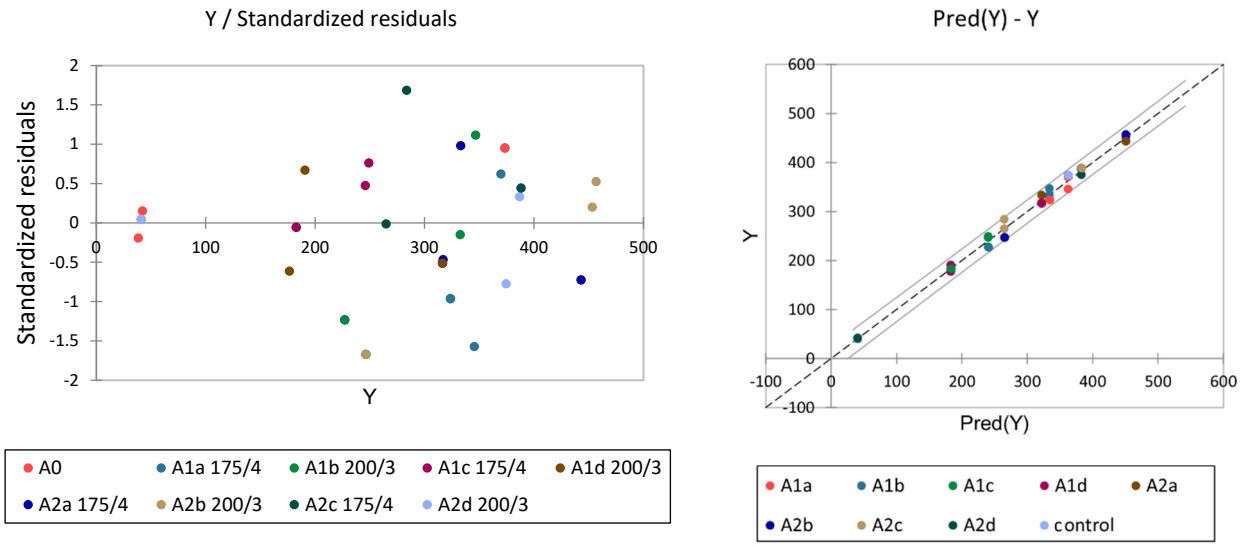

Figure 4 Residual plot (standardize residuals against predicted y).

Table 2 TPCs and deep-frying process.

Deep-fat fryers	Oil type	Frying conditions	Oil deterioration (h)	Number of deep-frying cycles
1	multi-component oil	175 °C/4 min	77	154
2	multi-component oil	200 °C/3 min	74	148
3	rapeseed oil	175 °C/4 min	26	52
4	rapeseed oil	200 °C/3 min	25	50

Zeleňáková et al. **[5]** examined the thermo-degradative changes of rapeseed and sunflower oils during deep-frying French fries (170 °C, 4 min). One of the investigated parameters was the TPCs value. Rapeseed oil found 3.3% of TPCs and the limit (24%) was achieved on the fourth day. The total time for the deterioration of deep-frying rapeseed oil was 23½ hours. On the contrary, in fresh sunflower oil on the first day was TPCs content 5.5% and the limit of 24% was reached on the third day. The total time for the deterioration of deep-frying sunflower oil was 17½ hours. The oil with higher oleic acid content achieved the 24% TPCs after 22 hours (at room temperature) and 26½ hours (in the refrigerator) of deep-frying. The low erucic acid rapeseed oil was less stable – 19 hours and 22½ hours, respectively **[42]**.

Table 3 Descriptive characteristics of acrylamide content (ug/kg) in French fries deep-fried in two types of oil and under different time-temperature conditions.

Sample	Parameter				
	$\bar{X}$	x_{min}	x_{max}	SD	v [%]
Control	40.33^{g}	38.00	42.00	2.08	5.16
1a	362.91^{bc}	345.50	373.45	15.19	4.19
1b	334.40^{cd}	323.73	346.73	11.59	3.47
1c	240.75^{e}	227.09	249.16	11.93	4.96
1d	183.35^{f}	176.56	190.77	7.13	3.89
2a	322.19^{d}	316.51	333.05	9.41	2.92
2b	451.13^{a}	443.12	456.94	7.17	1.59
2c	265.08^{e}	246.57	283.73	18.58	7.01
2d	383.24^{b}	374.66	388.15	7.46	1.95

Note: $\bar{X}$ – mean; X_{min} – minimum; X_{max} – maximum; SD – standard deviation; v – coefficient of variation. The different letters ^{a,b,c,d,e,f,g} listed with the mean values in the columns represent statistically significant differences between the observed varieties (p <0.005).

The highest acrylamide values in French fries fried in multi-component oil were measured in sample 1a at a temperature of 175 °C/4 min (362.91 µg/kg in immediate frying). The lowest value was found in sample 1d using a temperature of 200 °C/3 min (183.35 µg/kg after exceeding the TPCs). A statistically significant difference (p <0.005) was found between all samples from each other, except for when a significant difference was not found between the samples that were fried immediately 1a at a temperature of 175 °C/4 min and 1b at a temperature of 200 °C/3 min (p = 0.097). In the case of using rapeseed oil, the highest values of acrylamide were found in sample 2b deep-fried at a temperature of 200 °C/3 min (451.13 µg/kg in immediate frying) and the lowest value was found in the case of sample 2c deep-fried at a temperature of 175 °C/4 min (265.08 µg/kg after exceeding the TPCs). Statistically significant differences in frying French fries in rapeseed oil were found between all samples from each other (p <0.005). The decreased tendency of acrylamide in both types of oils and variants of temperature after exceeding TPCs compared to the state immediately after frying is confirmed for all samples. A complete visual presentation of the results is given in Figure 5.

In principle, it can be concluded that deep-frying influences the acrylamide content in potato French fries. The results also showed that the multi-component oil showed better thermal degradation properties expressed by the TPCs indicator and is more suitable for continuous frying.

Its deterioration (24% TPCs) occurred after 74 and 77 hours of deep-frying (148 and 154 cycles), depending on the combination of temperatures and time. Rapeseed oil reached the TPM limit after only 25 and 26 hours of deep-frying (50 and 52 cycles). The final content of acrylamide in French fries deep-fried in multicomponent oil at its deterioration was 183.35 and 240.75 µg/kg, respectively. In French fries deep-fried in rapeseed oil, these values were higher and detected in a shorter time.

Multi-component oil with a special composition is rich in essential fatty acids with an excellent balance of stable monounsaturated and polyunsaturated fatty acids. It does not produce toxins or contains allergens, nuts, soy, palm oil or GMO raw materials. Heat-resistant and long-lasting compared to classic rapeseed oil. High smoke point 220 °C and more, higher oxidation resistance **[43]**, **[44]**.

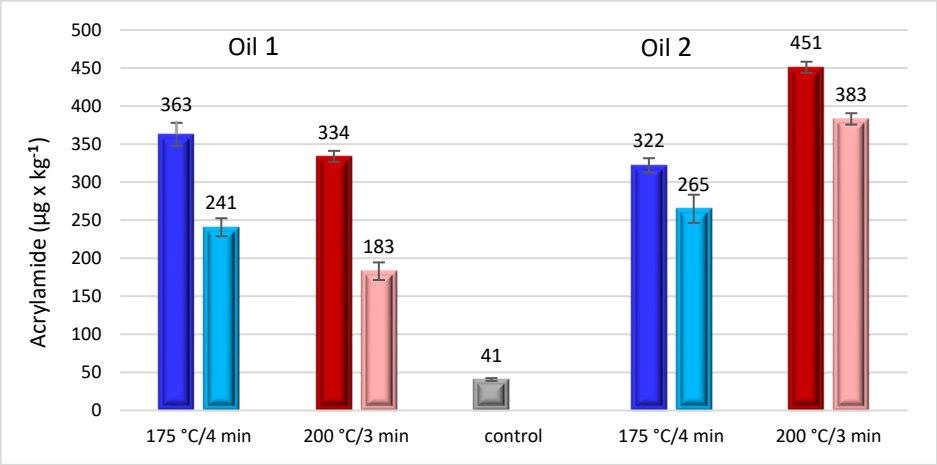

Figure 5 Visual presentation of acrylamide content (µg/kg) in individual samples with error bars. Note: Oil 1 – multi-component oil; Oil 2 – rapeseed oil.

The results from acrylamide were compared by ANOVA (Tukey test) whereby we performed pairwise comparisons of samples. To compare all possible simple and complex pairs of means, the Scheffe´s test was performed, which has a narrower confidence interval (Table 4), confirming the ANOVA results.

Table 4 Statistical evaluation of differences in acrylamide content (μg/kg) in French fries depending on the type of oil, the deep-frying conditions, and the total frying time by Scheffe´s test $P_{0.05}$.

F test					**366.02^{+++}**			
Sample	**1a**	**1b**	**1c**	**1d**	**2a**	**2b**	**2c**	**2d**
Control	+	+	+	+	+	+	+	+
1a		-	+	+	+	+	+	-
1b			+	+	-	+	+	+
1c				+	+	+	-	+
1d					+	+	+	+
2a						+	+	+
2b							+	+
2c								+

Note: - Statistically insignificant difference according to the Scheffe's test (p >0.05); + Statistically significant difference according to the Scheffe's test (p <0.05).

Figure 6 shows the percentage increase in acrylamide in individual French fries samples. Compared to the control, the most significant increase in acrylamide occurred in sample 2b, where the value of acrylamide increased 11 times. In samples 2a, 1b, 1a and 2d, the value of acrylamide was 7.9-9.4 times higher. The lowest increase of 4.5 times more compared to the control was in the 1d sample.

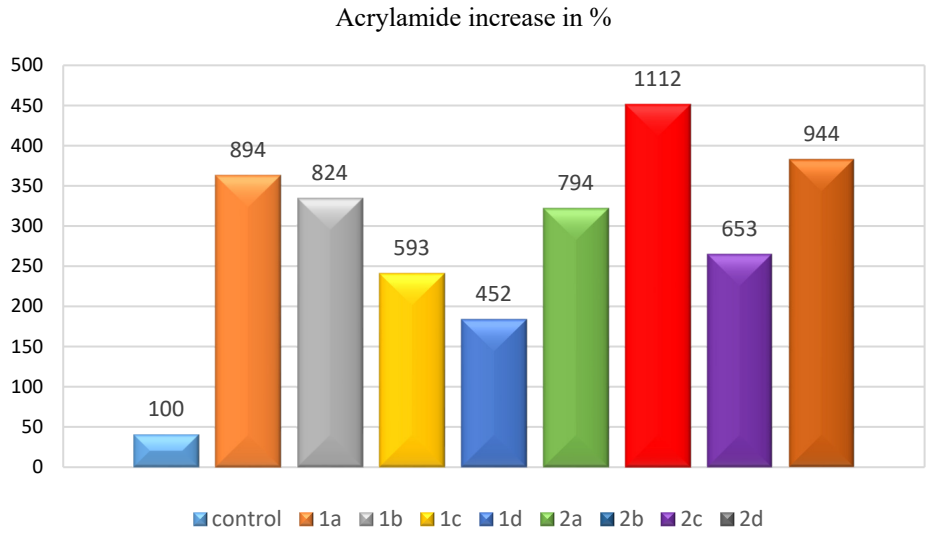

Figure 6 Percentage expression of acrylamide increase in French fries.

Regarding the frying conditions, it is known that both the temperature and time of frying determine the kinetics of a wide spectrum of oxidation products and acrylamide formation. This formation starts at temperatures above 120 °C and the maximum rate takes place at temperatures higher than 170-180 °C **[45]**, **[46]**. In our research, the same batch of French fries (100 g) was deep-fried, and the same frying conditions (175 °C/4 min and 200 °C/3 min) were applied.

The highest acrylamide content in rapeseed oil was formed at 200 °C and linearly occurred with an increment of temperature. The heat sensitivity of the Maillard reaction occurring between reducing sugars and asparagine may cause an increase of the acrylamide content **[47, 48, 49]**. Likewise, Yang et al., Lu et al. and Mariotti-Celis et al. **[50, 51, 52]** have reported that acrylamide formation was affected by an increment of frying temperature which agrees with our results.

In the case of multi-component oil, it was recorded for each pair of samples either immediately frying or deep-fried in oil after exceeding the TPCs, when a higher acrylamide content was detected at a lower frying temperature.

Ahrné et al. **[53]** measured lower acrylamide values in bread samples when baked at a higher temperature. They stated that the water content plays an important role in the degradation process of acrylamide. They reported that acrylamide concentration tended to decrease as crust temperature increased and water content decreased.

For acrylamide analysis, oil type and lipid oxidation profile plays an important role in the acrylamide concentration in the fried products **[54]**.

French fries are susceptible to acrylamide formation due to the high content of precursors in the tuber and the intensity of the heat treatment used. The chemical composition of potatoes varies, among others, according to cultivar, place of growth, agricultural practices, and maturity at harvest or storage conditions **[55]**.

Reducing the content of acrylamide in potato products can be achieved by choosing varieties with low sugar content, adequate storage and transport, suppression of germination, blanching, the addition of sodium diphosphate, treatment with asparaginase, coarser cutting and frying at a maximum temperature of 175 °C **[34]**, **[55]**, **[39]**.

Recent findings by Lee Kuek et al. **[56]** also confirmed that oil types significantly influenced the acrylamide formation in French fries during intermittent frying.

For instance, based on a real-food investigation, Granda and Moreira **[46]** have measured the concentration profiles of acrylamide in potato chips and have reported that a counteracting effect exists, in which the acrylamide formation rate rapidly increased at the early stage of frying and then gradually decreased after some time.

Many other scientific teams have been engaged in analysing the acrylamide content in food **[18]**, **[33]**, **[40]**, **[57-59]**.

In the context of the work's objectives, it is important not to forget the legislative requirements regarding the amount of acrylamide in food.

Potato-based products are one of the main contributors to acrylamide exposure **[28]** due to their acrylamide content and frequency of consumption. The benchmark level of the amount of acrylamide in French potato fries ready for direct consumption is 500 µg/kg, according to the current legislation. However, although acrylamide content does not exceed the current benchmark level, like in our case, the Commission Regulation 2017/2158 **[25]** requires food processors and food business operators in Europe to take measures that lead to the reduction of the presence of acrylamide in products according to the ALARA principle, while these measures are proportionate to the size and nature of the operations. Moreover, a mandatory maximum level of acrylamide in a wide range of foods is expected to be set soon.

Food establishments must implement these requirements to produce semi-finished potato products in catering facilities and households. There are still shortcomings in the last two mentioned.

CONCLUSION

French fries are fried or deep-fried small potato wedges that are widely consumed around the world. According to surveys, they are among the most popular side dishes, even though they are associated with an unhealthy eating style. Fried potatoes (French fries, chips) are susceptible to acrylamide formation due to the high content of precursors in fresh tubers and the intensity of heat treatment applied during frying. Only the highest quality potatoes can be used to make top-quality French fries. Constant innovations that improve and speed up processes, greener processes, revolutionary ideas and more inventive thinking, manufacturers can guarantee and provide gastronomic establishments with top-quality potato French fries with a low acrylamide content. The correct choice of raw material is very important because it affects the preparation of safe and high-quality potato French fries. The decreased tendency of acrylamide in both types of oils and variants of temperature after exceeding TPCs compared to the state immediately after frying is confirmed for all samples. The highest acrylamide values were measured in samples deep-fried in rapeseed oil at 200 °C/3 min in sample 2b (451.13 µg/kg when deep-fried immediately) and in sample 2d (383.24 µg/kg after exceeding TPCs). The lowest values of acrylamide were found in samples deep-fried in multi-component oil at a temperature of 200 °C/3 min in sample 1d (183.35 µg/kg after exceeding TPCs) and at a temperature of 175 °C/4 min in sample 1c (240.75 µg/kg after exceeding TPCs). Evaluation of established scientific hypotheses:

H1: We expect that acrylamide in French fries will rise at higher frying temperatures. This hypothesis was confirmed only partially. An increasing trend was confirmed only for rapeseed oil (oil 2). On the contrary, when frying French fries in multi-component oil, there was a slight decrease in the acrylamide content at a combination of 200 °C/3min compared to a temperature of 175 °C.

H2: After the first frying of French fries, the acrylamide content will be lower than after reaching the TPCs limit value, which means wear/burn-through oil. This hypothesis was rejected because the opposite trend was noted in all analyses.

H3: We expect differences in acrylamide content in French fries fried in rapeseed and multi-component oil. This hypothesis was confirmed.

In principle, it can be concluded that deep-frying influences the acrylamide content in potato French fries. The results also showed that the multi-component oil showed better thermal degradation properties expressed by the TPCs indicator and is more suitable for continuous frying. From a legislative point of view, it can be stated that

none of the analysed samples of French potato fries (semi-finished product, first deep-frying, limit value for TPCs) exceeded the set content of acrylamide (500 μg/kg). Determining the content of acrylamide in French fries and other food products is especially important concerning human health. The results presented in this article represent a valuable source of information for further research in the subject area. Variability of acrylamide content depends on many factors such as temperature (>120 °C), high carbohydrate content, low protein content, free asparagine, reducing sugars, pH, water content, ammonium bicarbonate, high concentration of competing amino acids. The acrylamide content can be effectively reduced by carefully selecting and storing raw materials and by changing procedures during the heat preparation of dishes. In conclusion, it should be emphasized that acrylamide is not the only quality indicator of potato French fries. No less important are sensory and textural characteristics, nutritional value (especially fatty acids profile), and thermo-degradative properties, which are accompanied by chemical reactions like oxidation, polymerization of triglycerides (TAGs), and hydrolysis.

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

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The study of nutritional value and microbiological characteristics of brine cheese with vegetable additive

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ABSTRACT

This article investigated brine cheeses' nutritional value and safety by adding vegetable additives (dry powder of white cabbage and coriander). Brynza brine cheese was used as the basis for the recipe. By the chemical composition of the cheese with vegetable, additives has a significantly higher protein content (26.27 g/100g), while the fat content is lower (14.98 g/100g). There is a high content of amino acids and fatty acids (PUFA 6%, MUFA 24%). During prolonged storage of brine cheese, water activity aw decreases in control from 0.997 to 0.990, mass fraction of moisture increases from 60% to 62.5%, in the brine cheese with vegetable additives aw from 0.998 to 0.991, mass fraction of moisture from 61.1% to 63.7%.The use of vegetable additives in the formulation of cheeses does not affect the deterioration of microbiological parameters compared to the control sample. As a result of experimental studies, the shelf life of brine cheese with vegetable additives is 8-10 days.

Keywords: brine, cheese, white cabbage, cilantro, water activity, shelf life

INTRODUCTION

The cheese market occupies a significant share of dairy products in the Republic of Kazakhstan of its high demand. Brine cheeses are the most prominent among other types of cheeses. Brine cheeses are made from different types of milk (cow, sheep, goat, buffalo) or their mixtures. They are ripened in brine, have a specific sharp-salty taste, and have a soft, flaky or slightly brittle consistency **[1]**. A crust on the surface does not characterize these cheeses. Brine cheeses include Brynza, Suluguni, Chanakh, Adygei, Georgian, Ossetian, Lori, Chechil, Aiman **[2]**.

Brynza is the most common brine cheese. It is produced from sheep or cow's milk by curdling with a lactic starter and rennet. The taste of brynza is sour milk, salty, the consistency is slightly brittle but not crumbly, and there is no pattern. Like all brine cheeses, it has no crust. The period of brynza ripening from pasteurized milk is 20 days, from raw milk – 60 days **[3], [4]**. The composition of brynza is well-balanced and has a particularly positive effect on the body. This product contains a lot of protein, which provides the body's cells with energy. The large amount of calcium, which is easily assimilated, helps to enrich the body with a daily rate of this mineral, and normalizes blood formation **[5]**, **[6]**.

The quality of raw materials and the processing technology determines cheese quality. The chemical composition, physical properties and microbiological parameters of processed milk determine the cheesiness of milk, i.e. its ability to clot, clot formation of proper consistency, as well as the ability to ferment and create the medium necessary for the development and activity of beneficial microorganisms, primarily lactic acid bacteria **[7]**, **[8]**.

Plant additives are appropriate to use as a source of biologically active substances in creating new technologies and formulations of milk products. One such plant additive is cilantro. Cilantro contains a lot of fiber, B vitamins and antioxidants. All the benefits are concentrated in the stem and leaves. The plant contains high amounts of minerals: iodine, and phosphorus. Potassium is high in cilantro, so it is often included in the diet of people with diseases of the cardiovascular system.

It contains biologically active components, mainly α-pinene, α-terpinene and limonene, as well as flavonoids, coumarins, phthalides and phenolic acids **[9]**, **[10]**. The addition of cilantro in food increases the content of antioxidants and has the potential to act as a natural antioxidant and thus prevent unwanted oxidative processes **[11]**, **[12]**. The results show that cilantro extract can be added to milk products as a natural food preservative to improve stability during storage **[13]**.

White cabbage (*Brassica oleracea* L. var. *capitata f. alba*) is a widely used green leafy vegetable of the cruciferous family, belonging to the Brassicaceae family **[14]**. White cabbage contains 16 free amino acids (among them tryptophan, lysine, methionine, tyrosine, histamine and others). Cabbage is rich in vitamins A, B1, B6, C, P, K, antiulcer vitamin U, salts of potassium and phosphorus, and trace elements: cobalt, copper, zinc, and magnesium. It contains sugars, fats, enzymes (lactose, protease, lipase), hormonal substances, and phytoncides **[15]**, **[16]**.

Cabbage leaves contain fibre, which prevents the development of atherosclerosis and improves the function of the gastrointestinal tract. The most important mineral salts are potassium salts, which activate the removal of excess fluid from the body, and sodium salts, which have the property of binding water **[17]**. Cabbage has anti-inflammatory properties. It has a stimulating effect on the body's metabolic processes, stimulates the production of gastric juice, and has a positive effect on cardiac activity. The product is useful for gout, kidney disease, cholelithiasis and ischemia **[18]**, **[19]**.

This work aims to study brine cheese's nutritional value and safety by adding vegetable additives.

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Scientific hypothesis

Incorporating white cabbage and cilantro into the recipe of brine cheeses increases the nutritional value, inhibits the growth of microorganisms, and does not adversely affect sensory properties.

MATERIAL AND METHODOLOGY

Samples

To produce brine cheese with vegetable additives, the following components are used:

- cow's milk, acidity not more than 19 T, density not less than 1030 kg/dm^3;
- White cabbage (*Brássica oleracea*) variety "Present", belongs to the cruciferous family;
- *Coriándrum sátivum* is an annual herbaceous plant of the cilantro genus (*Coriandrum*), of the Umbrella family (*Apiaceae*);
- Starter cultures of Bulgarian bacillus pure cultures, milk-enzymatic preparation of microbial origin "Renin".

Chemicals

Calcium chloride technical (E 509) (Labor Farma Limited Liability Partnership, Kazakhstan).
Chromocult Coliform Agar (Merck KGaA, Germany).
Chromocult Listeria Selective Agar (Merck KGaA, Germany).
Byrd-Parker agar (Sigma-Aldrich, USA).
Sabouraud Agar (Sigma-Aldrich, USA).
Kessler-GRM medium (Azimut, Russia).
Hexane (Labor Farma Limited Liability Partnership, Kazakhstan).
Ethyl alcohol (90%, Pharmacy 2010 Limited Liability Partnership, Kazakhstan).
Potassium hydroxide (Labor Farma Limited Liability Partnership, Kazakhstan).

Instruments

Microbial Colony Counter SKM-2 (Stegler Company, Russia).
Shaker (S-3L, producer (ELMI) Limited trade development, Latvia).
Drying chamber SNOL (Snol Company, Lithuania).
Mikmed-5 microscope (binocular) (LOMO Company, Russia).
Measuring flask (500 ml, producer (Altey Group) Limited liability company, Russia).
Armed HH-S4 water bath (Armed Company, Russia).
pH-meter pH-150MI (Measurement Technology Company, Russia).
Shimadzu Prominence LC-20 liquid chromatograph (HPLC, Shimadzu Corporation, Japan).

Agilent 7890A Gas Chromatograph (Agilent Technologies, USA).

Laboratory Methods

Determination of physicochemical and organoleptic indicators: Fat was determined by the methods specified in GOST 5867 **[20]**. Determination of moisture and dry matter was performed by GOST 3626 **[21]**. Determination of active acidity by GOST 32892 **[22]**. The method by GOST-32260 **[23]** was used for organoleptic evaluation of brine cheeses. Determination of amino acid composition was carried out by the method described in **[24]**. Fatty acid composition was determined by the method described in **[25]**.

Determination of water activity: The methodology for determining the water activity (a_w) is based on measuring the intensity of moisture exchange between the product surface and the environment by the product surface temperature during moisture evaporation and the temperature of the wet thermometer.

The water activity (aw) is determined by measuring and computing device. Calculation of the water activity (a_w) is made by the formula (2) **[26]**:

$$a_w = 1 - K\left[\frac{T_2 + T_3 + T_4}{3 - T_1}\right];$$

Where:

T_2, T_3, T_4 – product surface temperature, °C; T_1 – wet bulb temperature, °C; K – coefficient accounting for the barometric pressure in the measuring environment, which is equal: 760 mmHg - 0.070;755 mmHg - 0.069;750 mmHg - 0.068; 745 mmHg -0.067.

Preparation of samples for measurement was carried out as follows: samples of semi-hard cheese were cut in the form of a hollow cylinder 7 mm in length and 5 mm in diameter, equal to the diameter of the sensor of the device. It is necessary to note that tight contact of the product with the sensors must be guaranteed. The fourth sensor was wetted with distilled water and kept wet until the end of the measurement process **[26]**.

Determination of microbiological parameters: Determination of *Staphylococcus aureus, Salmonella, Listeria Monocytogenes,* yeasts and moulds of cheese products were determined following state standards of the Republic of Kazakhstan:

- GOST 30347-2016. Milk and dairy products. Methods of determination of *Staphylococcus aureus*.
- GOST 31659-2012. Food products. Method for the detection of bacteria of type *Salmonella*.
- GOST 32031-2012. Foodstuffs. Methods of detection of *Listeria Monocytogenes* bacteria.
- GOST 33566-2015. Milk and dairy products. Determination of yeasts and moulds.

Determination of coliform bacteria: Test procedure: 1 cm³ of the sample is inoculated into a test tube with 5 cm³ of liquid medium. The tube with inoculations is placed in the thermostat for 18-24 hours at 37 °C. After 24 hours, the tube is inspected, and the presence or absence of gas is visually determined. If there is gas formation, it is considered that coliform bacteria is found. If there is no outgassing, it is concluded that the coliform bacteria in the product is not detected, i.e. the product is safe for this indicator **[27]**.

Counting microbial colonies: Test procedures: 14 cm³ of nutrient medium is poured into a Petri dish and 0.1 cm³ of the sample is inoculated. After filling, the mixture is stirred thoroughly by gentle shaking to distribute the media evenly. After the medium has solidified in the Petri dish, it is turned upside down and placed in a thermostat at 30˚C for 72 hours. Results processing: after the time has passed, colony counting begins. The bottom of the Petri dish is divided into two to three sectors. In each sector, the number of colonies is counted. The formula calculates the number of mesophilic aerobic and facultatively anaerobic microorganisms X in 1 cm³ or 1 g of the product **[28]**.

$$X = n \cdot 10^m$$

Where:

n – the number of colonies counted on a Petri dish; m – the number of tenfold dilutions.

The arithmetic mean obtained for all dishes is taken as the final result of the analysis.

Description of the Experiment

Production of brine cheeses with vegetable additives: As a control sample, the traditional brine cheese "Brynza" without adding the vegetable mixture, following GOST R 53421-2009 "Brine cheeses" was used. Both control and experimental samples of brine cheeses were made in the milk enterprise "Aisha" (Semey city, Kazakhstan). The recipe composition of brine cheese is presented in Table 1.

Table 1 Recipe of brine cheese.

Ingredient	Consumption rate, kg/100 kg
Cow milk	100
Table salt	0.3
Water	20
Bacterial starter	0.4
Vegetable additives	0.3

Figure 1 Samples of brine cheese with plant additives.

Whole cow's milk with an acidity of 18-20 °T produces brine cheese. Milk must meet the physicochemical composition requirements specified by the cheese industry. In order to kill pathogenic bacteria and undesirable vegetative forms harmful to cheese microorganisms, milk is pasteurized at a temperature of (72-75) °C, holding (for 20-25) s. The pasteurized milk is then cooled to a temperature of 30 °C, and 1.5-2% starter is added. Starter cultures of Bulgarian bacillus pure cultures, milk-enzymatic preparation of microbial origin "Renin". The initial concentration of starter culture in brine cheese production was 2% (w/v) of the milk used for the cheese. Then rennet is added to the milk mass for milk coagulation during 35-40 minutes. The ready clot is cut into cubes of size 15×20 mm and is left at rest to fix the cheese grain for 5 min. Next, the cheese mixture is kneaded for 20-30 minutes. Then the whey is removed, and table salt at 300 g per 100 kg of the mixture and vegetable raw materials are added to the cheese mass. The mixture of cheese mass with a small amount of whey is evenly poured into pre-prepared forms. Then the cheese is self-pressed for 20-30 minutes. The cheese heads are turned over every 10 minutes. Then it is pressed for 25-40 minutes at a temperature of (18-22) °C. Next, the cheese is ripened for 24 h at (8-10) ºC. The finished cheese is packed in a shrinkable vacuum bag, labelled and sent for sale. The cheese is stored at a temperature of 8 ±2 °C, a humidity 85% 8-10 days (Figure 1).

Number of samples analyzed: To analyze the nutritional value and safety of brine cheeses, 30 samples of cheese were studied.

Number of repeated analyses: Each study was carried out 3 times, with the number of samples being 30, which amounted to 90 repeated analyses.

Number of experiment replication: The study was repeated three times, with the experimental data processed using mathematical statistics methods.

Design of the experiment: At the beginning of the experiment, we analyzed the organoleptic characteristics and physical and chemical properties of brine cheeses. The nutritional value, amino acid, fatty acid

composition, microbiological characteristics, and water activite were studied. Based on the data obtained, determine the recipe for brine cheese with adding vegetable additives.

Statistical Analysis

The results of measurements were analyzed using Statistica 12 PL software (StatSoft, Inc., Tulsa, OK, USA). The differences between the samples were evaluated using a one-way ANOVA, $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Plant proteins have a higher water-holding capacity compared to milk proteins. Therefore, the dose of plant components added to the cheese grain significantly impacts the curdling time and the clot's active and titratable acidity **[29]**, **[30]**. The main goal of brine cheese production is to obtain a quality and safe product. The organoleptic characteristics of produced samples are shown in Table 2 and compared with the organoleptic properties of Brynza brine cheese without additives. Physico-chemical characteristics of brine cheese in comparison with the control brine cheese "Brynza" from cow's milk are shown in Table 3.

Table 2 Organoleptic characteristics of brine cheese with vegetable additives.

Indicator	Brynza brine cheese (control)	Brine cheese with vegetable additives
Appearance	Low cylinder shape	Low cylinder shape
Taste and odor	Sour, with no extraneous flavors or odors	Sour, with no extraneous flavors and smells. With a slight taste of cilantro and cabbage greens
Consistency	Tender smeary, slightly crumbly. No pattern	Tender smeary, slightly crumbly. No pattern
Color	White, homogenous on the whole mass	White, homogenous over the whole mass. With flecks of plant additives throughout the mass

Table 3 Physical and chemical properties of brine cheese.

Indicator	Brynza brine cheese (control)	Brine cheese with vegetable additives
Mass fraction of moisture, %	62.1 ±0.71	63.7 ±0.85
Mass fraction of moisture in skimmed product, %	85.0 ±1.13	88.2 ±1.32
Mass fraction of table salt, %	2.60 ±0.04	1.25 ±0.02*
Mass fraction of fat in dry matter, %	80.1 ±1.4	76.6 ±1.0

Note: *$p < 0.05$.

At the next stage, we studied the microbiological parameters of brine cheese, which are presented in Table 4, the norms of which are regulated in the document TR CU 033/2013 "On the safety of milk and dairy products": Technical Regulation of the Customs Union: approved by the Commission of the Customs Union on October 9, 2013, No. 67 **[31]**.

Table 4 Microbiological analysis of brine cheese.

Name	Indicator	Result	Regulated parameter [25]
Brine cheese with plant additives	Coliform bacteria	Not detected in 0.001 g	Not allowed in 0.001 g
	Pathogens, including *Salmonella*	Not detected in 25 g	Not allowed in 25 g
	S. aureus	Not detected in 0.001 g	Not allowed in 0.001 g
	L. monocytogenes	Not detected in 25 g	Not allowed in 25 g

Cheese is a high-protein, biologically complete food product containing all essential amino acids in proteins. Nutritional and energy value of the brine cheese with vegetable additives compared with the brine cheese "Brynza" without additives are presented in Table 5.

Table 5 Nutritional and energy value of brine cheese with vegetable additives, %.

Indicator	Brynza brine cheese (control)	Brine cheese with vegetable additives
Nutritional value, g per 100g		
Protein	19.1 ±0.23	26.27 ±0.64*
Fats	21.6 ±0.36	14.98 ±0.22*
Carbohydrates	3.67 ±0.07	3.07 ±0.05*
Moisture	45.0 ±0.53	52.82 ±0.85*
Ash	3.10 ±0.05	2.86 ±0.05*
Energy value, in 100 g		
Kcal	271	252
KJ	1135	1054

Regarding the biological, nutritional and energy value of brine cheese, it is possible to recommend it for the diet of all age groups. To assess the biological value of cheese, we determined the amino acid and fatty acid composition. Analysis of the amino acid composition is presented in Table 6.

Table 6 Amino acid composition of brine cheese (mg/100 g protein)

Amino acid	Brine cheese with vegetable additives
Aspartic acid	1363.53 ±20.03
Glutamic acid	3964.26 ±55.51
Serine	1664.31 ±21.81
Histidine	4542.26 ±71.57
Glycine	236.52 ±2.79
Threonine	1163.13 ±21.80
Arginine	838.84 ±11.70
Alanine	496.49 ±9.42
Tyrosine	1385.00 ±10.84
Cysteine	505.94 ±6.19
Valine	4481.85 ±53.57
Methionine	739.33 ±7.34
Phenylalanine	407.80 ±5.56
Leucine	2026.40 ±26.89
Isoleucine	388.99 ±7.29
Lysine	324.49 ±4.11
Tryptophan	266.70 ±5.01
Proline	41.13 ±0.69

Analysis of the results shows a wide range of free amino acids in the experimental cheese. The protein of the cheese is well-balanced and contains all essential amino acids.

The results of the fatty acid composition of experimental brine cheese with vegetable additives are presented in Table 7.

The research results show that brine cheese with vegetable additives contains 6% polyunsaturated fatty acids and 24% monounsaturated fatty acids.

Table 7 Fatty acid composition of brine cheese with vegetable additives, %.

Fatty acid, %	Brine cheese with vegetable additives
Saturated fatty acids	
$C_{4:0}$ butyric acid	4.24 ±0.06
$C_{6:0}$ caproic acid	2.49 ±0.04
$C_{8:0}$ caprylic acid	1.39±0.03
$C_{10:0}$ caprinic acid	3.00 ±0.07
$C_{12:0}$ lauric acid	3.01 ±0.06
$C_{14:0}$ myristic acid	9.28 ±0.12
$C_{16:0}$ palmitic acid	26.92 ±0.50
$C_{18:0}$ stearic acid	12.75 ±0.21
$C_{22:0}$ behenic acid	0.52 ±0.01
$C_{20:0}$ arachidic acid	0.11 ±0.01
Monounsaturated fatty acids	
$C_{14:1}$ (cis-9) myristoleic acid	0.34 ±0.01
$C_{16:1}$(cis-9) palmitoleic acid	0.78 ±0.01
$C_{18:1n9c}$ oleic acid	22.74 ±0.33
Polyunsaturated fatty acids	
$C_{18:2n6c}$ linolic acid	4.07 ±0.03
$C_{18:3n3}$ linoleic acid	1.68 ±0.02

Determination of the shelf life of brine cheese

The main task in developing a new product is to determine the shelf life of the finished product. It is important to determine the microbiological parameters when validating the shelf life of brine cheese made of cow's milk **[32]**, **[33]**. The issues related to preserving quality and reducing food losses during long-term storage are among the most important tasks facing the processing industry workers. Determining the shelf life of cheese requires a detailed study of the influence of external factors (ambient temperature, relative humidity, etc.) on the change of cheese quality indicators **[34]**, **[35], [36]**. Currently, for all types of foodstuffs, there are standard storage times regulated by State Standard, which, however, do not consider the possible deviations of some parameters when changing storage conditions. In this regard, focusing only on the specified storage time of food products and not taking into account their state of moisture, it is impossible to accurately predict the high quality of food products during their storage **[37], [38]**.

Studies of cheese storage ability were conducted in laboratory conditions at storage temperature (8 ±2 °C) for 3, 5, 10, 12, and 15 days with a relative humidity of 80-85%. Brinza" brine cheese was used as a control.

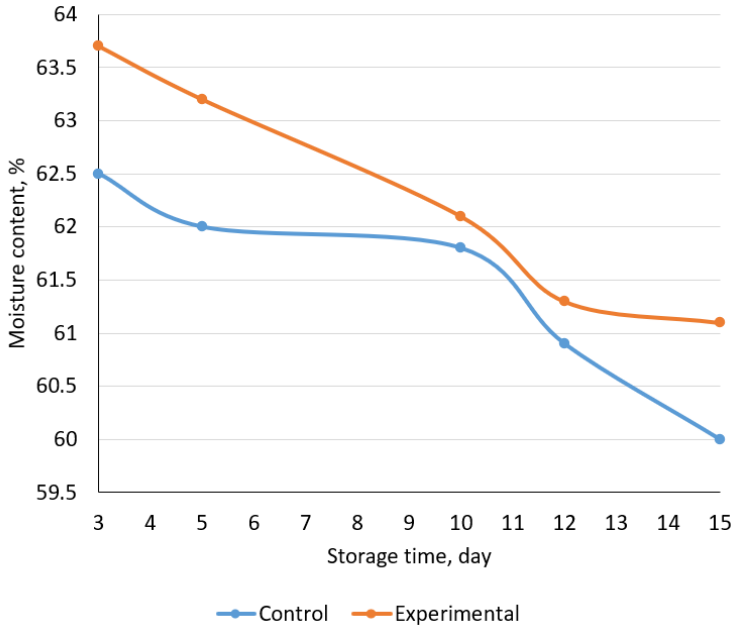

Figure 2 Changes in the mass fraction of moisture in brine cheeses depending on the storage time.

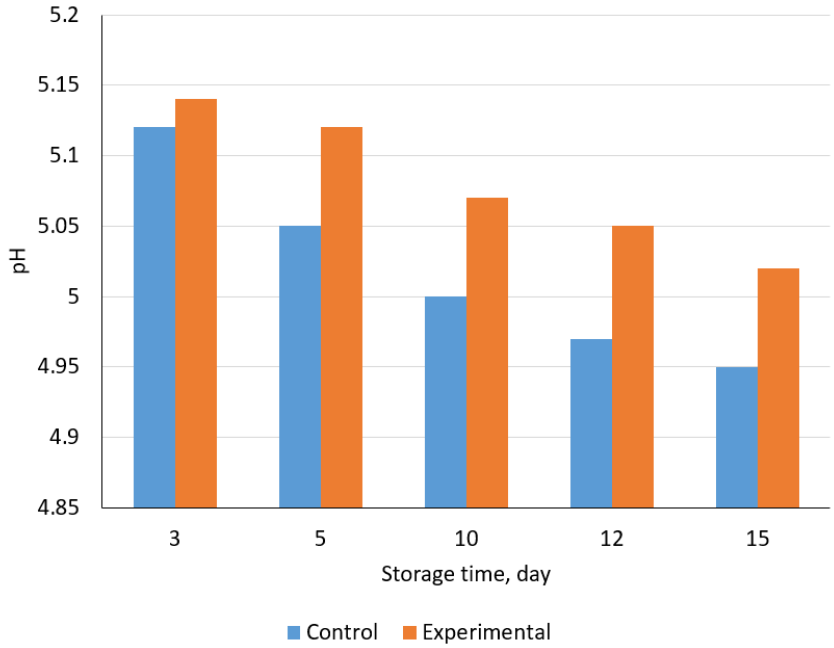

Figure 3 Changes in pH of brine cheeses depending on the storage time.

According to the result during storage, the moisture content of the experimental brine cheese compared to the control cheese did not significantly differ (Figure 2). However, during the storage of brine cheeses on the 10th day, there was a decrease in active acidity pH 5.12-4.95 (Figure 3).

WATER ACTIVITY

Water accounts for the largest proportion of fresh brine cheeses. Technological properties, consumer properties and shelf life of brine cheeses are determined largely by the properties of the contained water. The moisture in the product is associated with its dry weight, and the form and energy of the connection of this moisture are different.

As a component of food products, water significantly affects such important indicators as organoleptic and rheological properties, microbial spoilage, growth of pathogenic microorganisms and quality reduction as a result of physical, chemical and biochemical reactions **[39]**, **[40], [41]**. The product's vulnerability to bacterial spoilage depends on moisture and its physical state, which is estimated by the water activity aw. Determining water activity during product manufacture helps control the technological process and the yield and quality of the output products. In addition, water activity value shows microbial, enzymatic, chemical, and physical changes in food products **[42]**, **[43]**.

Both by the amount of moisture and water activity the following products are distinguished: products with high moisture (aw = 1.0-0.9); products with intermediate moisture (aw = 0.9-0.6); products with low moisture (aw = 0.6-0.0). The dried product's moisture interaction with air distinguishes the moisture as hygroscopic, equilibrium and free moisture **[44]**, **[45]**. Thus, by controlling the functional and technological parameters in the product and, in particular, the indicator "water activity", we can predict its ability to store, which will create "stability maps" of milk products and determine the optimal conditions for their storage. In this regard, we investigated the dynamics of changes in the water activity of brine cheese during ripening.

It is known that water activity and mass fraction of food products' moisture are among the main indicators determining such important properties as shelf life **[46]**, **[47]**. In this regard, studies were conducted to determine the optimal technological parameters of the studied products' water activity and mass fraction of moisture. The results of the study of the water activity of brine cheeses are presented in Figure 4.

It was found that during prolonged storage of brine cheese *aw* decreased in control from 0.997 to 0.990, the mass fraction of moisture increased from 60% to 62.5%, in the brine cheese with vegetable additives aw decreased from 0.998 to 0.991, the mass fraction of moisture increases from 61.1% to 63.7%.

The reduction of water activity is associated with table salt in the cheese product. The content of table salt reduces at the highest level of aw. This is due to the ability of sodium chloride to electrolytic dissociation, which increases by several times the effective concentration of particles **[48]**, **[49]**, **[50]**.

The main sugars in white cabbage are glucose and fructose. Regarding the glucose content (2.6%), white cabbage surpasses the most common vegetable crops: apples, oranges, and lemons. It surpasses potatoes (1.6

times) and beets, onions, and lemons for fructose content. Cilantro also contains small amounts of glucose, fructose and sucrose.

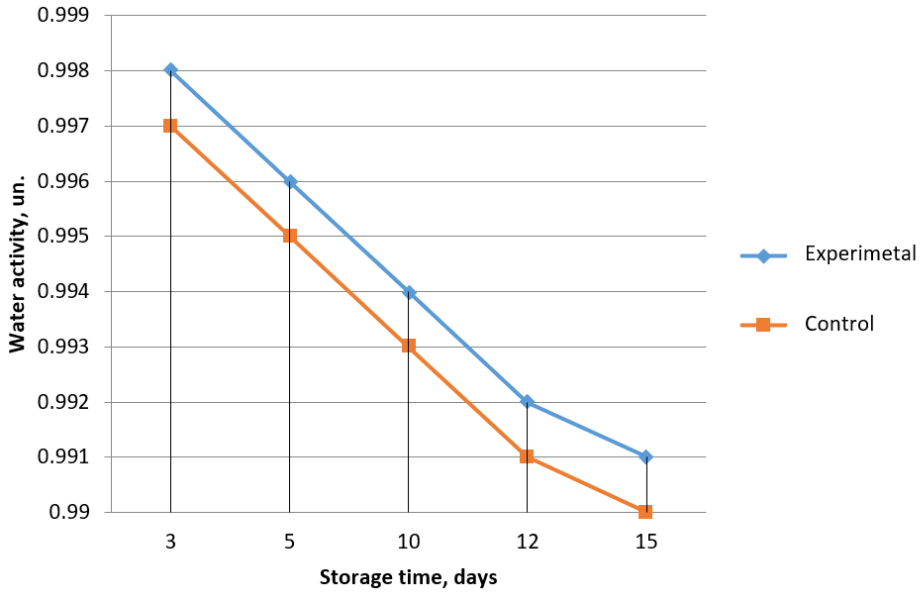

Figure 4 Change of water activity (a_w) of brine cheeses during storage.

The decrease in water activity is also due to sucrose, glucose and fructose, which can dissolve in the aqueous phase of the product, thereby increasing the osmotic concentration **[51]**, **[52]**. It is also possible to explain the reduction of water activity in cheese products by the fact that the reactions of the biochemical order occurring in products during storage are hydrolysis reactions, in which water retention occurs. As a result, there is a decrease in the free water content and accordingly, the indicator of water activity decreases.

For the microbiological safety criteria of brine cheeses with vegetables, additives were selected the following indicators: the titer of E. coli bacteria, the number of S.aureus bacteria belonging to the group of opportunistic pathogens, including Salmonella in 25 g of product (Figure 5).

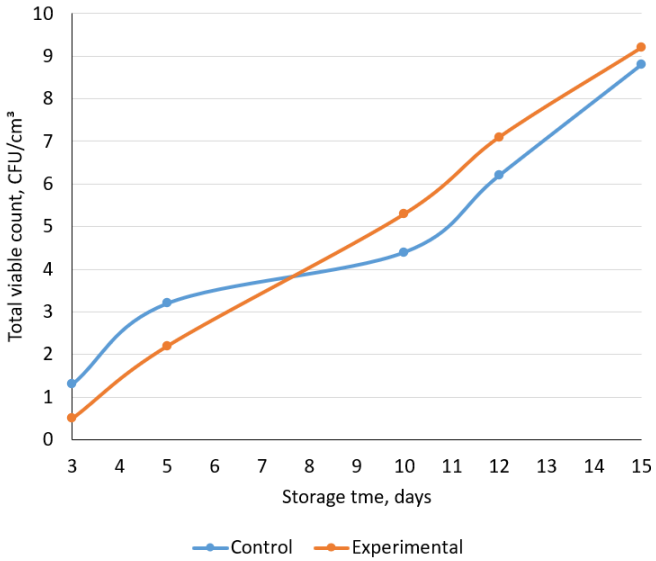

Figure 5 Changes in the total viable count of brine cheeses depending on the storage time.

When justifying the shelf life of new brine cheese, it is important to determine the content of microscopic fungi and yeasts, indicators of microbiological stability. It is known that fungi and yeasts can grow at low positive temperatures **[53]**, **[54]**. The development of mould fungi on the surface of brine cheeses decreases the marketable appearance and changes in protein and fat content since most fungi have lipolytic and proteolytic activities. The development of mold fungi in cheese is undesirable because it can lead to the penetration of toxins into the product to a depth of 2-4 cm **[55]**, **[56]**, **[57]**.

As a result of these studies, salmonella bacteria were not detected in any of the experimental samples. In experimental and control samples, the content of microscopic fungi and yeasts was determined, the total bacterial insemination in freshly produced cheese and the dynamics of their growth during storage. The results are presented in Table 8.

Table 8 Microbiological characteristics of brine cheese with vegetable additives.

Indicator	Storage time, day				
	3	5	10	12	15
Control brine cheese "Brynza"					
Total viable count, CFU/cm^3	1.3×10	3.2×10	4.4×10	6.2×10	8.8×10
Coliform bacteria, in 0.001 g of product weight	Not detected				
Pathogenic microorganisms, including *Salmonella* and *S. aureus*, in 25 g of product weight	Not detected				
Listeria monocytogenes, in 0.001 g of product mass	Not detected				
Yeast, CFU/g, in 0.1 g of product	-	-	1	1.3×10	2.9×10
Molds, CFU/g, in 0.1 g of product)	-	-	-	2	3.1×10
Brine cheese with vegetable additives					
Total viable count, CFU/cm^3	0.5×10	2.2×10	5.3×10	7.1×10	9.2×10
Coliform bacteria, in 0.001 g of product weight	Not detected				
Pathogenic microorganisms, including *Salmonella* and *S. aureus*, in 25 g of product weight	Not detected				
Listeria monocytogenes, in 0.001 g of product mass	Not detected				
Yeast, CFU/g, in 0.1 g of product	-	-	2	2.8×10	3.8×10
Molds, CFU/g, in 0.1 g of product)	-	-	-	2	3.1×10

It was found that during the entire storage period, microbiological parameters met the requirements of TR CU 033/2013 "On the safety of milk and dairy products. Obtained data of experimental samples of brine cheese with vegetable additives does not differ significantly from the control cheese "Brynza". This indicates that the use of vegetable additives does not affect microbiological parameters.

When conducting the organoleptic evaluation of the studied product at the end of the expected shelf life and similar freshly produced products, a slight change in the consistency of the product, which in general did not reduce the organoleptic assessment of its quality. On the 20th day, there was a slight decrease in the evaluation of organoleptic characteristics, the appearance of a sour taste, and slight bitterness.

CONCLUSION

This work has demonstrated the possibility of increasing the nutritional value of brine cheeses by adding vegetable additives (white cabbage, cilantro), rich in protein and fatty substances. An increase in protein, the balance of amino acids and fatty acids is noticeable in the experimental samples. Adding vegetables to brine cheese has led to an increase in protein content and a decrease in fat content and energy value. It can make the developed brine cheese a healthier option for individuals concerned about their fat and calorie intake. Both the control sample and the brine cheese with vegetable additives experienced decreased water activity and increased moisture content during storage. The addition of vegetables to the cheese does not result in a noticeable negative impact on its microbiological and physical characteristics. As a result of experimental studies, the shelf life of brine cheese with vegetable additives is 8-10 days.

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The risk-based control of the safety and quality of freshwater fish for sale in the agri-food market

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ABSTRACT

Scientifically substantiated and experimentally proven the feasibility of conducting proper risk-based control of the safety and quality of freshwater fish in the Kyiv region's agro-food markets following the regulatory document's requirements, developed by express, improved methods for determining freshness and microstructural examination of muscle tissue. At organoleptic assessmentUkrainian scaly carp, crucian carp, and pike perch were fresh, and rotan was of dubious freshness. Regarding pH value, Nesler number, and qualitative reaction to the content of ammonia and ammonia salts with 'Nesler's reagent, the meat of Ukrainian scaly carp, crucian carp, and pike perch corresponded to fish of a fresh degree. For rotan meat, its dubious freshness was established. In the photometric determination of the studied fish's freshness, the optical density of the supernatant correlated with the quality indicators for the content of ammonia and ammonia salts. An improved benzidine test with a filtrate from the gills of the mouth confirms the doubtfulness of the freshness of the fish. The studied fish samples corresponded to the standard indicators according to microscopic indicators and the number of mesophilic aerobic and facultative anaerobic microorganisms. By determining the chemical parameters of the studied fish, it was found that the mass fraction of water in meat was the highest in rotan (78.30 ±0.13%) and was accompanied by the smallest mass fraction of dry matter (21.70 ±0.09), the proportion of proteins (16.96 ±0.06), indicators of fat in meat (3.01 ±0.06%) and formed the lowest indicator of its relative biological value – 92.5%. In benign fish with organoleptic indicators, a microstructural study of muscle tissue revealed significant changes in its structure with atrophy of individual muscle fibers and growth in these areas of connective, mainly fatty tissue.

Keywords: fish, carp, pike perch, rotan, risk-based control, muscle, atrophy, agri-food market

INTRODUCTION

In Ukraine, fish and fish products are included in the list of strategically important food products. According to the World Food Organization (FAO), fish products are the third largest food producers in the world.

Aquaculture plays an important role in ensuring countries' food and nutrition security. Thus, the fish farming industry is an important component of the economy not only in Ukraine but also in the countries of Central and Eastern Europe. At the same time, the production of aquatic biological resources in Ukraine over the past decade has decreased to 90 thousand tons, which has led to an increase in the import of fish and fish products **[5]**, **[24]**, **[55]**.

Fish and fish products are necessary for complete human nutrition, as they are a source of complete proteins, vitamins, macro-, microelements, and other nutrients **[10]**, **[31]**, **[38]**, **[46]**, **[74]**. The development and globalization of import-export relations between countries against the background of technological progress in the field of production, intensification, and commercialization of the fishing industry, processing and

redistribution of food from fish, increase the level of consumer requirements for their safety and quality **[2]**, **[19]**, **[20]**, **[84]**, **[85]**. Therefore, the priority task for veterinary medicine specialists is to strengthen the proper risk-based control of the safety and quality of fish and fish products put into circulation **[1]**, **[13]**, **[15]**, **[22]**, **[23]**, **[26]**, **[32]**, **[49]**, **[52]**, **[53]**, **[85]**.

Satisfactory microbiological indicators must confirm the safety of fish products, and the residual amounts of salts of heavy metals (cadmium, zinc, copper, arsenic, mercury; decarboxylation products of amino acids – histamine and nitrosamines; pesticides, radionuclides) must not exceed the maximum allowable levels (MAL) **[7]**, **[28]**, **[34]**, **[40]**, **[45]**, **[50]**, **[63]**, **[69]**, **[82]**, **[83]**. A wide range of indicators to confirm the good quality of fish products is explained by the fact that fish is often the cause of serious food poisoning, sometimes fatal **[11]**, **[29]**, **[54]**. According to the literature data, freshwater fish caught from water bodies contaminated with untreated sewage and organic matter can be affected by pathogenic and conditionally pathogenic microflora. As a rule, signs of the disease in such fish are absent. Still, the presence of microorganisms leads to its rapid deterioration with the accumulation of histamine during storage and sale **[8]**, **[25]**, **[54]**, **[85]**. It is known that fish can be a carrier of pathogens of Asian human cholera, swine fever, erysipelas, tuberculosis, and Escherichia coli, very dangerous for humans, toxic infections, and toxicosis caused by *Clostridium botulinum, Clostridium perfringens*, bacteria of the genus *Salmonella, E. coli*, *Proteus,* leptospira, various coca microflora, etc. **[6]**, **[15]**, **[26]**, **[30]**. Harmful infections can occur when a product contains more than 106 cells of live toxigenic bacteria per 1 cm^3. Fish toxic infections include diseases caused by bacteria of the *Escherichia coli* group*, Salmonella*, *Bacillus cereus, and Clostridium perfringens*, typical representatives of the Proteus genus **[8]**.

The occurrence of food intoxication is associated with consuming fish products with enterotoxins secreted by certain types of microorganisms (coagulase-positive staphylococci, *Clostridium botulinum*). At the same time, they themselves may be absent, for example, after heat treatment. Recently, there have been more frequent reports of toxic food infections caused by opportunistic microflora, constantly found in water bodies and the body of fish. This is due to many circumstances, in particular, a violation of ecological relationships within bacterial associations, a change in the existing balance between the normal microflora in the human body, a decrease in the level of natural immunity, and the widespread use of antibiotics, to which many opportunistic bacteria are resistant. The possibility of occurrence of food-toxic infections caused by spores of bacteria of the genera *Citrobacter, Klebsiella, Pseudomonas, Aeromonas, Hafnia, Vibrioparahaemolyticus* has been proved **[6]**, **[12]**, **[21]**, **[70]**, **[78]**.

Therefore, when conducting a study of fish products, it is necessary to thoroughly and reliably confirm their compliance with the requirements established in regulatory documents to ensure their safety for life, health, property of citizens, and the environment **[52]**, **[53]**, **[54]**. Today, this is especially relevant due to the loss of validity from 01/01/2018 of the Resolution of the Cabinet of Ministers of Ukraine dated 05/10/2018, 1993, No. 46-93 “On standardization and certification” and “List of products subject to mandatory certification in Ukraine”, approved by order of the State Consumer Standard of Ukraine dated 01.02. 2005, No. 28, registered with the Ministry of Justice of Ukraine on May 04, 2005, 2005, No. 466/10746. This means that as of May 17, 2021, food products are not subject to mandatory certification in the State Certification System of Ukraine.

Thus, the nature of the dangers associated with fish consumption is global and falls within the scope of professional interests not only of veterinary and sanitary experts but also of human medicine doctors and all those associated with the production, processing, and sale of freshwater fish. Therefore, the purpose of the research is to assess the compliance of the studied freshwater fish (Ukrainian scaly carp, crucian carp, pike perch, rotan) from different producers in the Kyiv region, in terms of safety and quality, with the requirements of the State Standard of Ukraine (SSTU) 2284:2010 and approbation of modern express methods to identify the degree of its freshness.

Scientific Hypothesis

Will risk-based fish safety and quality control be ensured by improved express methods in the conditions of the agro-industrial market?

MATERIAL AND METHODOLOGY

The work was carried out from 2020 to 2021 at the Research Laboratory of Veterinary and Sanitary Expertise of Livestock Products, the Laboratory of the Department of Veterinary and Sanitary Expertise of the Institute for Postgraduate Education of Managers and Specialists of Veterinary Medicine, a research laboratory for complex ichthyopathological studies, laboratory of the Department of Safety and Quality of Food Products, Raw Materials and Technological Processes of the Bila Tserkva National Agrarian University.

Samples

The material for the study was freshwater fish, which was supplied for sale in the agro-food markets (department No. 1-3) in the Bila Tserkva, Kyiv region. The fish of the following species were studied: scaly carp, crucian carp, pike perch, and rotan. Freshwater fish was fresh. The selection of medium samples of freshwater fish has been carried out following the requirements of SSTU 7972:2015 “Fish and fish products. Acceptance rules, sampling methods” **[36]**.

Chemicals

Nesler's reagent - pure for analysis, manufacturer "Ural Plant of Chemical Products", Russia; hydrogen peroxide, benzidine hydrochloric acid - pure for analysis, manufacturer "Inter-Synthesis, Ukraine; potassium iodide, iodine crystalline, crystal purple, ammonium oxalate, sour fuchsin, hematoxylin, eosin – pure for analysis, Farmakom, Ukraine; immersion oil, glycerol, xylene, neutral formalin (10%), concentrated hydrochloric acid ($p = 1.19$ g/cm^3), sodium hydroxide, sodium hypochlorite, sodium sulfate, ammonia sulfate, ethyl alcohol with a mass concentration of 96%, "Farmak", Ukraine; Essential oil Clove (Oleum Caryophylli) 100%, natural, PC "Zolotonosha PCF", Ukraine; PCA media (Plate Count Agar); XLD (Xylose-Lysine Deoxycholate Agar; PALCAM agar; Endo, Beard-Parker agar medium, HiMedia, India

Animals, Plants and Biological Materials

Carp *(Cyprinus carpio),* Crucian carp *(Carassius gibelio),* Pike perch *(Sander lucioperca),* Rotan *(Perccottus glenii),* gills and muscle tissue.

Instruments

Potentiometer pH-meter NI 8314; photoelectric photometer series - Washer IW−8, FRIMED, Romania; binocular microscope MICROmed, KRÜSS, INVESTLAB, Germany; binocular microscope Euromex BioBlue S/N – EC 1800836 with Euromex Microscope Camera CMEX-5 PRO USB 3.0, Holland; sledge microtome MS-2 Primed, Russia; laboratory weights FEN-300-S, Helpix, Ukraine; water bath VB-4, Soxhlet apparatus, homogenizer for undermining shots, Laboratorna Tekhnika, Ukraine; drying oven SNOL-24/200 Thermo, "Thermoengineering", Ukraine.

Laboratory Methods

Organoleptic assessment of freshwater fish: general condition, appearance, colour, smell, taste - according to DSTU 2284:2010 “Live fish. General technical requirements” and GOST 7631-85 “Fish, marine mammals, invertebrates and products of their processing. Acceptance rules, organoleptic quality assessment methods, sampling methods for laboratory research” **[46]**.

Determining freshwater fish's physical and chemical characteristics (degree of freshness). The pH value was set according to DSTU ISO 2917-2001 **[60]**, the ammonia and salts content, and the Nesler number - GOST 7636-85. Fish freshness was determined using the photometric method **[18]** and an improved benzidine test **[17]**, and the water-retaining capacity of fish meat was determined using an improved method **[16]**.

Determination of microscopic and microbiological characteristics. Microscopy of smears-prints from the surface layers of fish muscle tissue was carried out according to DSTU 4895:2007 **[37]**; quantity of MAFAnM – DSTU ISO 4833:2006 **[66]**; count of coagulase-positive staphylococci (*Staphylococcus aureus*) – DSTU ISO 6888-1:2003 **[65]**, bacteria of the genus *Salmonella* – DSTU ISO 6579:2006 **[67]**, *Listeria monocytogenes* – DSTU ISO 11290-1:2003 **[64]**.

In the meat of the studied fish, mass particles of water and dry matter were determined according to DSTU ISO 1442:2005 **[61]**, protein – DSTU ISO 937:2005 **[59]**, fat – DSTU ISO 1443:2005 **[62]**, ash – GOST 26226–1995 **[35]**, energy value – following the guidelines of the State Research Institute of Laboratory Diagnostics and Veterinary Sanitary Expertise, relative biological value – using the test object of the ciliate Tetrahymena pyriformis **[68]**.

Histological studies (tissue sampling, fixation, wiring, placement in a compacting medium, making histological sections, their staining, and making histological preparations) of freshwater fish muscle tissue were carried out using modern histological methods **[48]**.

Description of the Experiment

Sample preparation: The fish of each species, in the amount of 30 individuals, was anesthetized, cleaned of scales, the skin in the back was cut off, muscle tissue was cut out with scissors in the amount of 15.0 ±0.5 g, then a combined sample of fish meat was obtained, and a point sample was isolated from it in the amount of 200 ±0.5 g for testing; for histological studies, pieces of muscle tissue were taken together with skin 1x1 cm in size in the area of the dorsal lateral muscle and intercostal oblique muscles.

Number of samples analyzed: 30 fish samples of each species were analyzed.

Number of repeated analyses: 30.

Number of experiment replication: 3.

Design of the experiment: The experiment planned to determine the quality and safety indicators of fish: organoleptic; physicochemical methods for determining freshness by generally acceptable methods and developed express ones; microscopic and microbiological; quality (water content, dry matter, protein, fat, energy value, relative biological value); morphological.

Statistical Analysis

The results obtained were calculated by the methods of variation statistics using an ASUS personal computer using MS Excel software packages, STATISTICA 7.0 (Statsoft) software. We determined the arithmetic mean (M) and the statistical error of the arithmetic mean (m), the probability of the difference between the arithmetic means of two variational series according to the probability criterion (p) and Student's tables. The difference between the values was considered probable $p < 0.05$; 0.01 and 0.001.

RESULTS AND DISCUSSION

When organoleptic evaluation (general condition, appearance, colour, smell, taste of meat and broth) of freshwater fish (Ukrainian scaly carp, crucian carp, pike perch, rotan) from different producers of the Kyiv region, it was found that it met the requirements of SSTU 2284:2010. In the studied fish, the scales are shiny, with a mother-of-pearl tint, it is difficult to pull out, and the mucus is transparent. The fish of the studied species is characterized by the natural colouration inherent in the Ukrainian scaly carp, crucian carp, pike perch, and rotan. The skin is elastic, and the fins are solid. Gill covers tightly close to the gill cavity. The eyes are convex, and the cornea is transparent and dirty grey. The muscle tissue is tight and tightly attached to the bones. In the cross-section, it has a characteristic colour for fish of each species. The abdomen is not deflated, and the anus is not protruded. When cooking, the smell and taste are specific for each type of fish, without putrefactive or other foreign odours and flavours. The broth is transparent, with drops of fat on the surface of the appropriate size, depending on the age and type of fish. The smell is pleasant, specific, and fishy, the muscle tissue is well divided into muscle bundles, and the taste of the broth and fish is pleasant, without bitterness and mustiness. According to organoleptic assessment, Ukrainian scaly carp, crucian carp, and pike perch corresponded to fresh fish. The results obtained coincide with the technical approaches of the authors Magas et al., according to the assessment of the main features of the organoleptic indicators of freshwater fish **[56]**. The authors point to a possible difference in the quality of carp meat in terms of organoleptic indicators, which depends on feeding, type of nutrition and environmental factors **[56]**. The rotan broth is somewhat cloudy, with a sour smell, which testified to the dubious degree of freshness of the fish of this species. Improved methods are being developed to determine the freshness of freshwater fish meat, Xiao and Zheng proposed a system for identifying freshwater fish meat of different freshness, based on multi-sensor synthesis using the BP artificial neural network method, providing a high degree of recognition **[86]**. Such studies often include the analysis of PH values, electrical conductivity, and odour, which are determined, combined and analyzed by fuzzy theory in comparison with reference samples **[87]**.

In implementing risk-based control of food safety and quality, effective studies should be applied to determine their suitability for consumption **[9], [72]**. Therefore, we have developed express methods for determining the degree of freshness of fish using a photometric method, an improved benzidine test, and determining the water-holding capacity of meat. These methods provide high reliability of research to control the safety and quality of fish sold in the Kyiv region's agro-food markets.

The method for determining the degree of freshness of fish was carried out by the photometric method using 2.0-2.2 g of a crushed sample of fish meat and infusion of the meat extract for 12-15 minutes. At the next stage, 1.0-1.2 cm^3 of Nesler's reagent was added to the filtered meat-and-breed extract (4.0-4.2 cm^3), kept in a tripod for 4-5 min, then centrifuged for 1-2 min at 2000 rpm, followed by measurement of the optical density of the colour intensity of the supernatant on a photoelectric photometer (in a cuvette absorbing light thickness of 1 cm at a wavelength of 455-460 nm).

When determining the water-retaining capacity of fish meat, fish meat samples were used in the amount of 100.0-150.0 g, ground in an electric meat grinder, and thoroughly mixed, preventing the loss of meat juice, ground minced meat was taken in the amount of 0.3-0.4 g, was placed on a polyethylene circle, then transferred to a circle of filter paper placed on a glass plate so that the minced meat sample was covered with a glass plate. A press weighing 1.0 kg was placed on it and kept for 9-10 min, after which a sample of fish meat was freed from filter paper and polyethylene disks, placed in pre-calibrated weighing bottles, weighed and dried in an oven at a temperature of 105-106 °C in within 4-5 minutes. The formula calculated the water-holding capacity of fish meat as a percentage.

When setting up an improved benzidine test, 2.0-2.2 cm^3 of a filtered extract from fish gills was used (the ratio of gills and distilled water was 1:5). The extract was infused for 12-14 minutes, 0.4-0.5 cm^3 of an alcohol

solution of benzidine with a mass fraction of 0.3% and 0.20-0.25 cm^3 of a hydrogen peroxide solution with a mass fraction of 2% were added. The colour intensity of the filtrate from the gills was determined: if it is of intense blue-green colour, and after 2-3 min it becomes dark brown, the fish is fresh; if it slowly turns into a light bluish-green colour, and after 3-4 minutes it becomes dark brown – the fish is of dubious freshness; if it remains unchanged, but after 5-6 minutes it acquires a dark brown colour – the fish is stale.
The results of the study of the physicochemical parameters of freshwater fish from various producers of the Kyiv region are presented in Table 1.

Table 1 Physical and chemical indicators of the meat of the studied fish.

Indicator	**Type of fish**			
	Carp *(Cyprinus carpio)*	**Crucian carp** *(Carassius gibelio)*	**Pike perch** *(Sander lucioperca)*	**Rotan** *(Perccottus glenii)*
pH value	6.65 ±0.02	6.59 ±0.02	6.88 ±0.03	7.15 ±0.03*
Nesler number	0.80 ±0.01	0.98 ±0.01	1.02 ±0.01*	1.25 ±0.01*
Qualitative reaction to the content of ammonia and ammonia salts	Colour			
	olive	olive	olive	intense yellow
The optical density of the colour intensity of the supernatant extract from fish meat, Bel	0.195 ±0.024	0.218 ±0.014	0.289 ±0.019*	0.684 ±0.032*
The water-holding capacity of fish meat, %	69.35 ±0.26	72.61 ±0.34	70.53 ±0.21	62.13 ±0.17*
Benzidine test (qualitative reaction for peroxidase)	colour			
	intense blue-green	intense blue-green	intense blue-green	light-blue-green

Note: $M \pm m$, n=30.* $p<0.05$ to carp indicators.

Analyzing the data in Table 1, according to the studied indicators, carp, crucian carp, and pike perch corresponded to fish of a fresh degree. Thus, the pH value of the meat-water extract of this fish was within the established standards – 6.65 ±0.02 units, 6.59 ±0.02, and 6.88 ±0.03 units, respectively; in the meat of rotan – 7.15 ±0.03 units (p <0.05), which indicated a violation of the shelf life of this fish. According to the Nesler number, carp, crucian carp, and pike perch corresponded to fresh-grade fish according to the requirements of regulatory documents (up to 1.0). However, in rotan, this indicator was 1.25 (p <0.05), which did not correspond to fresh-grade fish. Indicators of ammonia and salts' content correlated with optical indicators of the colour intensity of the supernatant using Nesler's reagent (in Bel). When conducting a study on a photoelectric photometer, the reliability of tests was 99.8%.

The water-holding capacity of rotan was the lowest – 62.13 ±0.17% (p <0.05), in the studied fish of other species – in the range from 69.35 ±0.26 to 72.61 ±0.34%, the reliability of the test results was 99.4%.
When the improved benzidine test was performed, the filtrate of the gills of carp, crucian carp, and pike perch was of intense blue-green colour, turning dark brown in 2-3 minutes (fresh fish); the filtrate from the gills of rotan turned slowly into a light bluish-green colour and after 3-4 min acquired a dark brown colour (fish of dubious freshness). The reliability of the test results was 99.5%.

Microscopic and microbiological characteristics of the studied freshwater fish

Microscopic examination of the superficial muscles of the fish (under the skin, in the region of the spine), in 10 fields of view of the 1st smear-imprint, stained according to Hram in the modification of Khuker, the number of microorganisms was counted, and the average value was obtained arithmetically.
The results of microscopic and microbiological studies are presented in Table 2.

Table 2 The results of microscopic and microbiological parameters of the studied fish of different species.

Indicator	Type of fish			
	Carp *(Cyprinus carpio)*	**Crucian carp** *(Carassius gibelio)*	**Pike perch** *(Sander lucioperca)*	**Rotan** *(Perccottus glenii)*
The number of microbial cells in 1 field of view of the microscope	6.0 ±2.0	7.0 ±2.0	8.0 ±2.0	14.0 ±2.0*
Quantity of MAFAnM, CFU/cm^3	(1.34 ±0.21) x 10^2	(1.74 ±0.25) x 10^2	(1.12 ±0.09) x 10^2	(2.84 ±0.16) x 10^3*
Bacteria of the *Escherichia coli* group (coliforms), in 0.001 g	not detected	not detected	not detected	not detected
Coagulase-positive staphylococci, in 0.01 g	not detected	not detected	not detected	not detected
Pathogenic microorganisms, including bacteria of the genus *Salmonella* and *Listeria monocytogenes*, in 25 g	not detected	not detected	not detected	not detected

Note: M ±m, n = 30. The standard amount of MAFAnM in fish meat is 5x10^4 CFU/cm^3; *p <0.05 to carp indicators.

The results of microscopic examination of smears-prints from the surface layers of the muscles of the studied carp, crucian carp, and pike perch corresponded to fresh fish - the number of microbial cells in the 1st field of view of the microscope was from 6 ±2.0 to 8 ±2.0. Relative to rotan, this indicator was 14.0 ±2.00 (p <0.05) microbial cells, which confirmed the conclusion about a possible violation of the shelf life of this fish, so rotan meat must be subjected to bacteriological examination to resolve the issue of its food use.

The amount of MAFAnM in the meat of carp, crucian carp, and pike perch was within the normative indicators, according to SSTU 2284:2010 – (1.34 ±0.21) x 10^2, (1.74 ±0.25) x 10^2, (1.12 ±0.09) x 10^2 CFU/g, respectively. However, in rotan meat, this indicator was somewhat higher and amounted to (2.84 ±0.16) x 10^3 CFU/cm^3 (p <0.05)

In the studied freshwater fish (carp, crucian carp, pike perch, rotan), no bacteria of the Escherichia coli group (coliform), coagulase-positive staphylococci, pathogenic microorganisms (*Salmonella, Listeria monocytogenes*) were found. Similar results were obtained by Onishchenko, who noted that the quality of fresh fish carcasses obtained in terms of bacteriological parameters, the number of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAM), contamination with bacteria of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* depends on the condition of the fish on sale. According to the results of the studies, it was found that the meat of fresh fish sold on the agro-food market, according to bacterial indicators, is of high quality and does not pose a risk to the consumer. From the muscle tissue of such fish, mesophilic aerobic and facultative anaerobic microorganisms were isolated in 6.6% of the studied carcasses. Also, bacteria of the group *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* were not detected. From fish meat of dubious freshness, the QMAFAM indicator was above the norm in 20.6% of samples, bacteria of the *Escherichia coli* group – 10.3% of *Salmonella* – in 6.6%, *Staphylococcus aureus* was not isolated **[73]**.

According to Khan, studies using polymerase chain reaction have established the prevalence of hemolytic *L. monocytogenes* in isolates from fish meat in 4.0% of the studied samples **[51]**. The presence of microorganisms in fish isolates that are openly sold on the market is also indicated by Marijani, who found the presence of six types of bacteria: *E. coli* – 40.0%, *Klebsiella* spp. – 26.0, *Salmonella* spp. – 24.0, *Shigella* spp. – 6.7, *Citrobacter* spp. – 6.5 and *Pseudomonas* spp. – 2% **[57]**.

Also, no live helminths and their larvae, dangerous to humans, were found in the studied fish.

The chemical composition of fish meat is not constant and can change depending on various factors. The chemical composition, energy, and relative biological value of the meat of the studied freshwater fish were determined. The results of the study are presented in Table 3.

Table 3 Chemical indicators and biological value of fish of different species.

Indicator	Type of fish			
	Carp *(Cyprinus carpio)*	**Crucian carp** *(Carassius gibelio)*	**Pike perch** *(Sander lucioperca)*	**Rotan** *(Perccottus glenii)*
Mass fraction of water, %	74.50 ±0.12	71.34 ±0.09	72.21 ±0.08	78.30 ±0.13*
Mass fraction of dry matter, %	25.50 ±0.08	28.64 ±0.07	27.79 ±0.06	21.70 ±0.09*
Mass fraction of protein, %	19.15 ±0.06	20.63 ±0.06	21.33 ±0.06	16.96 ±0.06*
Mass fraction of fat, %	4.84 ±0.06	5.99 ±0.06	4.31 ±0.06	3.01 ±0.06*
Mass fraction of ash, %	1.24 ±0.08	2.02 ±0.08	2.15 ±0.08	1.73 ±0.08*
Energy value, kJ	527.25 ±2.08	546.09 ±3.11	540.12 ±2.45	465.02 ±2.64*
Relative biological value, %	99.8	99.5	98.9	92.5*

Note: M ±m, n = 30. *p <0.05 to carp indicators.

Table 3 data indicate that the highest indicator of the mass fraction of water is in the meat of rotan 78.30 ±0.13% (p <0.05), the lowest is crucian carp (71.34 ±0.09%), in the meat of Ukrainian carp scales – 74.50 ±0.12%. Accordingly, the mass fraction of dry matter in rotan is 21.70 ±0.09% (p <0.05), crucian carp and pike perch – 28.64 ±0.07 and 27.79 ±0.06%, Ukrainian scaly carp – 25.50 ±0.08%. The obtained values of indicators of the mass fraction of water coincide with the results of studies by Martsenyuk indicating that the muscle tissue in three-year-old small-scaled carp contains 77.54% water and in two-year-olds – 75.1%.At the same time, the author notes that two-year-olds' muscle tissue is characterized by a higher fat content and confirms an interdependence between moisture content and fat **[58]**. Golovko obtained similar results of the water content in muscle tissue the mass fraction of which averages 75% **[47]**. According to Blazhekovikj-Dimovska, the meat of common carp from open water contains 76.03% water **[14]**.

When determining the mass fraction of proteins, it was found that their lowest indicator was in the meat of rotan (16.96 ±0.06%; p <0.05), and the highest was in pike perch (21.33 ±0.06%), in carp meat, it was 19.15 ±0.06%. Other researchers have obtained similar values. So, according to Golovko, protein content in spring-caught carp meat ranges from 16% to 18.8% **[47]**. Skibniewska et al. point to similar values of the indicator, the level of which depends on the technology of carp breeding and can range from 16.9 to 18.6% **[79]**. The mass fraction of fat in rotan was the smallest among the studied fish and amounted to 3.01 ±0.06%. In crucian carp, it was the highest (5.99 ±0.06%). Accordingly, the energy value of crucian meat is 546.09 ±3.11 kJ, rotan – 465.02 ±2.64 kJ (p <0.05). Blazhekovikj-Dimovska obtained similar values of the mass fraction of protein. The results of the studies show that the meat of common carp from open water contains 2.92% fat and 1.06% ash **[14]**. At the same time, Martsenyuk points to excellent values of the indicator, according to which the fat content in meat is 6.39 ±0.69 for two-year-olds and 3.09 ±0.57 for three-year-old carp (p >0.99) **[58]**. Authors Golovko et al. noted that carp meat's mass fraction ranges from 3.1% to 8%, classifying it as a medium-fat fish **[47]**.

An important indicator of the quality of freshwater fish is the relative biological value, which depends on the level of assimilation of meat proteins. Olifirenko et al. also point out the need for constant quality control of the resulting commercial fish and determining the biological value, which will provide a solution to the problems of modern fish farming: selection of fish species, stocking density, development of recommendations on technology and bionorms of cultivation fish, determining the timing of fishing, transportation and storage of fresh marketable pond fish **[71]**. The highest relative biological value of fish meat was in the Ukrainian scaly carp, which amounted to 99.8%, and the lowest in rotan (92.5%; p <0.05).

Thus, the research results indicate that the chemical composition of fish meat depends on the species and feed ration.

The energy value of fish meat directly depends on the chemical composition, especially the content of fats and proteins. In particular, the indicator of the mass fraction of proteins in the meat of pike perch and carp is 21.33 ±0.06 and 19.15 ±0.06%, respectively. The relative biological value of carp was 99.8%, and that of pike perch was 98.9%. This indicates that fish's energy and relative biological value depends on its species, food supply, and protein content. According to Martsenyuk, the calorie content of carp meat is 130.26 ±6.11 and 103.29 ±5.58 kcal/100 g for two and three-year-olds, respectively **[58]**.

Microstructural analysis

Fish meat is represented by muscle and connective tissues. The skeletal muscles of fish are formed by striated muscle tissue, the structural and functional unit of which is the muscle fiber. Muscle fibers are combined into myomers, separated by myosepta **[39]**, **[76]**, **[77]**. The muscle fibre composition includes sarcoplasm, myofibrils, and numerous nuclei surrounded by sarcolemma. For myofibrils, individual sections with different structures and optical properties, a transverse banding is characteristic, forming the actual banding of the fibers **[33]**. Actin myofilaments form light stripes (isotropic disks) with single characteristic refraction, and dark (anisotropic disks) are formed by myosin and, partially, actin myofilaments – with birefringence.

The muscle fibers of the skeletal muscles of fish differ in colour, shape, structure, and functional activity and, as a result, are divided into red and white. Red muscle fibers are located in the muscles superficially above the white ones. The taste of fish and its nutritional and biological value depends on the degree of adipose tissue development. Adipose tissue is formed from loose connective tissue containing fat cells – adipocytes. In the body of fish of different types, the localization of adipose tissue is somewhat different (under the skin, near the fins, in the tail, liver, in most fish – in the thickness of the muscles).

Fish oil largely determines adults' resistance to the effects of persistent fat-soluble toxins and can accumulate harmful substances **[81]**. Such hydrobionts pose a danger to humans when consumed.

The most valuable part of the fish is its body (from the head to the beginning of the anal fin), its main muscles: the longissimus dorsi, superficial and deep lateral muscles, and intercostals, in particular, the internal oblique muscle. Therefore, samples of these muscles were taken for microstructural analysis.

For histological examination, 30 samples of striated muscle tissue 0.5-1 cm^3 in size were taken from the superficial and deep lateral muscles, the internal oblique (intercostal) muscle of freshly caught freshwater fish. Muscle tissue was fixed with a neutral formaldehyde solution with a mass concentration of 10% at room temperature for 24 hours. After fixation, the material was washed with running water, dehydrated in alcohols of increasing concentration, and poured into celloidin. Sections 5-10 μm thick were made on a microtome and stained with hematoxylin and eosin by the Van Hizon method (picrofuchsin dye, which contains picric acid, has a differential property), according to modern histological methods **[48]**.

Microscopic examination of celloidin histological preparations of fish muscles was performed using a binocular microscope with a built-in video camera.

According to the results of their histological examination, it was revealed that the muscle tissue of the lateral muscles of the scaly carp (*Cyprinus carpio*) on fixed longitudinal sections of the superficial and deep muscles is characterized by a parallel arrangement of muscle fibers. Muscle fibers had a thin sarcolemma and well-defined transverse striation. There are no adipocytes in the sarcoplasm, but a small number were found in the perimysium. On transverse sections, both superficial and deep lateral muscle fibres had a rounded, oval, triangular shape with rounded edges, tightly adjacent. Staining of fixed histological preparations from muscle tissue with Weigert's hematoxylin and picrofuchsin occurred evenly. Microscopy at a magnification of 10 x 40 revealed the existing granularity in the structure of muscle fibers, due to the placement of myofibrils over the entire area of the fiber (Figure 1a). On transverse sections of muscle tissue in the perimysium of muscle bundles of the II order, single adipocytes were observed.

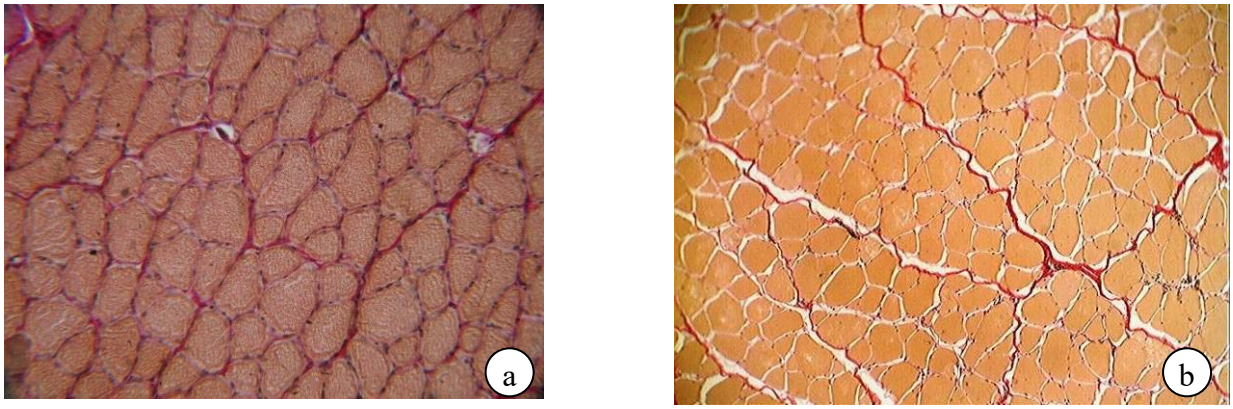

Figure 1 The structure of muscle tissue is normal. Note: a – superficial lateral muscle; b – internal oblique muscle. Cross-section, Van Hizon, Magnification: 400×.

According to the results of a microstructural study of the internal oblique muscle (intercostal), it was found that the areas bordering the deep lateral muscle had the usual structure of muscle tissue. Muscle fibers were round and polygonal in shape, placed close to each other, had a uniform colour, and the nuclei were localized on their periphery. The oblique muscle fibres formed well-defined bundles (Figure 1b).

Consequently, no changes in their structure were noted during the study of some muscle samples. That is, it was characteristic of the fish of this species **[80]**.

However, histological examination of the muscle tissue of individual samples revealed some changes. So, in scaly carp, classified as a quality fish according to organoleptic parameters, significant structural changes were noted in the internal oblique muscle (intercostal). On longitudinal sections, curvature, thinning of muscle fibers, compaction, and stratification were noted. In some areas, the thickening of muscle fibers and their ruptures were observed. Such fibers had no transverse striation. In addition, some fibers were lysed. In such areas, large voids were noted due to the growth of adipose tissue (Figure 2), lymphocytes, fibroblasts, collagen fibers, and a large amount of intercellular fluid.

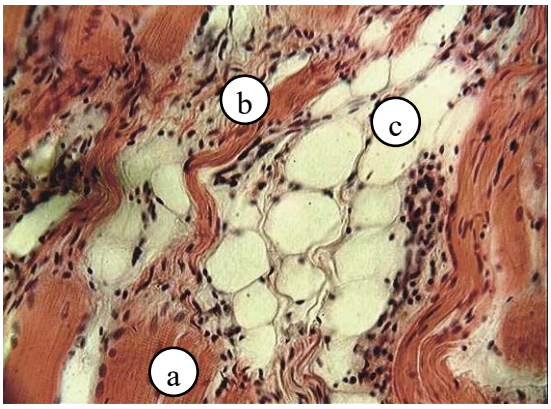

Figure 2 Replacement of the internal oblique muscle fibres with connective tissue. Note: a – muscle fibers; b – atrophied muscle fibers; c – connective tissue (longitudinal section, hematoxylin-eosin; Magnification: 400×).

On transverse sections of muscle tissue in muscle fibers, a loose placement of myofibrils was observed, and in some areas – voids (Figure 3a), endomysium, and perimysium – thickened. Muscle fibers had a heterogeneous colour: inside the fiber – light and dark on its periphery.

Many destroyed fibres were noted in the bundles of muscle fibers (Figure 3b), inside - with dystrophic and destructive changes, and fibers with the rest of myofibrils next to the sarcolemma (Figure 3c).

The perimysium between the fiber bundles is massive and thickened, with dense collagen strands and many adipose tissue cells. Scientists have also noted changes in muscle fibers' structure in other fish species, particularly zander. In studies of striated muscles by the authors Grushko found an increase in the distance between muscle fibers and fixed fibers in which the transverse banding barely appeared. At the same time, the deformation and weakening of muscle fibers were established. The reasons for deviations from the norm in histological preparations were: weakening of muscle fibers – 50%, necrosis and violation of the structure of

muscle fibers – 10% **[43]**. Thus, with qualitative organoleptic indicators of fish, significant structural changes can occur in its muscle tissue.

In particular, dystrophic and destructive changes lead to the atrophy of individual muscle fibers. There is a scattered placement of thinned, atrophied fibers among fibers with a relatively preserved structure or hypertrophied ones. The areas of muscle tissue that have undergone lysis are filled with connective tissue, mainly adipose tissue. Therefore, it can be assumed that changes in the structure of fish muscle tissue are due to the influence of unfavourable growing conditions, in particularly stressful situations **[4]**, the presence of harmful toxic substances, in which there are violations of the regulation of metabolic processes **[75]**, which leads to a decrease in biosynthetic processes and increased fat formation **[81]**, violation of the hydrochemical regime **[41]**. The study by Grushko et al. also confirms, pointing to the establishment of a wide range of pathologies identified during histological studies of the internal organs of fish, which is probably associated with persistent environmental pollution due to human economic activity **[44]**.

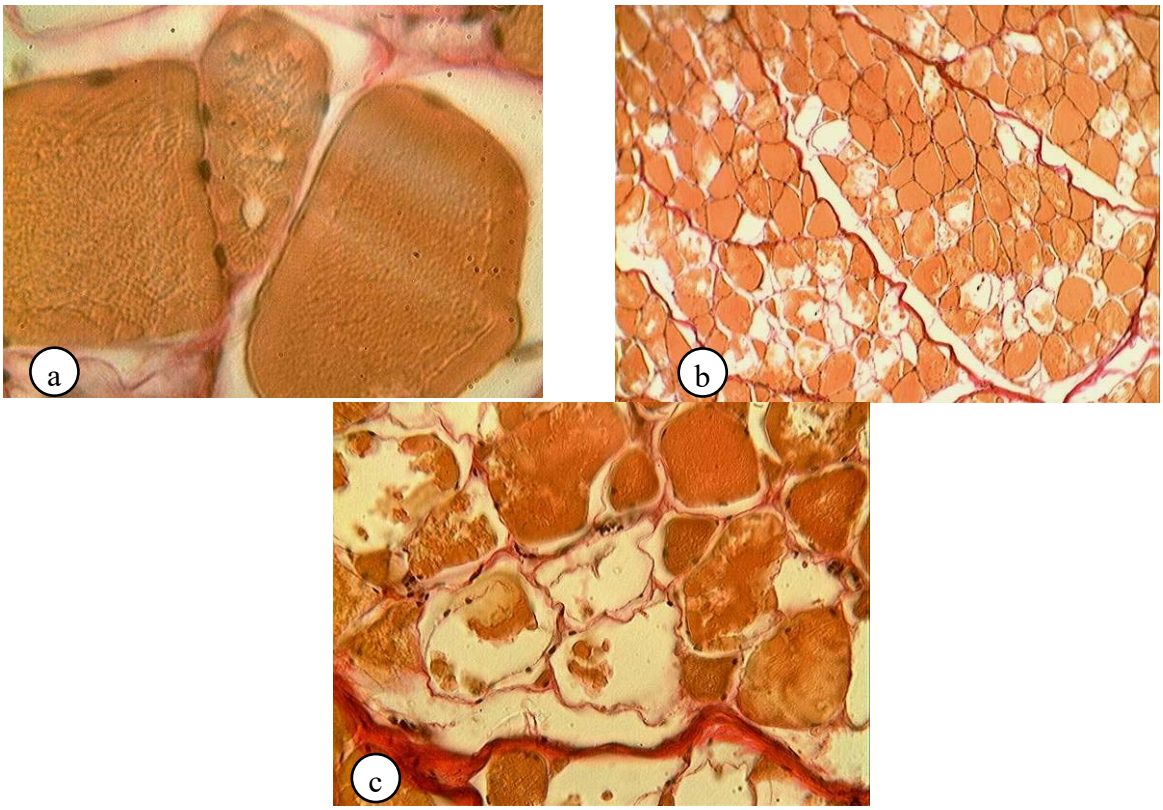

Figure 3 Structural changes in the intercostal muscles of scaly carp. Note: a – voids between myofibrils (cross-section, Van Hizon; Magnification: 1000×); b, c – atrophied muscle fibers (cross-section, Van Hizon; Magnification: 100× and 400×).

The possibility of the influence of the ecological situation, especially chronic intoxication of aquatic organisms with organochlorine, oil and other pollutants, can cause serious physiological changes in various body systems. In this regard, it is essential to assess the current state of fish according to biological criteria, the most important of which is histological **[42]**. Damage to the organs and tissues of fish can be observed without external signs of intoxication; in such cases, pathomorphological changes are the only indicator of the harmful effects of toxic substances **[3]**. The histological method of research allows, at the cellular and tissue level, to find out the depth of the pathological process in each fish and assess the damage to the entire herd in the reservoir **[27]**.

CONCLUSION

According to organoleptic (general condition, appearance, colour, smell, taste of fish and broth) and physicochemical parameters (pH value, Nesler number, qualitative reaction to ammonia and ammonium salts, optical density, the water-holding capacity of fish meat, improved benzidine test), scaly carp, crucian carp, pike perch corresponded to the indicators of fresh fish, and rotan – dubious. Ukrainian scaly carp, crucian carp, and pike perch met the requirements of regulatory documents (DSTU 2284:2010) in terms of microscopic indicators and the number of MAFAnM; in rotan, the indicators of the number of microbial cells in the field of view of the microscope and the number of mesophilic aerobic and facultative anaerobic microorganisms were slightly higher. When determining the chemical parameters and biological value of fish of various types, it was found that the largest mass fraction of water was found in the meat of rotan (78.30 ±0.13%), the smallest in crucian carp (71.34 ±0.09%), and the average values had the meat of the Ukrainian scaly carp (74.50 ±0.12%). In rotan, the mass fraction of dry matter was 21.70 ±0.09%, crucian carp, pike perch – 28.64 ±0.07 and 27.79 ±0.06%, respectively, carp – 25.50 ±0.08%. The mass fraction of proteins is the smallest in rotan meat (16.96 ±0.06%), the largest – pike perch (21.33 ±0.06%), and in carp meat, this figure was 19.15 ±0.06%. The lowest fat content was in rotan meat (3.01 ±0.06%), and crucian carp (5.99 ±0.06%) was the highest. The energy value of crucian meat was the highest and amounted to 546.09 ±3.11 kJ, in rotan – the lowest (465.02 ±2.64 kJ). The relative biological value of Ukrainian scaly carp meat was 99.8% (the highest indicator), rotan – was 92.5% (the lowest indicator). In the muscle tissue of fish, recognized by organoleptic indicators as benign, histological examination of its structure revealed significant changes with atrophy of individual muscle fibers and growth, mainly of adipose tissue. In risk-based control of the safety and quality of freshwater fish, state veterinary inspectors were asked to use the developed express methods for determining the degree of its freshness (photometric determination of optical density, determination of the water-holding capacity of fish meat, staging an improved benzidine test), reliability 99.8%, 99.4 and 99.5% respectively.

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Investigation of the yield of biologically active substances during the ultrasound and electro-discharge extraction of medicinal herbs of the foothills of the North Caucasus

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ABSTRACT

Biologically active components are present in plants in small quantities. There are many different extraction methods, which can be used for their extraction. In this scientific work, extracts of three plants (common origanum, peppermint and garden sage) were prepared in three different ways: water extraction, ultrasound extraction and electro-discharge extraction. The dynamics of saturation of extracts with flavonoids, essential oils and organic acids for each case were studied within 48 hours after the experiment's start. The conducted studies have confirmed the effectiveness of electro-discharge extraction in comparison with ultrasound and in comparison with water extraction. Forty-eight hours after the start of the experiment, 7-15% more organic acids, flavonoids and essential oils were observed in extracts of the studied plants obtained after electro-discharge treatment than in water extracts. A similar dynamic can be traced in the assessment of all indicators. At the same time, 80% readiness of extracts in the case of electro-discharge treatment was observed already 30 minutes after the start of the experiment. Similar indicators (80% of the maximum) were achieved after 24 hours of water extraction and after 2 hours with ultrasound treatment. Thus, the electro-discharge treatment allows you to obtain higher-quality and more enriched active substance extracts in a much shorter time. At the same time, electro-discharge treatment has a significant list of disadvantages described in detail in this article.

Keywords: herbal extract, water extract, ultrasound extract, electric pulse extraction, medicinal herbs

INTRODUCTION

Plant-based ingredients are frequently used in the food industry to give foods distinctive flavors and aromas. For example, lemongrass, ginseng, green tea, and eleutherococcus are often used in the production of energy drinks; bay leaf, onion, pepper, parsley, cumin, dill, horseradish, garlic are used in the production of sauces, sausages, crackers; anise, vanilla, peppermint, tarragon, etc. are used in the confectionery and alcohol industry **[1]**, **[2]**, **[3]**, **[4]**. Many biologically active components are present in plants in small quantities, so there is a need for their isolation and/or concentration. One of the ways to solve this problem is the extraction process **[5]**, **[6]**. Extraction is a method that allows you to extract flavoring components from vegetable raw materials. Extracts are concentrated products of liquid (liquid extracts and tinctures), soft (thick extracts) or solid (dry extracts) consistency obtained from vegetable raw materials or animal materials **[7]**, **[8]**. Currently, when obtaining plant extracts, the technology of long–term infusion of raw materials with an extractant is widely used, as which in most cases, alcohol solutions with a mass fraction of alcohol of 40-80% are used. Water infusions of vegetable raw materials are also made by pouring boiling water and holding it at a temperature of 70-80 °C for 4-6 hours **[9]**. This method of processing plant raw materials does not allow the maximum use of extractive substances. It obtains extracts enriched with substances of carbohydrate and protein nature, micro- and macroelements, flavouring and tannins, vitamins, organic acids, glycosides and other compounds. Therefore, their selective ability

must be considered when choosing the extraction method and the extractant **[10]**. In the food industry, water, alcohol, hexane, acetone, and liquefied carbon dioxide are usually used as extractants **[11]**. The extractant plays a significant role in the extraction of biologically active substances. It must have the ability to penetrate through the cell walls and selectively dissolve biologically active substances inside the cell, after which the latter must pass through various hard shells and go beyond the plant material. In addition, it should have low toxicity (the use of dichloroethane, benzene, pyridine, etc. is unacceptable), dissolve essential oils and oleoresins, have a low boiling point and be removed from the extract as wholly as possible by distillation **[12]**, **[13]**. The raw materials for obtaining extracts are fresh or dried parts of plants: bark, roots, stems, wood, leaves, petals, inflorescences, and seeds **[14]**. Often extracts from the same plant are completely different in composition, action and aroma.

As a rule, the diffusion rate in the solid phase limits the extraction of biologically active substances (BAS) from natural materials of plant origin. Therefore, intensifying the processes of obtaining extracts for food production is of great practical importance. This is because, firstly, the extraction processes are lengthy; secondly, the stability and organoleptic characteristics of the finished products significantly depend on the quality of the extracts. This fact makes it necessary to research the development and improvement of technology for obtaining extracts from plant raw materials, providing for the directed regulation of their properties.

Currently, there is extensive experience in the introduction of new methods that intensify mass transfer in a solid-liquid system, which are based on methods for measuring vibrations, pulsations or vibrations of various amplitudes, frequencies and intensities **[15]**, **[16]**, **[17]**.

The ultrasound extraction method is often used in the food industry. Regulation of the BAS extraction process using ultrasound is of interest for scientific research concerning abnormal processes occurring during ultrasound water treatment. The main advantage of ultrasound technology is the impact of specific factors inherent in ultrasonic vibrations: the cavitation effect, the formation of micro-flows and the effect on the diffusion permeability of the tissue of the extracted material. In this regard, using ultrasound to obtain extracts is of great interest **[18]**, **[19]**.

Along with many methods of intensifying the BAS extraction process, electro-discharge treatment is promising. The high-voltage electro-discharge treatment is based on the phenomenon of the electrohydraulic effect. The principle of the appearance of the electrohydraulic effect is the phenomenon of a sharp increase in the hydraulic and hydrodynamic effects and the amplitude of the shock action during the implementation of a pulsed electric discharge in an ion-conducting liquid, provided that the pulse duration is maximally shortened, the pulse front is as steep as possible, and the pulse shape is close to the aperiodic **[20]**. The main operating factors of the electrohydraulic effect are:

– high and ultra-high pulsed hydraulic pressures, leading to the appearance of shock waves with sonic and supersonic speeds;

– significant pulsed movements of fluid volumes, occurring at speeds reaching hundreds of meters per second;

– powerful pulsed cavitation processes capable of covering relatively large volumes of liquid **[21]**;

– infra- and ultrasonic radiation; mechanical resonant phenomena with amplitudes that allow mutual exfoliation of multicomponent solids from each other;

– powerful electromagnetic fields (tens of thousands of oersted);

– intense pulsed light, thermal, ultraviolet radiation;

– multiple ionization of compounds and elements contained in the liquid **[22]**.

Electric discharge technology with the use of rectangular pulses of nanosecond duration reduces the processing time of raw materials from 12-36 hours to 10-15 minutes, and has a number of additional advantages, such as:

– disinfection of the extract during treatment with high-voltage pulses **[23]**;

– the possibility of using non-toxic extractants due to the high extraction efficiency with water, etc. **[24]**.

Thus, this work aimed to study the effectiveness of the ultrasonic and electric pulse method of extracting medicinal herbs from the Foothills of the North Caucasus.

Scientific Hypothesis

The method of extraction using ultrasound and electro-discharge treatment will allow for obtaining high-quality extracts in a shorter time than the classical method of obtaining water extracts. And at the same time, such extracts will be more enriched with useful elements than water extracts.

MATERIAL AND METHODOLOGY

Samples

Extracts from medicinal herbs of the Foothills of the North Caucasus: garden sage (*Salvia officinalis*), common origanum (*Origanum vulgare*)and peppermint (*Méntha piperíta*) were prepared with conventioanls and intense methods and used as experimental samples. Garden sage, common origanum and peppermint were collected in Stavropol Region near the Strizhament mountain in July-August 2021.

Chemicals

We used reagents of recognized analytical purity and distilled water. The following chemicals were used in work: Ethanol, Sodium hydroxide, Sodium carbonate, Aluminum chloride, and Ascorbic acid. All chemicals above were purchased by LenReactive LLC (Sants Petersburg, Russia) and were of analytical grade quality.

Animals and Biological Material

The following herb collections were used as objects of research: common origanum (according to GOST 21908 – 76), peppermint (according to GOST 21908 – 93), garden sage (according to GOST 1994 – 43) **[25]**, **[26]**, **[27]**. The content of some micro- and macroelements contained in these plants is presented in Table 1.

Common origanum has soothing, anti-inflammatory, antibacterial, analgesic, diuretic, anthelmintic, and insecticidal properties. The content of essential oil is not less than 0.8%; humidity is not more than 14%; total ash is not more than 12%; blackened and browned leaves are not more than 5%; other parts of the plant (flowers and pieces of stems) are not more than 13%; particles passing through a sieve with holes of 0.5 mm in size are not more than 10%; organic impurity not more than 3%; mineral impurity not more than 0.5%.

Peppermint is a perennial herbaceous plant with a height of 25-60 cm. The stem and the entire plant are bristly-hairy or smooth. Essential oil not less than 1%; humidity, not more than 14%; total ash not more than 14%; ash insoluble in 10% hydrochloric acid solution, not more than 6%; blackened leaves not more than 6%, stems not more than 10%; particles passing through a sieve with 0.5 mm holes, not more than 8%; organic impurity not more than 3%; mineral impurity not more than 1%.

Garden sage is used not only in cooking, but also in medicine. It helps to normalize the work of the cardiovascular system, reduce blood pressure, and improve cerebral circulation. Garden sage contains up to 2.5% essential oil, 4% condensed tannins, ursolic and oleanolic acids, phenolic carboxylic acids, vitamins, macro- and microelements, diterpenes, bitter substances, 5-6% resinous substances, flavonoids, coumarin esculetin, etc.

Table 1 The content of some macro- and microelements in the plant raw materials used, mg.

Chemical element	Common origanum	Peppermint	Garden sage
K	19.80	569	22.90
Ca	12.40	243	40.90
Mg	2.10	80	9.20
Fe	0.63	5.08	0.80
Mn	0.12	1.176	99.20
Cu	0.49	329	15.50
Zn	0.34	1.11	97.40

Instruments

The amount of flavonoids in plant raw materials was determined photometrically after reacting with aluminium chloride on a photocolorimeter KFK-3 (ProfMT, St. Petersburg, Russia). Studies of the kinetics of accumulation of biologically active substances were carried out on the Bruker MaXis Impact mass spectrometer using Target Analysis software (Bruker, Belgium)

Laboratory Methods

During the experimental studies, the following methods were used to determine individual analytes:

Definitions of extractive substances: 1 g of raw materials, crushed and sifted through a sieve with holes with a diameter of 1 mm, is placed in a conical flask, and 50 ml of the solvent specified in the standard technical documentation for this type of raw material is poured. The flask is closed with a stopper, weighed with an error of no more than 0.01 g and left for 1 h. Then the flask is connected to a reverse refrigerator, heated to a boil, and a weak liquid boiling is maintained for 2 h. After cooling, the flask with the contents is again closed with the same stopper, and weighed and the mass loss is supplemented with the same solvent. The contents are thoroughly shaken and filtered through a dry paper filter into a dry flask with a capacity of 150-200 ml. 25 ml of filtrate is transferred to a porcelain cup with a diameter of 7-9 cm, pre-dried at 100-105 °C to a constant weight and weighed on analytical scales, evaporated dry in a water bath, dried at 100-105 °C for 3 hours, then cooled in desiccator and quickly weighed **[28]**.

The percentage of extractive substances (x) in absolutely dry raw materials is calculated by the formula (1):

$$x = \frac{m\ 200 \times 100}{m_1 \times (100 - w)} \quad (1)$$

Where:
m – is the mass of the dry residue in the cup, g; m_1 – is the mass of raw materials, g; w – is the loss of raw materials during drying, %.

Determination of flavonoid content in raw materials: The analytical sample of raw materials is crushed to the particle size, passing through a sieve with holes of 1 mm. The exact weight (1 g of raw materials) is placed in a flask with a capacity of 100 ml, 30 ml of 70% alcohol is added, attached to a reverse refrigerator and heated in a boiling water bath for 30 minutes. After cooling to room temperature, the contents are filtered through a paper filter into a 100 ml volumetric flask (for complete extraction of flavonoids, extraction is repeated twice by the above method, and the extracts obtained are filtered into the same volumetric flask through the same filter). The filter is washed off with 70% alcohol, and the filtrate volume is brought to the mark with the same alcohol. The resulting solution will be called "solution A". 4 ml of "solution A" is placed in a measuring flask with a capacity of 25 ml, 2 ml of a 2% solution of aluminium chloride in 95% alcohol is added, and the volume is brought to the mark with 95% alcohol. After 20 minutes, the optical density is measured on a photocolorimeter KFK-3 (ProfMT, St. Petersburg, Russia) at a wavelength of 410 nm in a cuvette with a layer thickness of 10 mm **[29]**.

Determination of organic acid content: The total content of organic acids is determined by titration using indicators. An aliquot of the infusion equal to 100 ml is placed in a 250 ml flask, and 100 ml of distilled water is added and titrated with a 0.1 M sodium hydroxide solution in the presence of phenolphthalein and methylene blue indicators until a purple-red colour appears in the foam **[30]**.

Mass spectrometry: The elemental content of organic compounds in the model samples was determined by mass spectrometry with inductively coupled argon plasma on a quadrupole mass spectrometer Bruker MaXis Impact with Target Analysis software (Bruker, Belgium). The detection limit in a water solution is 1 mcg/l. Studies of the kinetics of the accumulation of biologically active substances were carried out on the Bruker MaXis Impact apparatus. Bruker MaXis Impact is a device designed for mass spectrometric analysis. The device detects and identifies known compounds, their metabolites, and unknown compounds in a wide range of masses from 20 to 40,000 Da (drugs, pesticides, etc.).

The content of essential oils: The content of essential oils was carried out by distillation from vegetable raw materials with water vapour of essential oil and subsequent measurement of its volume according to GOST 24027.2-80 **[31]**.

Description of the Experiment

Sample preparation: Medicinal herbs growing in the North Caucasus were selected as the objects of the study: garden sage (*Salvia officinalis*), common origanum (*Origanum vulgare*), peppermint (*Méntha piperíta*).

Following the technical requirements, the feedstock was dried to an air-dry state, then crushed and divided into separate fractions with a particle size of 5.0 mm. The samples of various fractions were extracted with water.

It should be noted that an increase in the temperature of the extractant is undesirable for essential oil raw materials since essential oils are largely lost when heated. An increase in temperature is advisable when extracting from roots, rhizomes, bark and leathery leaves. Hot water, in this case, contributes to a better separation of tissues and rupture of cell walls, thereby accelerating the course of the diffusion process. The production of acidified water extracts was carried out using both traditional and innovative methods. Traditional methods widely used in the production of extracts are the method of water extraction and ultrasound treatment. Obtaining a water extract using the method of water extraction was carried out as follows: selection and preparation of raw materials (degree of grinding 5 mm) was carried out, soaking in warm water at t = 40 °C for 15 minutes, then heating in a water bath to a temperature of t = 60 °C for two hours, then cooling and infusing. By ultrasonic treatment, the water extract was obtained as follows: the selection and preparation of raw materials was carried out (the degree of grinding is 5 mm), soaking in warm water at t = 40 °C for 15 minutes, ultrasound treatment for 30 minutes, then infusion. Extraction using high-voltage electro-discharge treatment was carried out as follows: selection and preparation of raw materials were carried out (degree of grinding 5 mm), soaking in warm water at t = 40 °C for 15 minutes, treatment for 10 minutes (360 discharges, 10 kV, 0.01 microfarads), then infusion.

Number of samples analyzed: 3
Number of repeated analyses: 3
Number of experiment replication: 1
Design of the experiment: The scheme of the experiment is shown in Figure 1.

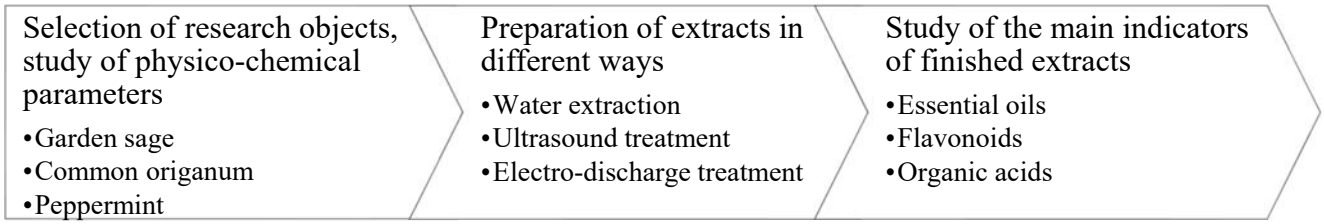

Figure 1 The scheme of the experiment.

Statistical Analysis

Statistical processing of experimental data. The obtained results were processed using the statistical package PASW Statistics 18, version 18.0.0 (SPSS Inc., USA). Verifying the normality of the distribution of signs was carried out using the Kolmogorov-Smirnov criteria. The critical significance level when testing statistical hypotheses in the study were assumed to be 0.05. The organic compounds' elemental content results were processed using the Target Analysis software package.

RESULTS AND DISCUSSION

On the first stage, after the extraction processes, we could observe the differences between groups in color (Figure 2). As can be seen on the example of Common origanum extracts, electro-discharge treatment caused the most intense colorization of the water during extraction. However, to make an objective conclusion we carried out complex experimental research.

Figure 2 Aqueous extracts of Common origanum obtained with water extraction, ultrasound (US), and electro-discharge (ED) treatment.

To obtain a complete picture of the effectiveness of various extraction methods (water extraction, ultrasound treatment and electro-discharge treatment), we analyzed the content of organic acids, essential oils and flavonoids in extracts at various time intervals:

– 15 minutes after the start of the experiment (the end of the soaking stage),
– 30 minutes after (the end of the electric discharge treatment),
– after 2 hours (end of ultrasound treatment),
– after 8 hours (intermediate value),
– after 24 hours (intermediate value),
– after 48 hours (the end of the experiment).

The results of the research are presented in Figures 3, 4 and 5.

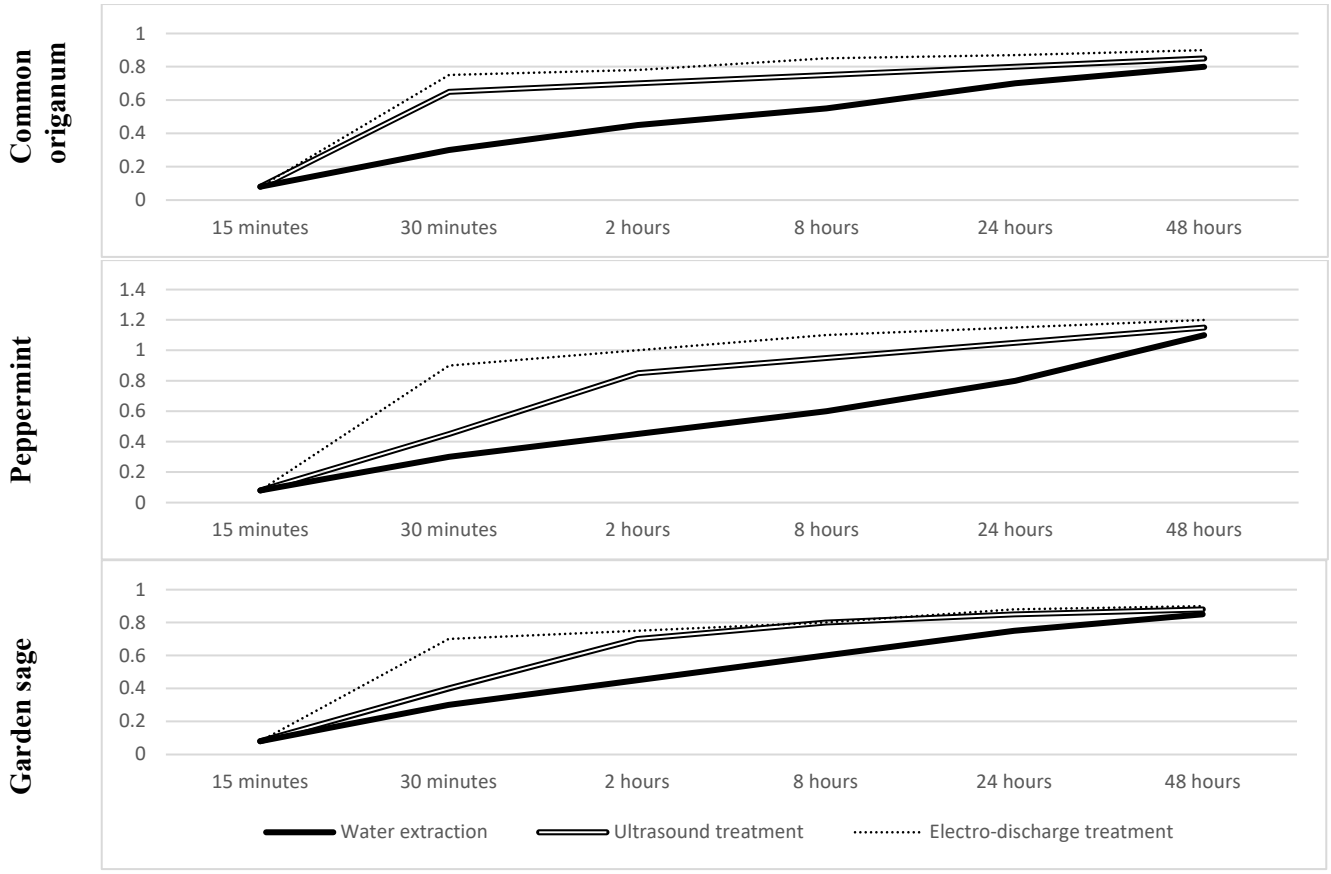

Figure 3 Essential oil content (%) in Common origanum, Peppermint and Garden sage.

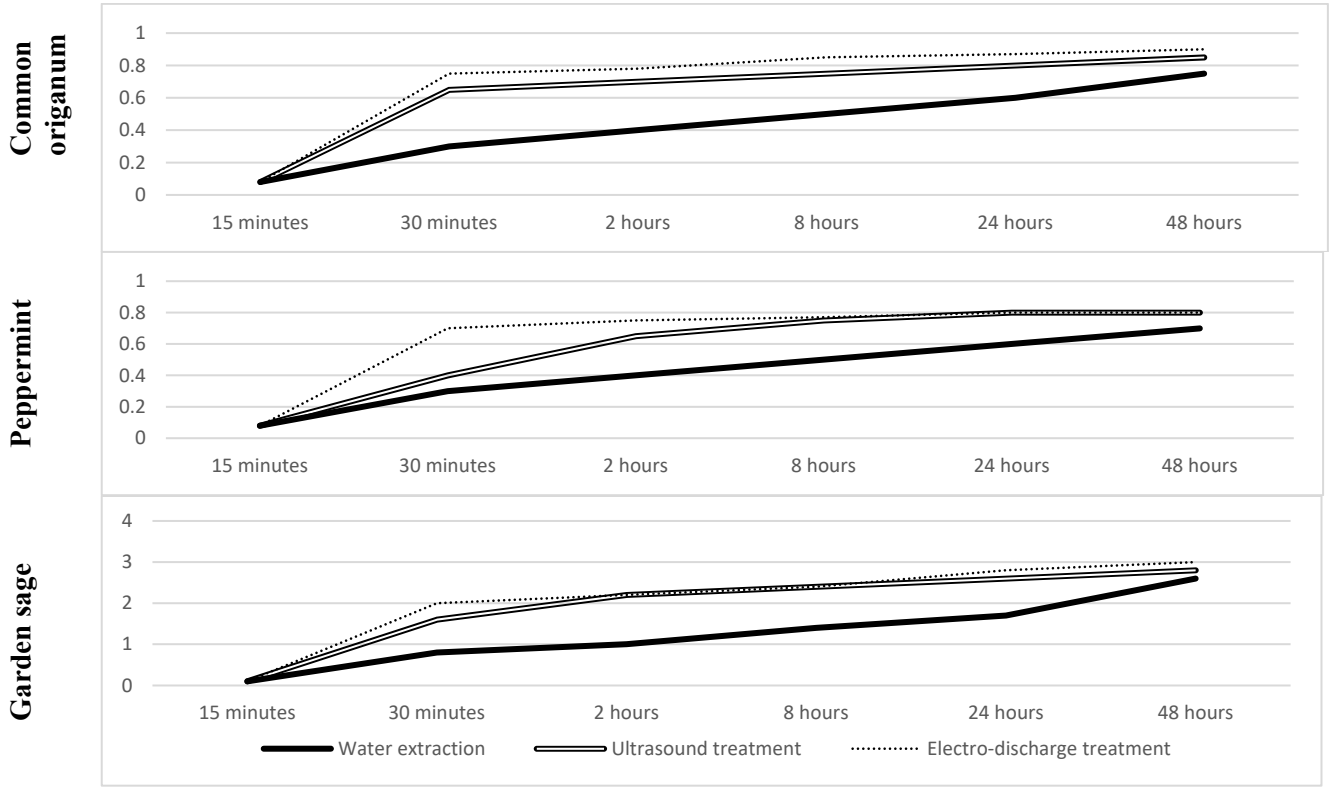

Figure 4 Flavonoid content (%) in Common origanum, Peppermint and Garden sage.

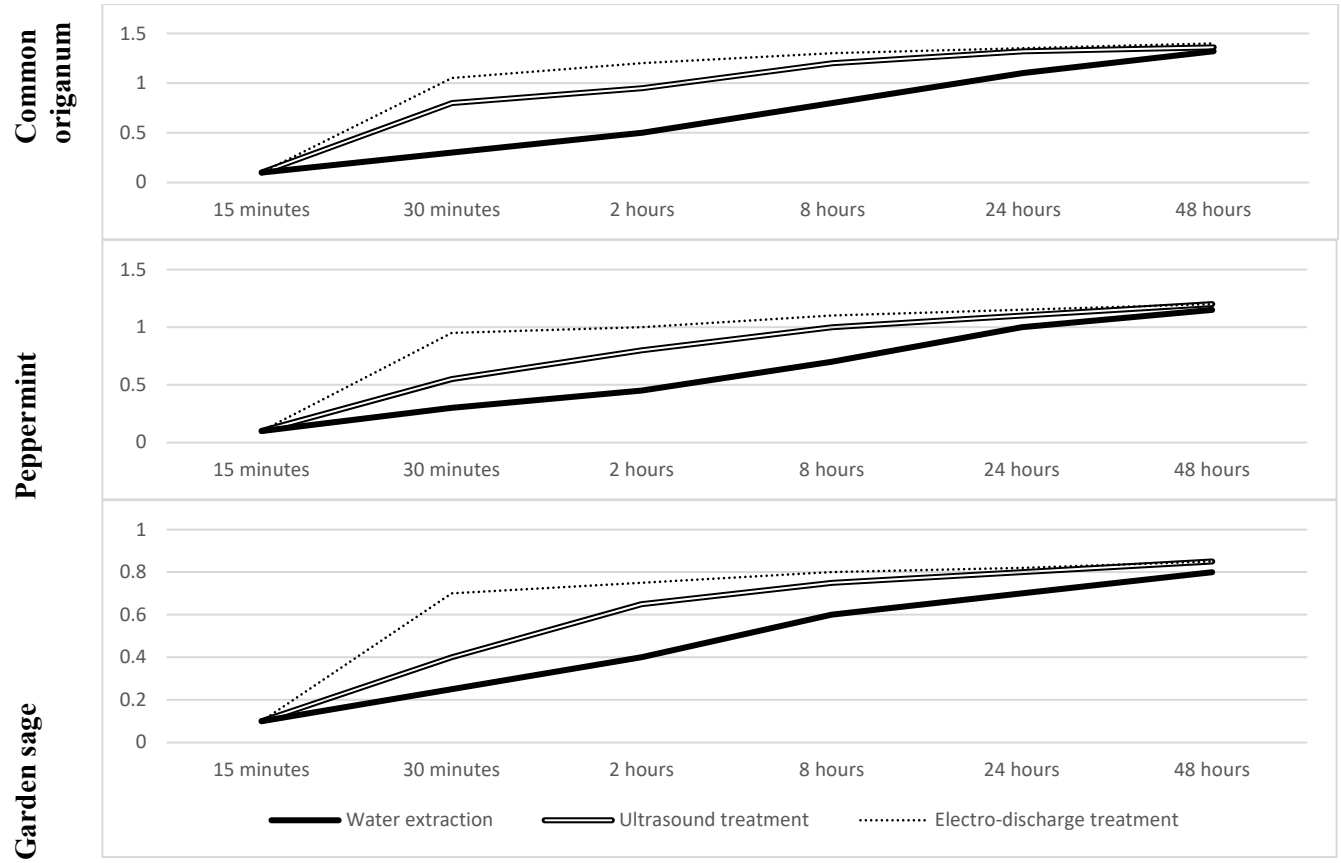

Figure 5 Organic acids content % ml of 0.1n NaOH in Common origanum, Peppermint and Garden sage.

The results of the conducted studies show a unified picture of the transition of useful substances into liquid extracts. So, after 48 hours, the maximum content of essential oils, flavonoids and organic acids is reached, while the indicators of water extracts are, on average, 7 – 10% lower than extracts obtained by electro-discharge and ultrasound treatments. At the same time, 80% of the maximum value is reached after 24 hours in the case of water extraction, after 2 hours with ultrasound treatment and after 30 minutes with electro-discharge treatment. Similar dynamics can be traced in the evaluation of all indicators.

The obtained extracts are largely enriched with useful micro- and macroelements and vitamins. They are promising to increase the body's resistance to adverse environmental factors **[32]**. In addition, the obtained extracts have a pleasant, harmonious taste and aroma, which means they can produce various food products **[33]**. Using modern technologies, such as ultrasound and electro-discharge extraction and producing extracts and biologically active additives will allow for more productive use of natural plant resources **[34]**. When extracted by different methods, the accumulation rate of organic acids in extracts varies **[35]**. This is explained by the fact that under the intense influence of ultrasound or electrical discharges on solid particles, strong turbulent currents and hydrodynamic micro-flows occur, contributing to substance mass transfer and dissolution **[36]**. Interestingly, these phenomena are observed both in the surrounding liquid and directly inside solid particles **[37]**. In electro-discharge treatment, a large amount of energy is accumulated in local areas, and then its instant release. These processes make it possible to intensify various chemical and technological processes. In particular, there is the appearance, development and explosion of cavitation regions, additional grinding of solid fractions, and the rupture of sorption bonds **[38]**. As confirmed by the studies of other authors, during electro-discharge treatment, water's redox potential may change, and various physical and chemical changes in the processed raw materials may occur **[39]**. Ultraviolet radiation that occurs during electro-discharge treatment has a detrimental effect on the microflora of the treated liquid, which, ultimately, allows to increase in the shelf life of the irradiated extract **[40]**. Table 2 shows the study results of dry substances in ready-made extracts of common origanum, peppermint and garden sage, depending on the extraction method. The maximum values are presented 48 hours after the start of extraction.

Table 2 Dry matter content in prepared extracts.

Method of extract production	Common origanum, %	Peppermint, %	Garden sage, %
Water extraction	2.87 ±0.15	2.95 ±0.15	4.25 ±0.22
Ultrasound treatment	3.07 ±0.16	3.15 ±0.16	4.53 ±0.23
Electro-discharge treatment	3.3 ±0.16	3.2 ±0.16	4.75 ±0.24

The following unique phenomena occurring during electro-discharge treatment of liquid have been experimentally identified and confirmed:

− Reduction of microbiological contamination of the liquid **[39]**;
− Softening of water, precipitation of calcium and magnesium salts **[39]**, **[40]**;
− Reduction of suspended solids concentration **[41]**;
− The transition of the value of the redox potential of water to a stable negative range of values **[42]**;
− Change of pH values towards the neutrality of the medium **[43]**.

Preparation of the extract using electro-discharge treatment allows you to obtain a high-quality extract within 30 minutes after the start of the experiment (15 minutes of soaking, 10 minutes of processing, and cooling). Ultrasonic exposure makes it possible to obtain a high-quality extract 2 hours after the start of the experiment (15 minutes of soaking, 30 minutes of processing, cooling and infusing). Thus, it can be concluded that the efficiency of ultrasonic and electric discharge extraction is unambiguous compared to the traditional technology of preparing an aqueous extract **[44]**.

The next stage of the study was a comparative analysis of the kinetics of the accumulation of biologically active substances in ready-made common origanum extracts (Table 3). The highest accumulation of extractive substances is observed in the third sample obtained using electric discharge technology. The lowest content is observed in the first sample obtained by classical extraction with an aqueous extract. The obtained conclusions are also confirmed by the results of other studies **[45]**.

Despite the obvious advantage of electro-discharge treatment over ultrasound, a number of disadvantages of this technology are also obvious. In particular, the energy costs for conducting experiments with electro-discharge treatment turned out to be almost 7 times higher than for ultrasonic treatment **[46]**. In addition, the electro-discharge treatment's light and sound accompaniment require a specially equipped room with high sound insulation properties **[46]**. The costs for the purchase and maintenance of electro-discharge equipment are 12-18 times higher than similar costs for the maintenance of an ultrasonic radiator **[47]**. In addition, electric discharge treatment requires a careful approach to choose the treatment mode due to the significant heating of the liquid **[48]**. And this means that food processing enterprises using electro-discharge equipment must have a technologist-adjuster in the staff of the main employees.

Table 3 Results of the study of the kinetics of accumulation of biologically active substances (p <0.05 for all groups).

No.	Characteristics of the main peaks		Relative intensity, %			Approximate content, grams per 100 g of sample		
	Weight	Gross formula	Water extraction	US treatment	ED treatment	Water extraction	US treatment	ED treatment
1	445.099	C19H17N409	8.84	8.85	35.72	0.95	1.43	2.11
2	383.1125	C21H1907	8.06	9.77	15.8	0.89	1.58	0.93
3	365.1033	C22H13N402	11.79	14.96	25.98	1.24	2.42	1.54
4	219.0264	C9H805Na	100	100	86.6	9.85	16.20	5.124
5	215.0162	C6H807Na	11	20	100	1.45	3.24	5.91
6	203.052	C6H1206Na	81.72	7.61	41.49	7.42	1.23	2.45
7	171.0992	C7H1603Na	13.35	9.6	1	1.21	1.5	0.06
8	156.042	C8H7NONa	12.17	5.84	5.41	1.14	0.95	0.32
9	140.0706	C7H10N02	13.11	9.31	14.32	1.23	1.51	0.85
10	118.0863	C5H12N02	14.31	9.41	36.29	1.22	1.52	2.15
11	104.107	C5H14NO	77.31	66.15	63.2	7.16	10.72	3.74

So, when using electro-discharge equipment, a well-chosen liquid treatment mode is particularly important, in which the environment will not be overheated **[49]**. It is known that an increase in temperature above 60°C is undesirable for aqueous plant extracts **[50]**. First, this is explained by the irreversible decomposition of biologically active substances that are part of extractive substances **[51]**. In addition, a significant part of essential oils is lost when heated **[52]**. It is known that the content of essential oils provides the finished extracts with antispasmodic, antifungal, antiviral, sedative, wound healing, toning, relaxing, anti-stress, soothing, hypo- or

hypertensive effects **[53]**. In addition, with significant heating, partial gelatinization of starch, and peptization of substances occurs **[54]**. Starch gelatinization is breaking the intermolecular bonds of starch molecules in the presence of water and heat, which allows the sites of hydrogen bonds to attract more water. In this case, starch granules are irreversibly dissolved in water. Water acts as a plasticizer **[55]**. The consistency of the extracts, in this case, becomes thicker and slimier and further work with them becomes much more difficult **[56]**.

CONCLUSION

Intensive exposure to ultrasound or electrical discharges on solid particles in a liquid leads to strong turbulent currents and hydrodynamic micro-flows promoting mass transfer and substance dissolution. As a result, intensive mixing of elements is achieved both in the total volume of the liquid and inside individual cells. Ultimately, this leads to an increase in the internal diffusion coefficient. The conducted studies have confirmed the effectiveness of electro-discharge extraction in comparison with ultrasound and comparison with water extraction. 48 hours after the start of the experiment, 7-15% more organic acids, flavonoids and essential oils were observed in extracts of the studied plants obtained after electro-discharge treatment than in water extracts. A similar dynamic can be traced in the assessment of all indicators. At the same time, 80% readiness of extracts in the case of electro-discharge treatment was observed already 30 minutes after the start of the experiment. Similar indicators (80% of the maximum) were achieved after 24 hours of water extraction and after 2 hours with ultrasound treatment. Thus, the electro-discharge treatment method proposed for extraction accelerates the time of obtaining the extract by 4 times compared to ultrasound. The dry matter content in the finished extracts also varied significantly (p <0.05). The finished extract of Common origanum as a result of electro-discharge treatment contained 3.3% of essential substances (ultrasound treatment – 3.07%, water extraction – 2.87%). As a result of electro-discharge treatment, Peppermint extract contained 3.2% of essential substances (ultrasound treatment – 3.15%, water extract – 2.95%). As a result of electro-discharge treatment, garden sage extract contained 4.75% of essential substances (ultrasound treatment – 4.53%, water extract – 4.25%). In addition to the high production speed of finished extracts and the higher quality of finished extracts, electro-discharge treatment has a detrimental effect on the microflora of the treated liquid. This ultimately allows you to increase the shelf life of the irradiated extract. The obvious drawbacks of electro-discharge treatment include increased energy consumption, high noise level, and the need to select the treatment mode that prevents excessive liquid overheating carefully.

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Utilization of different yield regulation methods of the vine for production of wines of higher designation protected of origin

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ABSTRACT

Grape yield regulation is a method used to improve grape quality parameters. Experiments were carried out in 2021 on the grapevine (*Vitis vinifera* L.) wine varieties 'Feteasca regala' and 'Sauvignon blanc', focusing on the effect of two different methods of grape yield regulation on its selected parameters and must sugar content. The first method used was cluster thinning, leaving one bunch on the shoot. The next method used was cluster tipping when we removed the terminal part of each bunch. Yield reduction was carried out in the period between pea-sized berry phenophase (BBCH 75) and bunch closure phenophase (BBCH 77). The operations were carried out manually. Cluster thinning did not lead to a statistically significant difference in bunch weight compared to the control in any of the studied varieties. We observed a statistically significant ($p < 0.05$) decrease in the average bunch weight in the variant cluster tipping. The 'Feteasca regala' hectare yield was 32.25% lower in the cluster thinning than the control. The hectare yield in the cluster thinning variant was reduced by 46.61% compared with the control. Cluster thinning variant of the Sauvignon blanc variety had a 19.13% lower yield than the control variant. The cluster tipping variant had a 29.03% lower yield than the control variant. In the case of the cluster thinning method, we observed a greater decrease in grape yield compared to the cluster tipping method. The obtained results indicate that cluster tipping method is preferable to the cluster thinning in terms of the profitability of grape production. The must sugar content was statistically significantly ($p < 0.05$) increased in all the yield reduction variants. The variety 'Feteasca regala' had the highest sugar content of the must in the cluster thinning method, 19.42 kg/hL. The highest sugar content of 'Sauvignon blanc' was 21.33 kg/hL in the variant with cluster tipping. This shows that regulating the grape yield can improve the quality parameters of the grapes. On the other hand, it may lead to a decrease in yield per hectare below the break-even point. The justness and intensity of the method used must be carefully considered.

Keywords: Grapevine, Feteasca regala, Sauvignon blanc, Yield regulation, Sugar content

INTRODUCTION

Several factors influence the quality of grapes. It is mostly shaped by the variety's genotype, the site's environmental conditions and the used agro technique **[1]**. Low night temperatures can positively influence grape quality during ripening, which commonly occurs in cooler viticultural areas during grape ripening **[2]**. By yield reduction, we can positively influence the ripening of grapes and create the conditions for improving their qualitative parameters. Although we will lose a significant part of the harvest, giving up part of the yield in years with high grape yields is sometimes a necessity. For the production of quality wines, there are national limits on the maximum yield per hectare for the production of quality wines, which can be resolved by reducing the yield. In order to make viticulture economically efficient, we must keep the vineyard fully engaged and apply modern cultivation technologies. The selection of a suitable site, the choice of the right rootstock-variety combination and the implementation of adequate agrotechnical measures are also important **[3]**.

Grape yield control is a technique mainly used in wine-growing countries with cooler climates. Cooler climates provide fewer days for the crop to ripen sufficiently, so it is advisable to promote optimal ripening of the grapes **[4]**. Mawdsley et al. **[5]** argue that the effect of grape yield regulation is always weaker than that of climatic conditions during vegetation. Yield regulation can positively prevent some fungal diseases and reduce the severity of disease, especially *Botrytis cinerea*. The given fact relates to a looser arrangement of berries in the bunch, especially when using a cluster tipping method **[6]**, **[7]**. A smaller grape crop improves grape ripening **[8-10]**. It can improve grape quality and wine quality parameters **[11]**.

Sugar content belongs to the main quality parameters of grape. It is a changeable parameter with a less stable spatio-temporal progression **[12]**. Sugars are the basic elements of alcoholic fermentation. The two dominant sugars in grape berries are glucose and fructose. Simultaneously, there is a low concentration of sucrose, maltose, xylose and other sugars **[13]**. As grape berries ripen, sucrose from the leaves accumulates as glucose and fructose in the berry vacuoles **[14]**. Most studies have confirmed in yield-controlled variants higher sugar content and lower titratable acidity in must, increased content of polyphenolic compounds and increased intensity of berry colour **[9]**, **[15]**, **[17]**. One of the benefits may also be an increase in the total phenolic content of the grapes or berry skin **[18]**, **[19]**. Grapes from bush-regulated variants have higher antioxidant activity **[20]**, generally associated with higher polyphenol content **[21]**. Condurso et al. **[22]** observed a positive effect of grape yield regulation on the quantity and species composition of aromatic compounds in wine.

Important aspects of grape yield reduction are the intensity of the intervention and the timing of the intervention. The intensity of the intervention is directly related to the economics of vine cultivation. Strong yield control can lead to more significant benefits in the quality composition of the grapes. On the other hand, increasing the intensity of grape regulation reduces the quantity of grapes produced, which can lead to lower profitability. An intervention implemented at different stages of berry development may affect some quality parameters of grapes **[23-25]**. When choosing the right date for regulating grape reduction, we have to base our decision on the dynamics of berry growth. The most significant berry growth is observed 30-40 days after fertilization of the inflorescences. If we intervene in this period, all the assimilates are directed to the clusters left on the vine. The intervention results are the increase in berry size and cluster density, which, although it increases the overall yield per cluster, reduces the overall quality and health of the clusters. The most suitable period for yield control is the stage between the phenophase of pea-sized berries and berry softening **[26]**. Too radical yield regulation can negatively affect the wine's character. Yield reduction in a vineyard whose growth is very strong leads to high content of IBMP (3-isobutyl-2-methoxypyrazine), which is a manifestation of an imbalanced state of the bush **[27]**.

We aimed to prove the validity of performing grape yield regulation by experiments.

Scientific Hypothesis

In our study, we investigate hypothesis:

a. Grape yield regulation (cluster thinning, cluster tipping) reduces the yield.
b. A smaller quantity of yield can lead to the improvement of wine grape qualitative parameter as sugar content.

MATERIAL AND METHODOLOGY

Samples

The experiments were held in the wine-growing village Vráble, belonging to the Nitra wine-growing region. The planting was established in 2006. The main soil unit in the vineyard site is pseudo glacial brown earth. The vineyard is cultivated with full black fallow and is managed on the Rhine-Hesse line with 1 stalk. The row spacing is 1.2 m, the rows are spaced 1.5 m apart. The pruning of the fruiting wood follows the Guyot pruning principle, leaving one 10-bud and a 2-bud reserve trunnion. The varieties 'Feteasca regala' and 'Sauvignon blanc' grafted on rootstock SO4 were used in the experiment.

- Location: Nitra wine-growing region, Vráble wine-growing district, Vráble wine-growing village.
- Varieties: Variety I: Feteasca regala (FR) – Romanian white wine grape variety with middle-sized clusters and thin berry skin; Variety II: Sauvignon blanc (SB) – French white wine grape variety with small to medium-sized compact clusters and hard berry skin **[38]**.

Chemicals

No chemicals were used for the experiment.

Instruments

Laboratory scales EMB 6000-1 (Kern, Germany).

Laboratory Methods

Cluster weight (g): The weight of the clusters was measured using laboratory scales.

Grape yield (g/bush): Grape yield was calculated by multiplying the cluster weight by the number of bunches on the vine.

Hectare yield (t/ha): We calculated the hectare yield by the conjunction of the grape yield and the number of grapevines present on an area of 1 ha (1):

$$10000/(1.2 \times 1.5) = \text{number of bushes per 1 ha} \times \text{grape yield per vine} = \text{yield per ha} \quad (1)$$

Sugar content of must (kg/hL): To determine the sugar content of the must we used a Czechoslovak standardized must meter according to STN 25 7621.

Estimated yield (EUR): We calculate the yield per hectare using an economic formula (2):

$$\text{Yield} = \text{yield (kg/ha)} \times \text{realisation price (EUR/kg)} \quad (2)$$

Description of the Experiment

Number of samples analyzed: 6

Number of repeated analyses: 18

Number of experiment replication: 1

Design of the experiment: In 2021, the growing season was 40 days shorter than the long-term normal **[28]**. The cold beginning of the growing season caused a delayed onset of budding and flowering of the vines. Due to the high temperatures above the long-term average in the summer months, grape ripening was not delayed. The year 2021 was below average annual precipitation. The cumulative sum of sunshine duration reached normal values during the grape ripening period **[29]**, **[30]**. On 15th July, a severe hailstorm occurred in the study area, damaging developing grapes and causing an increased incidence of berry rot. During the experiment, we observed the effect of two different methods of grape yield reduction (cluster thinning, cluster tipping) on selected parameters. In each variant, we treated 15 grapevines. The interventions were carried out on 8th August 2021, between the phenophases of pea-sized berries (BBCH 75) and bunch closure (BBCH 77). The cluster thinning method consisted of leaving one bunch on the shoot. In the case of the cluster tipping, we did not remove any bunches, but we shortened all bunches by about half their length. We harvested a grape on 28th September 2021.

A B

Figure 1 Example of yield regulation of grapevines variety SB, before (A) and after (B) yield regulation - removal of whole bunches

Statistical Analysis

The statistical program XLSTAT v.2021.4.1 (Addinsoft, France) was used for analyses of the obtained data. The Shapiro-Wilk test was used to distribute the data at the statistical significance level of $p = 0.05$. ANOVA – Tukey test was used to test whether there was a statistically significant difference between the samples ($p = 0.05$).

RESULTS AND DISCUSSION

Cluster weight (g)

The average cluster weight in the control variant of 'Feteasca regala' was 119.7 g. In the variant with cluster thinning, we measured an average cluster weight of 120.9 g. In this intervention, we did not remove parts of the bunches. Therefore, the average cluster weight is almost identical to the control. In the case of cluster tipping, we observed a decrease in the average cluster weight to 85.1 g. The difference between the variant with cluster tipping and the control variant is statistically significant (p <0.05).

In the control variant of the 'Sauvignon blanc' variety, we measured an average bunch weight of 175.5 g. This cluster weight is well above average compared to the ampelographic characterisation of the variety **[35],** and the difference represents an increase of up to 62.25 %. In the whole bunch removal variant, we measured an average cluster weight of 180.2 g. This average cluster weight confirms the high above-average nature of this parameter in the experiment. Cluster tipping reduced the average cluster weight to 116.5 g. A decrease in cluster weight in a cluster tipping variant was statistically significant (p <0.05) compared to the control.

Table 1 Average cluster weight (g).

Sample	Mean ±SD	Min	Max	CV (%)
FR K	119.7 ±18.3a	93	145	15.3
FR A	120.9 ±17.6a	96	141	14.5
FR B	85.1 ±19.9b	59	117	23.4
SB K	175.5 ±33.8a	126	234	19.3
SB A	180.2 ±28.6a	130	219	15.9
SB B	116.5 ±21.6b	71	139	18.5

Note: FR – Feteasca regala, SB – Sauvignon blanc, K – control, A – whole bunch removal, B – bunching, SD – standard deviation, Min – minimum, Max – maximum, CV – coefficient of variation; a, b means rows with different letter are statistically different (Tukey test, p <0.05).

Grape yield (g/bush) and hectare yield (t/ha)

Feteasca regala

The highest yield for the variety 'Feteasca regala' was found, as expected, in the control variant, at 2234.0 g/bush with a hectare yield of 10.14 t/ha. In the cluster thinning variant, we recorded an average yield of 1798.0 g/bush of grapes with a hectare yield of 8.2 t/ha, which is 19.13% lower average hectare yield than in the control variant. In bunching, one-third to one-half of the length of the bunch is removed. As we remove the lower part, which is less voluminous, while giving room for the development of the berries left behind, the reduction in yield may not be more than one-third of that of the untreated variant. The lowest yield was recorded for the variant with one bunch per shoot, i.e. 1431.5 g/bush. This value represents a reduction of 32.25% compared to the control variant. The differences between the variant with the cluster thinning variant and the control variant were statistically significant (p <0.05). The yield decrease for the cluster tipping variant was not statistically significant (p >0.05) compared to the control variant.

Sauvignon blanc

In the case of 'Sauvignon blanc', we found the highest yield in the control variant, namely 2687.5 g/bush, giving a yield per hectare of 11.85 t/ha. The second highest grape yield and yield per hectare were recorded in the bunching variant, with 1561.0 g/bush of grapes with 8.41 t/ha as a yield per hectare. This means a yield reduction of 29.03% compared to the control variant. Removing whole bunches decreased the average yield of grapes to 1025.0 g/bush with 6.35 t/ha as a yield per hectare. This is almost half the decrease compared to the control variant; therefore, the profitability of such an intervention may already be negatively affected. The differences between the experimental and control variants were statistically significant (p <0.05).

Table 2 Average grape yield (g/bush).

Sample	Mean ±SD	Min	Max	CV (%)
FR K	2234.0 ±172.5a	2112	2356	6.3
FR A	1431.5 ±58.7b	1390	1473	22.2
FR B	1798.0 ±250.3ab	1621	1975	2.6
SB K	2687.5 ±170.4a	2567	2808	7.7
SB A	1025.0 ±227.7b	864	1186	4.1
SB B	1561.0 ±41.0b	1532	1590	13.9

Note: FR – Feteasca regala, SB – Sauvignon blanc, K – control, A – removal of whole bunches, B – bunching, SD – standard deviation, Min – minimum, Max – maximum, CV – coefficient of variation; a, b means that rows with a different letter are statistically different (Tukey test, p <0.05).

Table 3 Average grape yield per hectare (t/ha).

Sample	Yield per hectare	Yield decrease (%)	
FR K	10.14	-	-
FR A	6.87	3.27	32.25
FR B	8.20	1.94	19.13
SB K	11.85	-	-
SB A	6.35	5.50	46.61
SB B	8.41	3.44	29.03

Note: FR – Feteasca regala, SB – Sauvignon blanc, K – control, A – removal of whole bunches, B – bunching, SD – standard deviation, Min – minimum, Max – maximum, CV – coefficient of variation; a, b means that rows with a different letter are statistically different (Tukey test, p <0.05).

Sugar content of must (kg/hL)

In all variants studied, we found statistically significant differences (p <0.05) in must sugar content between the yield reduction variants and control variants. The lowest sugar content was measured in the control variant in all measurements. The control variant of 'Feteasca regala' had an average sugar content of 18.17 kg/hL, in the control variant of 'Sauvignon blanc' we measured an average must sugar content of 19.67 kg/hL. In the whole cluster removal variant of 'Feteasca regala' we measured a sugar content of 19.42 kg/hL. The 'Sauvignon blanc' variety had a must sugar content of 20.33 kg/hL in the whole cluster removal variant. A cluster tipping of 'Feteasca regala' led to an increase in the average sugar content to 19.00 kg/hL. The 'Sauvignon blanc' variety had a must sugar content of 21.33 kg/hL in the tipping variant.

Table 4 Sugar content in must (kg/hL)

Sample	Mean ± SD	Min	Max	CV (%)
FR K	18.17 ± 0,29a	18.00	18.50	1.59
FR A	19.42 ± 0,14b	19.25	19.50	0.74
FR B	19.00 ± 0,50ab	18.50	19.50	2.63
SB K	19.67 ± 0,29a	19.50	20.00	1.47
SB A	20.33 ± 0,29b	20.00	20.50	1.42
SB B	21.33 ± 0,29b	21.00	21.50	1.35

Note: FR – Feteasca regala, SB – Sauvignon blanc, K – control, A – removal of whole bunches, B – bunching, SD – standard deviation, Min – minimum, Max – maximum, CV – coefficient of variation; a, b means that rows with a different letter are statistically different (Tukey test, p <0.05).

Estimated yield (EUR)

Yield reduction decreases the revenue by the labour costs necessary for its implementation, which amount to 0.05 €/bush. At the same time, the quantity of grapes is reduced. On the other hand, it is a good way of increasing the quality of the grapes, which can increase their price. In order to achieve a yield analogous to that of the control variant, we are forced to increase the realisation price of the grapes in the case of the variants with yield reduction.

At a grape price of 0.50 €/kg, the revenue per hectare in the control variant FR K would be 5 070 € . In order to achieve the same yield per hectare, we would have to thinning clusters at a price of 0.78 €/kg in the FR A variant. In FR B, we would have to increase the realisation price of the grapes to 0.65 €/kg. In the control variant SB K, at a grape price of 0.50 €/kg, we would obtain a revenue per hectare of 5 925 €. We would have to increase

the grape price to 0.98 €/kg to achieve the same yield in SB A. In variant SB B, we would need to increase the realisation price of grapes to 0.74 €/kg.

Our aim was to prove the validity of performing grape yield regulation by experiments. The cluster weight in our experiment did not change demonstrably in variant of cluster thinning. Other authors have also come to similar findings on the different varieties **[31]**. In an experiment, Zhuang et al. **[32]** found that removal of whole bunches in different years may lead to a different decrease in bunch weight. We observed lower cluster weight in the case of cluster tipping compared to the control. Our finding is contradicted by studies in which the effect of subsequent berry enlargement increased bunch weight to a level analogous to the control or in some cases surpassed this weight **[33]**. Authors have reported an increase in the berry weight of tipping bunches **[34]**, **[35]**, **[36]**, **[37]**.

Pospíšilová et al. **[38]** quantify the average hectare yield of 'Feteasca regala' at 10 tons or more, with which the observed yield of 10.14 t/ha fully corresponds. For the variety 'Sauvignon blanc', the author quotes an average yield of 8.50 t/ha, whereas the yield of 11.85 t/ha calculated by us is significantly higher than this value. The above-average yield in the control variant may be related to the high number of grape vines per ha (up to 5 5554), the above-average rainfall during intense berry growth, and the high water-holding capacity of the soil in the locality.

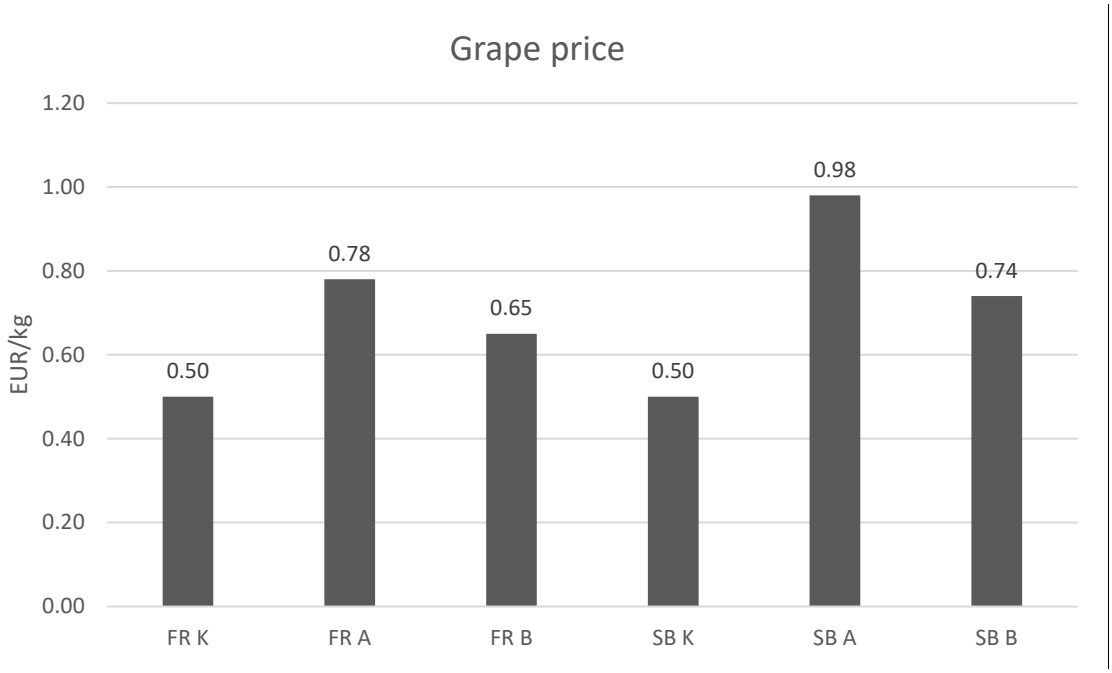

Figure 2 Graphical representation of the realisation prices of the grapes needed to achieve the same yield as the control. Note: FR – Feteasca regala, SB – Sauvignon blanc, K – control, A – removal of whole bunches, B – bunching.

The regulation of the grape yield usually leads to a decline in yield. Kok **[31]** investigated the effect of cluster thinning on selected parameters of the cultivar 'Sauvignon blanc'. A cluster weight was not demonstrably affected, but in the case of yield, there was a decrease of 37.50 %, which is analogous to the value we measured. Mančík (2017) **[39]** in his experiment evaluate the effect of cluster tipping on the quantitative and qualitative parameters of the cultivar 'Erilon'. Compared to the control, cluster tipping reduced yield by 39.80%. This decrease is significantly higher than in our case, with a 19.13 % decline in yield per hectare for 'Feteasca regala' and a 29.03 % decline in yield per hectare for 'Sauvigon blanc'. Our results indicate that the response of the varieties to the yield reduction is different, which may partly explain the differences in our results. The difference in sugar content of grapes measured by refractometer between the control and the cluster tipping variant is reported by the Mančík **[39]** to be only 0.35 kg/hL, which is less than the difference we measured in our experiment.

A yield decrease was also observed by Ruffo Roberto et al. **[7]** in an experiment with the cultivar 'BRS Vitoria' under the cluster thinning method. Fazekas et al. **[40]** in the cultivar 'Kékfrankos' recorded a yield decrease, but a greater yield decline was caused by cluster tipping when compared to cluster thinning. In our results, cluster tipping caused a smaller yield decrease than cluster thinning, which is explained by the different intensities of regulation.

On the other hand, many authors have obtained an inconclusive difference in yield of the studied variants compared to the control **[35]**, **[37]**, **[41]**. Some authors reported a slight increase in yield after grape yield reduction **[34]**, **[36]**. The increase in yield may be due to the low intensity of grape regulation in conjunction with the

bunching method. Another reason for the increase may be the implementation of the intervention at an earlier date and the subsequent response of vine by compensating for the lost part of the bunches in the form of an increase in berry volume and weight.

Sedlo **[42]** argues that there is no correlation between average sugar content and profitability. The vine grower is paid primarily for the grapes' quantity, not their sugar content. The situation is different if he decides to produce high-quality wines. In that case, a certain level of the sugar content of the grapes must be achieved, and the maximum hectare yield must not be exceeded. The author further reports that harvests below 5.5 t are not sufficiently profitable. We conclude that the interventions we have carried out can make production profitable despite the reduction in the hectare yield.

We found a statistically significant (p <0.05) increase in the total sugar content in all the studied variants. The conclusions of other authors are different. On the one hand, some authors of studies have reported an increase in sugar content **[4]**, **[8]**, **[11]**, **[34]**, **[43]**, **[45]**, **[46]**. On the other hand, many studies did not observe significant differences in the content of total sugars or an increase in °BX **[7]**, **[18]**, **[46]**. The ambiguous results may be due to the method used, the intensity of yield reduction, and the date of intervention, but also to varietal variability and vineyard vintage.

Říhová **[47]** describes an experiment in the Krasnodar region of Russia, where the effect of defoliation and yield regulation on must and wine quality was studied. The used varieties were 'Merlot', 'Cabernet Sauvignon' and 'Syrah'. The measures positively affected the sugar and alcohol content of the wine. We can agree with this study since we observed a positive effect of yield reduction on sugar content in our experiment.

De Barros et al. **[9]** investigated the effect of bunch yield reduction on the quality of 'Malbec' grapes. The experiment was carried out in Brazil. In the experimental plots, grape ripening improved after the intervention. The polyphenol content of the berries increased. First, the experiment took place in different climatic conditions to those of central Europe. Nevertheless, we can confirm some of the claims. In the grape yield reduction variants we have studied, grapes ripened better.

Pavloušek **[26]** argues that the cluster tipping method suits blue wine varieties. We applied to bunch to white wine varieties, and the results of this intervention suggest that cluster tipping may be a suitable method for yield reduction in this group of varieties as well.

CONCLUSION

In this work, we evaluated the effect of grape yield regulation of wine grape varieties on its selected parameters. We did not find statistically significant (p >0.05) changes in cluster weight in the sample of cluster thinning compared to the control. As expected, we observed a statistically significant (p <0.05) decrease in the case of cluster tipping, but the number of clusters on the bush compensated it. Our measurements showed statistically significant (p <0.05) differences in grape yield between the control and the regulated variants. The obtained results clearly show a decrease in yield in the case of the regulated variants. The statistically significant (*the calculated hectare yields of grape also confirmed p <0.05) decrease in yield*. A regulation of grape yield led to an increase in must sugar content compared with the control variant. The differences were statistically significant (p <0.05) in all the studied variants. The decrease in the quantity of grapes after the yield regulation and the increase in labour costs necessary for its implementation should be compensated for in the form of an increase in the realisation price of grapes. Based on the results obtained, it can be concluded that the regulation of the yield is important in improving its quality parameters. In order to confirm our results, it is necessary to conduct experiments over several vintages. The removal of whole bunches and bunching positively affected the sugar content of the must. After evaluating one growing year, we cannot determine which method of yield reduction is more suitable for the varieties we studied. Each variety responds differently to yield regulation, and the viticulturist must consider not only the improvement in grape quality parameters achieved but, above all, the profitability of the intervention. The most appropriate way to achieve profitability after the yield regulation has been regulated is to produce wines in the category of wines of higher protected designation of origin.

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

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Mathematical modelling of quality assessment of cooked sausages with the addition of vegetable additives

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ABSTRACT

We studied the physicochemical composition and functional and technological properties of plant additives - wheat fibre with pumpkin pectin (WFwPP). The nutritional value of cooked sausages was increased when fibre was added to the recipe. We have replaced fatty pork meat with up to 5% WFwPP. Supplementation with fibre improves product digestibility "in vitro". We have used mathematical modelling, linear, flat, and spatial estimation models developed in a radial scheme, polygon, and polyhedron to optimize the content of essential amino acids. We have developed a new recipe for the composition of cooked sausage with wheat fibre and pumpkin pectin with the optimal proportion of main ingredients: beef grade I – 30%, fatty pork – 50%, WFwPP – 5%, water. Compared to the control sample, the finished product's organoleptic characteristics improved. The basis of the mathematical model for assessing the quality of the developed cooked sausage with wheat fibre and pumpkin pectin was chosen flat model of the polygon, taking into account the time of preservation of product quality, which was assessed as a result of regression analysis. The quality assessment results of the developed products using a computer program for calculating the area of quality profiles with subsequent graphical visualization are consistent with the organoleptic studies, which confirms the reliability of the results and the adequacy of the developed mathematical model.

Keywords: herbal supplement, pectin, chemical composition, biological value, quality indicators, shelf life

INTRODUCTION

One of the most promising ways to meet the human body's physiological needs and vital functions is the production of food products of high biological value, including sausages. Therefore, the relevance of finding new approaches to forming consumer characteristics in cooked sausage recipes through using new ingredients, namely fibre with pumpkin pectin, valuable for functional and technological characteristics, is obvious **[1]**.

It is determined that an effective and promising direction of individual protection of the population from the accumulation of radionuclides in the body is the use of dietary fibre and substances of natural origin that do not have side effects on the body and show a pronounced radioprotective effect **[2]**. Such substances, in particular, include pectin – organic compounds that have the property of forming gels in the presence of organic acids and sugars **[3]**, **[4]**; able to form insoluble complexes with metals in the digestive tract, which are not absorbed but excreted from the body. Food pectin is used as a filler for dairy, confectionery, and meat products, which has many valuable properties of therapeutic and protective action **[5]**, **[6]**. In Germany, boiled sausages are also produced with various herbal additives, allowing the final product to form with a delicate aroma of spicy plants, paprika, etc. **[7]**.

A promising direction in the meat industry is producing products that combine meat and herbal additives capable of biological and nutritional value regulations, taste conservation, and help to balance nutrition to avoid

obesity, heart disease, and allergies **[8]**, **[9]**. Thus, the study of the properties of cooked sausages with the addition of wheat fibre enriched with pectin pumpkin, determining the effective structural composition of the product and its evaluation, is the relevance of the analysis.

This scientific work aims to substantiate the structural composition of newly cooked sausages using wheat fibre with pumpkin pectin by conducting organoleptic evaluation and mathematical modelling of quality assessment of developed products **[10]**. To achieve this goal, it is necessary to solve the following tasks: to systematize mathematical models for assessing the quality of the studied products depending on such features as clarity of presentation, depth and volume of information, possible branching of its flows; based on experimental evaluation of the nutritional and biological value of new recipes for cooked sausages to identify the rational content of additives with wheat fibre and pumpkin pectin (WFaPP).

Scientific hypothesis

An increase follows the development of mathematical models for assessing product quality in quantitative and qualitative characteristics of the studied process, which can be reflected with a high degree of clarity by building linear, planar, and spatial models. It is possible to show the patterns of change of each evaluation parameter over time.

MATERIAL AND METHODOLOGY

Samples

The following were used for experimental research:

• grade I boneless beef, in which muscle tissue with a mass fraction of connective and fatty tissue does not exceed 6%, producer Agro firm "Polyssia LTD", Kyiv region, Ukraine;

• boneless semi-fat pork, in which muscle tissue with a mass fraction of fatty tissue from 50% to 85%, producer Agro firm "Polyssia LTD", Kyiv region, Ukraine;

• wheat fibre "Poltermyshung Roth Superior" LLC "TD Lagis" with pumpkin pectin from LLC "Garbuz LTD", country of manufacture Ukraine – according to the product specification, table salt according to DSTU 3583:2015 **[11]**, containing 20.0% pumpkin pectin and 80.0% crushed wheat bran;

• multi-component minced meat, the composition of which includes de-veined semi-fat pork, grade I beef with a vegetable additive in the ratio of 3, 5, 7%;

• white sugar according to DSTU 4623:2006 **[12]**;

• sodium nitrite according to GOST 32781:2014 **[13]**.

Cooked Okrema sausage DSTU 4436:2005 **[14]** was used as a control sample.

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 and Biological Material

The meat of bulls obtained after slaughter under the age of 12 months and the meat of pigs under the age of 9 months were selected for research (Agro firm "Polyssia LTD", Kyiv region, Ukraine). Enzyme preparation of plant origin - "in vitro", (LLC "Alex", Kyiv, Ukraine)

Instruments

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 indicator characterizing the chemical composition of sausage products was determined according to standard methods:

• the mass fraction of moisture was determined by the drying method according to DSTU ISO 1442:2005 **[13]**;

• fat content was determined by the Soxhlet method according to DSTU 8380:2015 **[14]**;

• the proportion of protein was determined by the Kjeldahl method **[50]**;

• the degree of ashing using the Velp Scientifica DK6 device to determine the mass fraction of ash. The weight method was used according to DSTU ISO 936:2008 **[15]**.

• the study of active acidity was carried out by determining the pH according to DSTU ISO 2917:2001 **[16]**.

• mass fraction of protein - according to the Lowry method when using Folin's reagent, as a result of which a compound is formed that gives a blue colour to the protein solution **[17]**;

• fibre content - by the weight method of Kürschner and Hanek when boiling a portion of the product with an acid mixture in a reflux flask followed by filtering the solution and washing the precipitate with a hot acid mixture, water, and ether **[18]**;

• the content of pectin substances - by the titrimetric method, which is based on alkali titration of previously selected and prepared pectin substances before and after hydrolysis; • amino acid composition - by the method of ion exchange chromatography **[19]**;

• the moisture-binding capacity of product samples - by the Grau-Hamm press method in the modification of V. Volovynska and B. Kelman **[20]**;

• plasticity of minced meat - by the method of pressing according to the area of the meat stain on filter paper; content of micro toxin patulin - by liquid chromatography with spectrophotometric detection **[21]**;

• content of heavy metals - by atomic absorption method **[51]**;

• the content of radionuclides - by the gamma spectrometric method.

• the temperature of the samples was determined using a TH310 Milwaukee thermometer.

• samples were weighed using AXIS BDM 3 scales.

Description of the Experiment

Sample preparation: Samples were selected and prepared according to DSTU 7992:2015 **[22]**. On whole (uncut) sausage products, all samples' appearance, colour, and surface condition met the requirements of regulatory documents. The polyamide shell was dry, strong, and elastic, without mucus stains and mould deposits, without damage, which tightly adhered to the minced meat. The consistency when pressing with a finger on the surface of the sausages was dense. Sausage products cut into thin pieces were characterized by the following indicators: the appearance and pattern on the cut were typical for cooked sausages in samples with 1%, 3%, and 5% of the studied vegetable additive. In the process of preparing suspensions and emulsions, a weight of 2 g containing 0.5 g of water or vegetable oil, 1 g of the drug was composed; mixed to a homogeneous consistency, and transferred to glass centrifuges with a volume of 30 ml; placed in a thermostat with temperature t = 74 °C – 76 °C; kept for 15 minutes; the tubes were cooled with cold water to room temperature and centrifuged in an OPN centrifuge at 1500 rpm for 15 min. Microbiological studies of products were carried out on finished products immediately after preparation, as well as after 4, 8, 10, 12, and 15 days of storage in the refrigerator (+0 °C – +6 °C). The swelling coefficient was determined when the mesh was placed in water or oil for 15 minutes, after which it was removed, and the liquid was allowed to drain for 20 minutes and weighed.

Number of samples analyzed: Four types of sausages with different moisture content and shelf life were used to study samples.

Number of repeated analyses: The study was repeated 5 times, with the experimental data processed using mathematical statistics.

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, a study of the physicochemical parameters of the studied herbal supplement was carried out, namely, the amino acid composition of its proteins and mineral and vitamin composition. Based on the obtained indicators, the energy value, coefficients: protein, protein-water, fat-water, nutritional saturation, potential biological value, and the essential amino acid composition of the protein were calculated; as an indicator of excess content and index of essential amino acids. In the second stage, the development and substantiation of the technology of boiled sausages were carried out. The third research stage characterised finished cooked sausages according to organoleptic, physicochemical, biochemical, microbiological, and rheological indicators. The experimental results were conducted using the mathematical model of the polygon according to the characteristics presented in Figure 1.

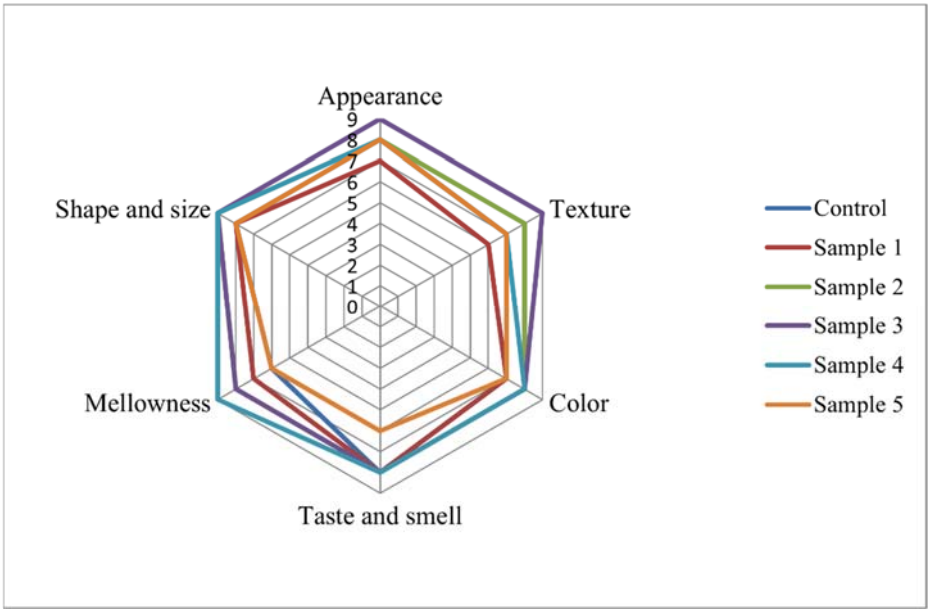


Figure 1 Organoleptic evaluation of recipes for cooked sausages with different amounts WFAPP: No. 1 – 1 %; No. 2 – 3 %; No. 3 – 5 %; No. 4 – 7 %; No. 5 – 9 %.

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. 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

In developing and substantiating the mathematical model for assessing the quality of cooked sausages with the proposed ingredients, the following main properties of it were used clarity of presentation, depth and volume of information, and possible branching of its flows. Similar experiments are described in many scientific works, but they were conducted to evaluate the quality indicators of dairy products **[23]**, bakery products **[24]**, and canned meat **[25]** with a long shelf life. Linear, flat, and spatial estimation models were developed according to such evaluation criteria.

In the linear beam model, the parameter l_0 corresponds in value to the reference indicator of the quality of this product, which the maximum possible units can conditionally represent. The values $l_i = x_i = l_0 \cos\alpha_i$ correspond to the values of accurate production indicators according to the selected evaluation criterion or for the family of indicators X_i derived from the main reference. In several scientific papers, which are related to the modelling processes related to the production of sausage products, various models are presented, in which up to 10 main parameters were used **[26]** without taking into account reference indicators or only one reference indicator **[27]**, due to which the reliability of the results, which were obtained rather difficult to verify. The authors of the paper **[28]** carried out a modelling process where only 2 parameters were used. The reliability of such results may be quite imprecise.

In the presence of two prevailing or basic quality indicators, it is convenient to use a radial flat model in which reference indicators are presented on coordinate axes x, and y. When the number of prevailing or basic quality indicators is three, it is convenient to use a radial spatial model (Figure 2), in which the values of real indicators according to the selected evaluation criteria correspond to the families of indicators X_i, Y_i, and Z_i, which are derived from the main reference parameters x, y, z and are estimated at up to 10 units. Similarly, for this scheme, as a criterion for evaluating the radial spatial model, it is advisable to choose the following parameters.

$$\begin{cases} k_x = \dfrac{IL\cos I \pm_i}{n} \\ k_y = \dfrac{IL\cos I?_i}{n} \\ k_z = \dfrac{IL\cos Il_i}{n} \end{cases} \quad (1)$$

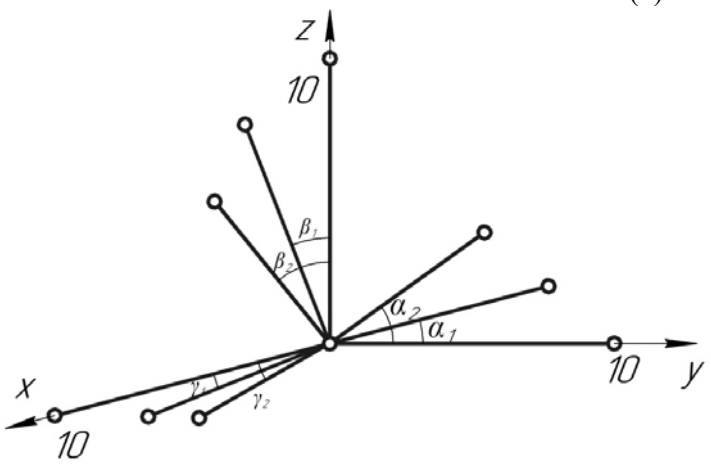


Figure 2 Radiation spatial model of product quality.

Features of the developed radial models of product quality are:
- effective assessment of extensive methods of quality assessment;
- it is advisable to evaluate up to 3 methods;
- requires large enough arrays of experimental research;
- ease of assessment.

When the rays of the shades, which reflect the prevailing or basic indicators of product quality, are set aside from a single centre, the boundary points form a shape in the form of a polygon. The number of angles in this figure shows the reference parameters used to assess the quality of certain products. Similar attempts to conduct a similar type of modelling are described in several manuscripts, but in our opinion, they had several shortcomings; in one case, only the parameters of the standard were taken into account **[29]**, and in the next series of experiments organoleptic evaluation was not carried out **[30]**, and in the third case, the physical and chemical composition was investigated. Only one reference parameter was taken into account **[31]**.

For simplifying the geometric construction of such figures, it is advisable to conduct them depending on the even (Figure 3 a) or odd (Figure 3 b) number of angles. In the case of estimating the basic reference parameters by a certain equal value, for example, up to 10 units, the constructed regular polygon corresponds to the ideal product; if to achieve the "ideal" it is enough to use a given number of reference parameters. Estimation of similar parameters using different values, for example, from 1 to 6 with a step of 2 units **[32]**, from 1 to 4 with a step of 2 units **[33]**, and from 1 to 3 with a step of 1 **[34]**, and also build irregular polygons cannot correspond to perfect products. One cannot agree with the statement that to achieve the "ideal" it is enough to use only a limited number of reference parameters in the range from 1 to 3 **[35]** or from 3 to 30 **[36]** or only to take into account the parameters of the input raw materials **[37]** such statements should be verified by conducting simulations using software with visualization of the results.

Deferring the numerical values of the accurate indicators of product quality for the corresponding rays, we obtain a figure inside the regular reference polygon. Comparing the areas of the presented figures allows us to estimate the approximation of the developed products visually to an ideal condition on the selected reference indicators. Therefore, for this scheme as an evaluation criterion, it is advisable to choose the following parameter.

$$k_s = \frac{S_{Dz}}{S_{Dl}} \quad (2)$$

Where:

S_n – cross-sectional area according to the actual condition of the product; – the area of an ideal section on reference or absolute indicators of production quality.

The formula can find the area of an ideal section:

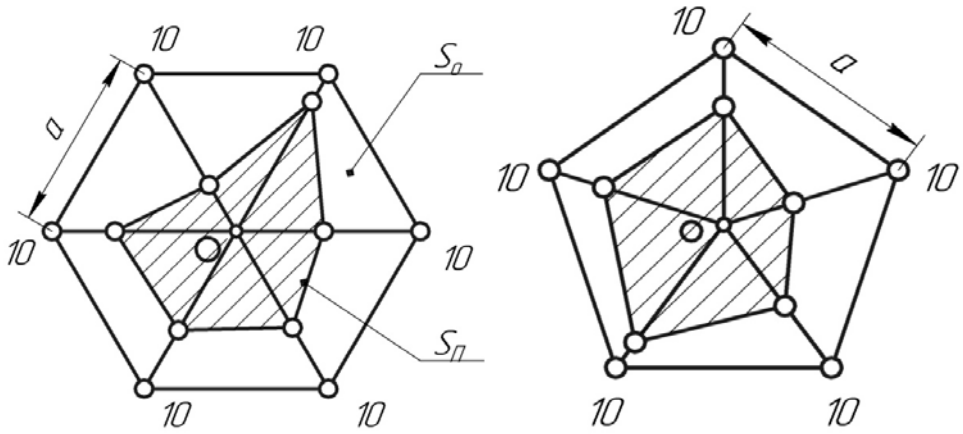
$$S_{Dl} = \frac{ma^2}{4tg\left(\frac{180}{m}\right)} \quad (3)$$

Where:

m – is the number of sides of the polygon; a is the value of the side face of the polygon (Figure 3).

Features of the developed flat mathematical model of product quality in the form of a polygon are:

- clarity of assessment;
- the ability to evaluate any number of parameters;
- the ability to adjust the accuracy of the assessment;
- relatively simple assessment with a small number of areas of assessment.



a b

Figure 3 Polygon models of product quality assessment by even (a) and odd (b) number of reference parameters.

To estimate the retention time of certain quality indicators of the product, it is advisable to implement the spatial scheme of the mathematical model in the form of a polyhedron, which is presented in Figure 4.

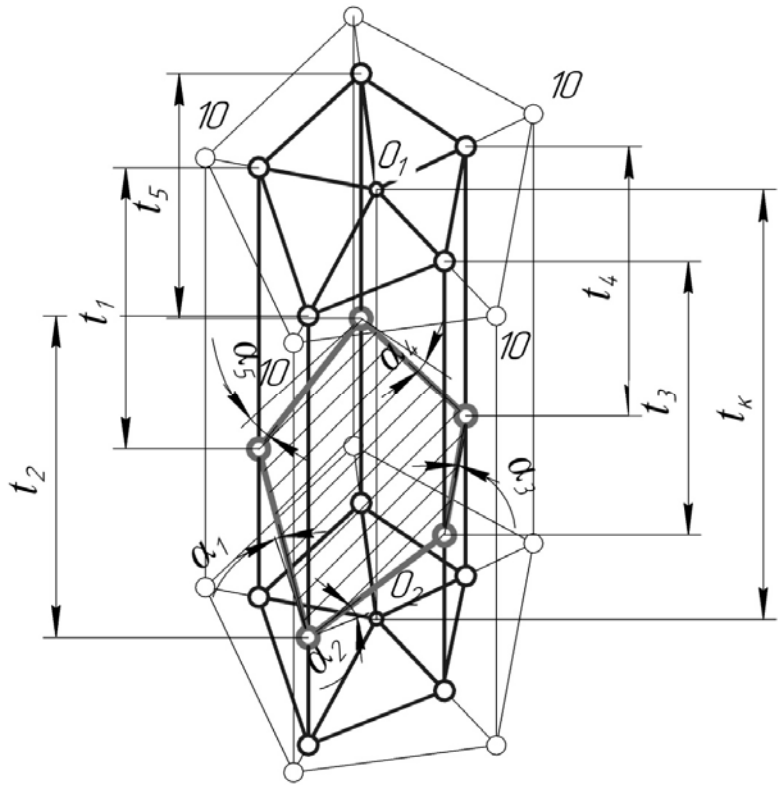


Figure 4 Spatial mathematical model of product quality in the form of a polyhedron.

According to this scheme, polygons make up the area of the base and the time of preservation of product quality – the height of the polyhedron. In the case of estimating the basic reference parameters by a certain equal value, for example, up to 10 units, the constructed correct polyhedron corresponds to the ideal product for a given number of reference parameters. The height of the polyhedron corresponds to the control period of storage of the product as a whole t_k .

Similar studies are described in the scientific works of L. Bal-Prylypko, which are devoted to the design of technological equipment **[38]**, **[39]**, the production of sausage products **[40]**, **[41]**, the development of technological schemes for the production of various products of the food industry **[42]**, but without taking into account time parameters that can affect the quality indicators of the final products. Deferring the numerical values of the real indicators of product quality for the corresponding rays, we obtain a figure inside the reference regular polyhedron. The height of each face of the constructed figure t_i corresponds to the product's shelf life according to certain quality parameters. This figure reflects the quality of the developed product in the spatial form: the approximation of the real polyhedron to the correct corresponds to its approximation to the "ideal".

Among the parameters that assess the quality of products, we can specify the following:

The average shelf life of quality parameters:

$$t_c = \frac{ILt_i}{m} \quad (4)$$

The average angle of deviation of the faces of a real polyhedron from the faces of the reference; - angles of deviation of the truncated base depending on the shelf life for certain quality parameters, where m is the number of sides of the polygon.

$$I \pm_c = \frac{ILI\pm_i}{m} \quad (5)$$

Comparing the volumes of the presented figures allows us to visually assess the approximation of the developed products to the ideal state according to the selected reference indicators. Therefore, for this scheme as an evaluation criterion, it is advisable to choose the following parameter.

$$k_v = \frac{V_{Dz}}{V_{Dl}} \quad (6)$$

Where:

$V_{Dl} = S_{Dl}a‹ \ldots t_k$ – the volume of the reference prism; t_k – control period of product storage;

$V_n = \frac{S_{Dz}}{\cos I\pm_c}a‹ \ldots t_{NI'}$ – the volume of the truncated prism according to the real quality parameters;

t_i – shelf life of products according to certain real quality parameters.

Features of the developed spatial mathematical model of product quality in the form of a polyhedron are: – the ability to take into account the shelf life of products for individual quality parameters; – clarity of assessment with a large number of quality parameters; – high accuracy of assessment; – the complexity of analytical calculations. Given the latter shortcoming, the flat model of the polygon was chosen as the basis of the mathematical model for assessing the quality of the developed cooked sausage with wheat fibre and pumpkin pectin (WFwPP), taking into account the time of preservation of product quality, which was evaluated by regression analysis. The results of the assessment of organoleptic characteristics of cooked sausages with vegetable additive WFwPP on a 5-point scale are shown in Figure 5.

During the tasting evaluation of boiled sausages, depending on the studied factors, it was found that partial replacement of raw meat in minced cooked sausages with vegetable supplement wheat fibre with pumpkin pectin does not reduce their organoleptic characteristics. We have analyzed the results of other scientific works in which the process of production of cooked sausages **[43]**, sausages **[44]**, and semi-finished products **[45]** with the addition of plant materials.

Supplementation with WFwPP in sausages from 7% to 9% led to a deterioration of the structure in the sausage section. The tasting of sausages allowed us to determine the product's smell, aroma, and taste, as well as the absence of foreign odours and tastes in the variants of samples with meat substitution from 1% to 5% of WFwPP. The increase in the concentration of WFwPP led to the appearance of pumpkin flavour. According to the consistency index, when pressing portions of sausages, a dense consistency was felt in 3% and 5% WFwPP. Increasing the concentration of plant additives from 7% to 9% led to a more rigid consistency and unsatisfactory organoleptic characteristics. An analysis of scientific works was carried out, which were devoted to the

introduction of various concentrations of plant additives into the composition of various meat and dairy products **[46]**, in particular, pectin from carrots and apples **[47]** and fibre from various bowls of cereal **[48]**.

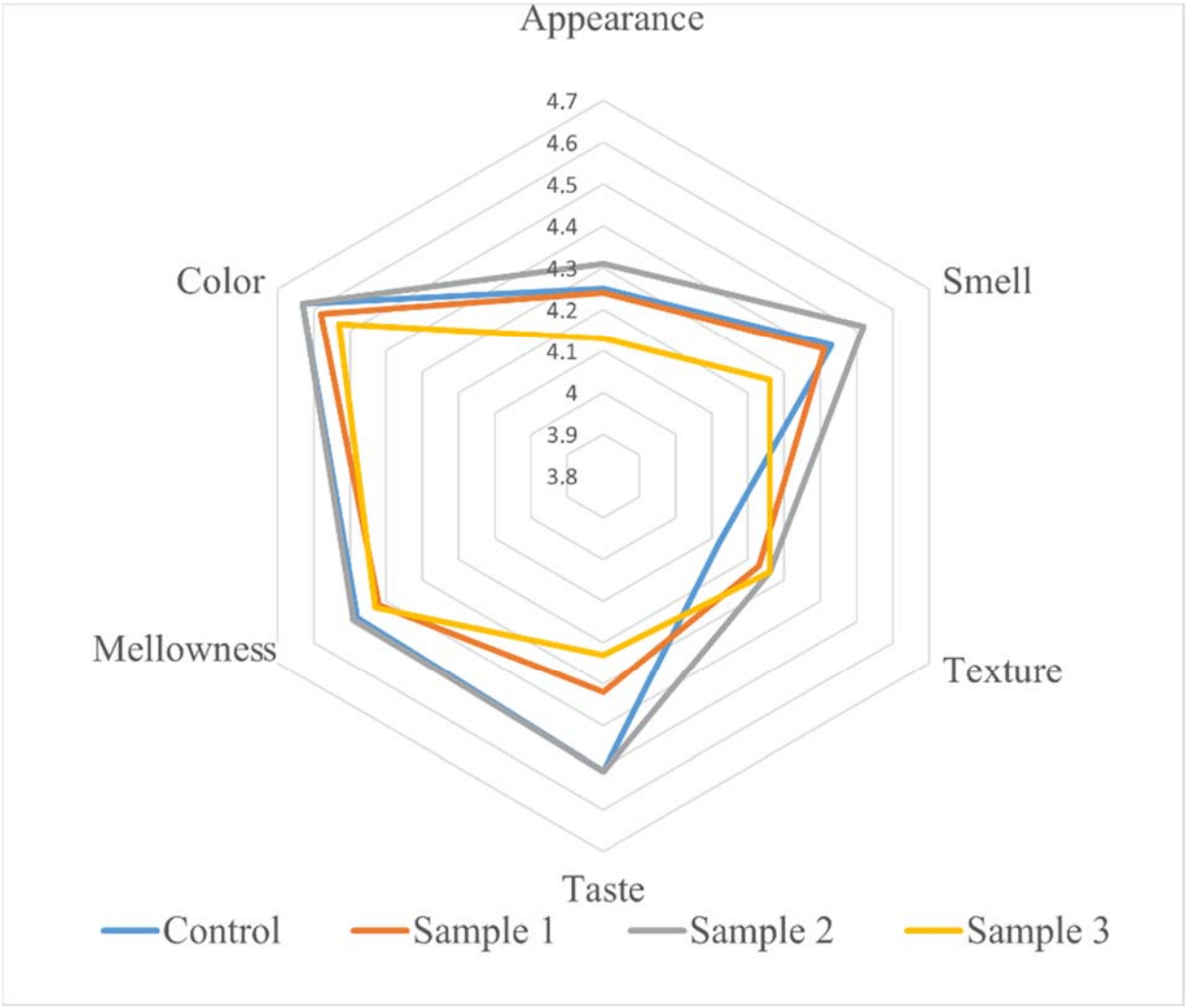


Figure 5 Profile of organoleptic parameters of cooked sausage samples with different content of WFwPP.

Considering the results of our experiments, we can say that an increase in the concentration of plant raw materials by more than 5% will lead to the appearance of a carrot or apple aftertaste, which in turn worsens the quality of finished products.%

Analysis of the aftertaste of sausages showed the best result regarding tenderness and juiciness in the sample with 5% WFwPP. In particular, the overall score of the experimental samples was in samples No. 1 – 4.44; No. 2 – 4.51; No. 3 – 4.41 points against 4.49 – In control. According to the results of the scores, the best sample is No. 2, with the content of WFwPP in 5%.

These studies also found that cooked sausages, besides the stuffing of various amounts of vegetable additives WFwPP on organoleptic indicators, had a pleasant taste, smell, colour, and texture. In second place was the quality indicator sample No. 3 with 7% WFwPP. The indicators of the control sample and No.1 with 3% of WFwPP were almost identical. The general analysis of the conducted research showed that the use of cooked sausages with vegetable additive in the amount of 5% by weight of minced sausage, namely wheat fibre with pumpkin pectin, does not reduce their quality in organoleptic parameters, and this product meets the requirements of regulatory and technical documentation. Comparative analysis of the evaluation of sausages according to the developed recipes 1, 2, and 3 with the control product and when used as a criterion for the ratio of the respective areas of the polygon is presented in Figure 6.

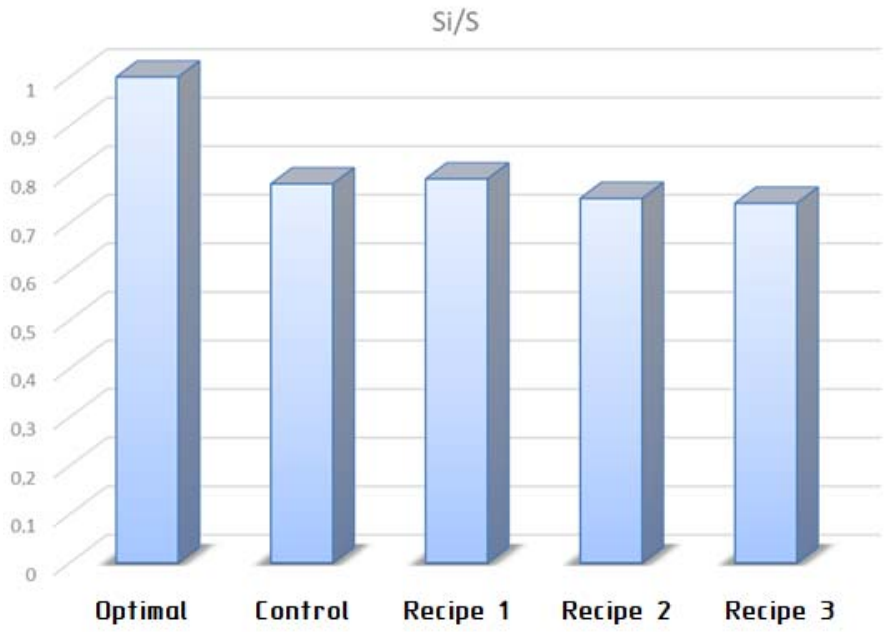


Figure 6 Comparative analysis of control and experimental samples of sausages.

The quality assessment results of developed cooked sausages using a computer program for calculating the area of quality profiles with subsequent graphical visualization are consistent with previous organoleptic studies by the classical method, confirming the research results' reliability.

The results of research on the appearance of the experimental and control samples are presented in Figure 7.

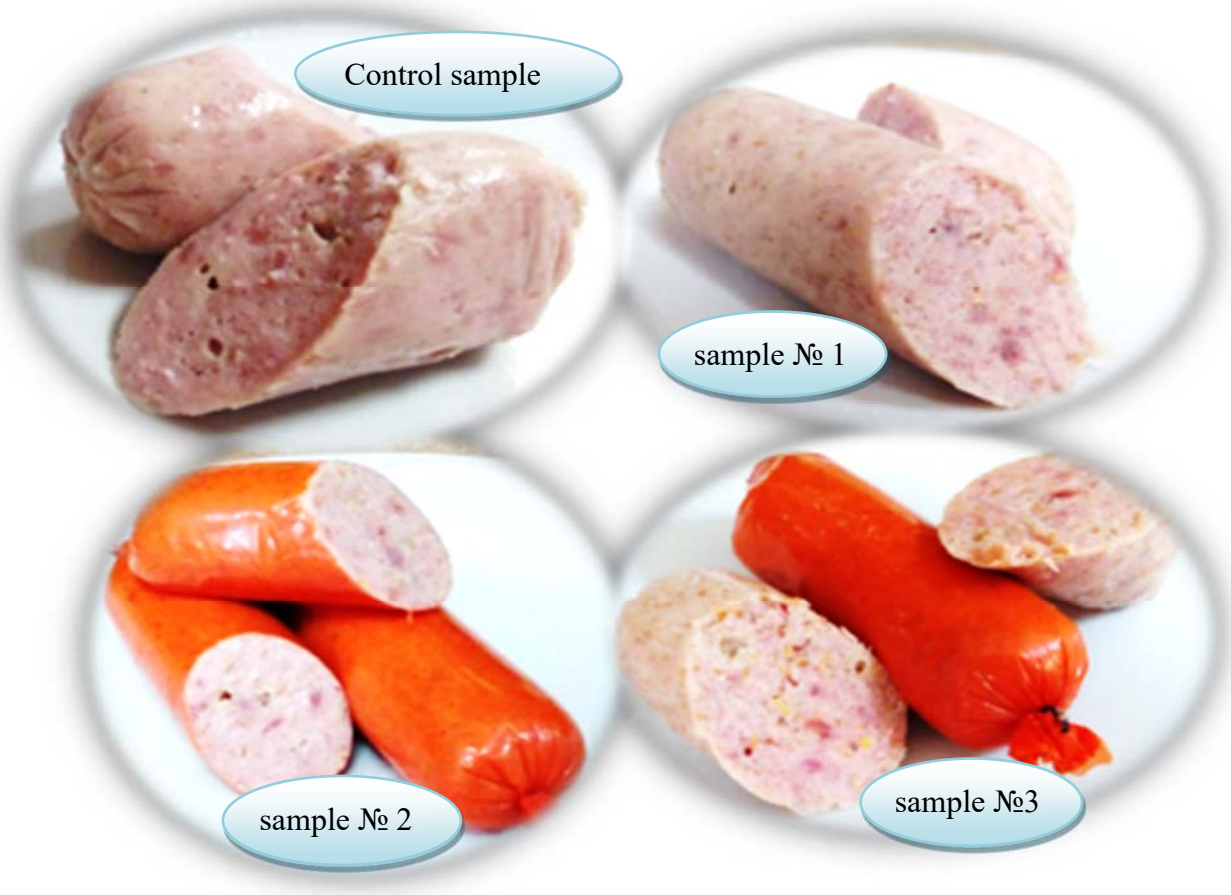


Figure 7 Appearance of samples of newly cooked sausages and control sample**.**

%

Analyzing Figure 7, which shows the appearance of the organoleptic indicators of the newly finished cooked sausage, it can be concluded that sample No. 1 had a loose, loose consistency; sample No. 2 has a dense consistency, proper plasticity and juiciness, and sample No. 3 has a denser consistency and unsatisfactory organoleptic indicators. Thus, using wheat fibre with pumpkin pectin in the amount of 5% ensures the consistency of the finished new sausage, which meets the requirements of the relevant regulatory documentation.

The final product must meet the basic food safety requirements applicable in the country of destination **[49]**.

Prospects for further research are related to the modelling of nutritional value indicators of similar sausage products and quality control of finished sausage products, which includes the selection of control points according to the system of risk analysis, dangerous factors, and control of critical points of HACCP, as well as optimization of production processes and determination of rational equipment parameters for production of sausage products from land and waterfowl meat.

CONCLUSION

Taking into account the main properties of the mathematical model for evaluating the quality of cooked sausages with the proposed ingredients: clarity of presentation, depth, the volume of information and possible ramifications of its flows, an appropriate systematization was carried out, and linear, flat and spatial evaluation models were developed in the form of a ray diagram, a polygon and a polyhedron.

The use of this type of model made it possible to evaluate the extensive methods of quality indicators effectively and to evaluate the corresponding number of parameters with high accuracy, to regulate the accuracy of the assessment with a small number of reference parameters, and take into account the duration of the shelf life of finished products according to individual quality parameters.

The results of the evaluation of the quality of the developed products according to the polygon model revealed that the replacement of raw meat increased by up to 9% WFwPP in the recipe of cooked sausages reducing the intensity of taste and aroma, giving a specific taste, negatively affects the juiciness. According to such indicators as consistency, color, smell, taste, and appearance were the best experimental samples using 5% WFwPP; according to the overall score, the best results are obtained when making these additives in the amount of 3% to 5%.

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Evaluation of laying hen breeding conditions on the farm and egg quality in the cage and cage-free systems in the period after the peak of laying

Ján Petrovič, Martin Mellen

ABSTRACT

The study aimed to examine laying hens in the cage and cage-free breeding systems, the quality of table eggs and energy consumption in the hall after the peak of laying. In the research, the following were investigated and statistically evaluated welfare of laying hens Bovans Brown was monitored in three different rearing systems based on resources and animals. The research was designed into the post-peak laying period, at the age of laying hens from 34 to 47 weeks and a rearing system of enriched cages on deep litter and in aviaries. Statistical analyses of the measured data of the established indicators were performed with the SAS program package, version 8.2, for statistical characteristics, significance, and correlation relations. The proportion of laying hens dying was lower in aviaries compared to cages and on deep litter ($p \leq 0.05$); in cages and on deep litter was comparable ($p > 0.05$). The weight of laying hens was comparable ($p > 0.05$). Feed consumption per hen, day, and egg was highest on deep litter ($p \leq 0.05$). The proportion of eggs with a cracked shell and contaminated with dropping was highest on litter ($p \leq 0.05$). Energy consumption in the hall expressed per layer and day was comparable in all three breeding systems ($p \leq 0.05$). Among some selected indicators of laying hen welfare, egg quality and energy consumption in the hall during breeding and correlation relations ($p \leq 0.05$) were statistically significant within individual breeding systems. The question of laying hen welfare and improving cage-free systems because of the adopted legislation banning breeding in a cage system requires further research to adopt best practices regarding resource-based, management- and animal-based parameters. Based on the results about welfare conditions, including energy consumption in halls and egg quality, it is an open question for comprehensive, interdisciplinary research.

Keywords: laying hen, breeding system, quality, welfare, egg

INTRODUCTION

EU legislation **[1]** banned the breeding of laying hens in conventional cages, but breeding in enriched cages is still possible. The use of enriched cages varies in individual European Union member states. Some large investments in this breeding system have shifted the decision in favour of the cage-free system. In recent times, large retailers and food companies have seen a shift towards sourcing eggs and egg products from egg producers from cage-free farming systems by 2025.

Laying hens' health and welfare significantly impact egg production and farm economics. From this point of view, ensuring the good living conditions of laying hens can help producers achieve economic benefits. Europeans prefer stricter animal welfare standards and are willing to pay more for products with high animal welfare. This is leading major food businesses and retailers to commit to using only eggs produced in cage-free systems by 2025 at the latest. These include retailers such as Tesco in the UK and Central Europe, Camst in Italy, Monoprix

and Carrefour in France, international food services such as Sodexo and Compass Group, and food multinationals such as Nestlé **[2]**, **[3]**. Some member states and regions of the European Union have even introduced a legislative ban on cages in egg production. Austria has banned enriched cages from 2019, Wallonia and the Netherlands from 2021. The Netherlands has allowed the so-called "colony cages". These larger cages are suitable for breeding, usually 40 to 80 laying hens. Germany has announced a ban on enriched cages from 2025. Farmers who have switched to enriched cages to comply with European Union legislation have made significant investments to meet the changed system requirements from conventional to enriched cages. Cage-free systems can offer laying hens to perform their natural activities **[4]**. Egg producers are currently debating ways to diversify the market supply and the replacement of litter eggs with enriched cage eggs is likely to create "value line" (or cheapest) production volumes **[5]**. The acceptability of eggs from the litter system to the general public varies from country to country **[6]**. The most recent manifestation of the trend that led to changes in selection criteria for laying hens are accelerated changes in housing systems and bans on management practices such as beak trimming in several countries of the European Union (Austria, Germany, the Netherlands, etc.). The traditional approach, which focused only on the economic aspects of egg production, has shifted **[7]**. European legislation sets the rules for three alternative breeding systems (cage-free), namely barn, free-range and organic. Breeding systems are marked by law on each individual eggshell and on the egg packaging with the code system defined in Commission regulation **[8]**. The relevant legal provisions for alternative systems are set out in several European Union legislation. There is a lot of research on using animal-based measures to assess animal welfare **[9]** (and others from recent years).

The biggest initiative is the Welfare Quality® project. This project differed from the views of the European Food Safety Authority in that its philosophy was not to identify factors that lead to good or bad living conditions. The Welfare Quality® project focused primarily on animal-based measures that can be monitored and used during a single farm visit by an independent observer to assess current levels of welfare, i. e. at a specific time **[10]**. Consumers are interested in the origin of poultry products. Surveys confirm, as shown by the Eurobarometer results, that most people support the improvement of the living conditions of laying hens in production systems **[11]**. Consumers' perception of animal welfare can influence product purchasing decisions. Up to 43% of consumers claim to consider animal welfare and protection when making a food purchase **[10]**. Egg production, defined as the number of eggs laid in each period, or the laying rate (the number of eggs laid divided by the number of days in a given period), is the main criterion of the selection scheme, which is evaluated for the laying hen. Egg production is influenced by the environment, mainly seasonality, but also depends on the genetic component **[12]**. Genetic variability in egg production has contributed to the current production level in laying hens capable of laying more than 300 eggs per year **[13]**. The table egg is considered an available source of protein, and it provides about 314 kJ or 75 $kcal.egg^{-1}$. Eggs are a high-quality human protein source due to their high digestibility and balanced amino acid composition. It is not restricted by the prohibitions of most religions and is, therefore a staple human food product that is commonly consumed worldwide. Asia is the world's leading producer (53.3% of world production in 2018), ahead of the European Union (10% of world production) and the United States (8.6%) **[14]**. China alone accounted for 32% of global production in 2018 **[15]**. Annual world consumption is about 150 eggs per year per capita. European consumption averages 217 eggs per year and per capita, with a large difference between countries (from 141 to 183 eggs in Greece and Poland to 301 in Denmark). The French annual consumption of 218 eggs per year per inhabitant in 2018 is like the European consumption, which corresponds to an average daily consumption of 30 $g.day^{-1}$, which corresponds to 60% of the consumption part of 60 g of eggs **[14]**.

The study aimed to examine, compare and evaluate the research results on the welfare of laying hens from cage and cage-free breeding systems, the quality of table eggs, and the economic indicator of energy consumption in the hall in the period after the peak of laying.

Scientific Hypothesis

The resources for the living conditions of laying hens are improved in cage-free rearing systems on deep litter and in aviaries in terms of performing natural activities compared to the cage system.

Egg damage in cage-free, deep litter and aviary systems is higher than in cage systems.

The economic costs of laying hens and egg production are higher in a cage system than in cage-free systems on deep litter and aviaries.

MATERIAL AND METHODOLOGY

Samples

The research was investigated and statistically evaluated:

- the welfare of laying hens in three different breeding systems expressed in number in the dead laying hens and the weight of laying hens, feed and water consumption recorded in the farm records during the duration of the research,
- egg weight, and damaged eggs with cracked shells and contaminated with dropping monitored on the farm in three different breeding systems during the duration of the research,
- energy consumption in the hall on the farm in three different breeding systems from the farm records during the research duration.

Chemicals

Chemicals were not used in these experiments.

Animals, Plants and Biological Materials

The object of investigation was laying hens of the Bovans Brown hybrid line reared in three different systems, namely in enriched cages with an initial state of 30,892 pcs, on deep bedding with an initial state of 11,130 pcs, and in aviaries with an initial state of 27,958 pcs. The research was situated in the period after reaching the peak of laying at the age of hens from 34 to 47 weeks during three calendar months, namely in August, age of hens from 34 to 38 weeks, in September, age of hens from 39 to 43 weeks and in October, age of hens from 43 to 47 weeks.

Figure 1 Housing laying hens on deep litter (Petrovič, 2022).

Figure 2 Laying hens in aviaries (Petrovič, 2022).

Figure 3 Enriched cage system (Petrovič, 2022).

Instruments

Instruments for indicators based on management and animals: A check was carried out daily with records of the state of the water meter and, electricity meter, feed consumption, based on which the consumption value for the previous day was calculated and recorded. The functionality of the ventilation system and other electrical equipment were checked, i. e. feeding and hall lighting equipment. After entering the breeding area, the laying hens were checked and the dead individuals were collected, which were stored in the waste container for dead animals. The laying hens were weighed weekly, the results of which were compared with the standard recommended by the producer of the hybrid combination.

Instruments for egg quality indicators: KERN PCB scale with an accuracy of d ±0.001 g was used to weigh the eggs. Damaged eggs with cracked shells and soiled with dropping were assessed visually during egg sorting in the egg sorting department of the hall.

Laboratory Methods

Research and evaluation of welfare conditions from the observation of deviations from the normal behavior of laying hens in the practical conditions of a breeding farm in a cage and two different cage-free breeding systems according to selected indicators based on resources, management and animals was carried out according to the **[16]**.

The number of dead laying hens and the weight of the hens: The number of dead laying hens was assessed daily based on evidence in the farm records in each monitored breeding system and confirmed by the veterinarian during the research period following legislative measures **[17]**.

Based on the number of dead laying hens per day, the proportion of dead laying hens from the total number of laying hens on the same day was calculated according to the formula (the proportion of dead laying hens, %):

$$\% = \frac{\text{number of dead laying hens (pcs)}}{\text{the number of laying hens in the breeding system (g)}} \times 100 \quad (1)$$

The weight of the laying hens was determined by weighing at weekly intervals on a digital scale, type Salter 1102 GNBLDR, with an accuracy of d ±1.0 g and a maximum weight of 5 kg.

Feed consumption was determined daily by checking consumption using a strain gauge installed on the strength of each hall and recorded values using a digital scale, type DGT Weight Transmitter.

The formula was used to calculate feed consumption per laying hen and day:

$$\text{Feed consumption per laying hen and day (g)} = \frac{\text{total feed consumption (g)}}{\text{number of laying hens (pcs)}} \quad (2)$$

Feed consumption per egg was determined daily by calculation according to the formula:

$$\text{Feed consumption per egg (g)} = \frac{\text{total feed consumption (g)}}{\text{number of eggs (pcs)}} \quad (3)$$

Water consumption per laying hen and day was determined daily based on checking water consumption on a water meter, type Enbra EV-1, and calculating according to the formula:

$$\text{Water consumption per laying hen and day (ml)} = \frac{\text{total water consumption (ml)}}{\text{number of laying hens (pcs)}} \quad (4)$$

Description of the Experiment

Sample preparation: Preparation of the samples based on welfare and animals – taking over from the farm record data. Preparation of the egg quality samples taken from the farm record data.

Number of samples analyzed: Number of samples for the evaluation of the welfare of laying hens – the number of dead laying hens was evaluated from the total number of hens during the research for 92 days in cages, 92 days on deep litter and 92 days in aviaries.

Number of samples for evaluation of the weight of laying hens – a sampling of the weight of laying hens was carried out weekly from the total number of laying hens during the 92-day research period based on the same

number of randomly selected pieces in each of the three months of August, September, and October, i. e. total n = 14 in cages, n = 14 on deep litter and n = 14 in aviaries.

Number of samples for evaluation of feed and water consumption – the number of samples of feed and water consumption was evaluated daily during the duration of the research based on the total feed consumption of the number of laying hens, i. e. 92 days in cages, 92 days on deep litter and 92 days in aviaries.

Number of samples for evaluation of egg weight – from the total number of eggs laid, the number of eggs weighed from randomly selected eggs was from 30 to 31 pieces during each of the three calendar months during the duration of the research, i. e. total n = 92 in cages, 92 on deep litter and 92 in aviaries.

Number of samples for evaluation of damaged eggs, i. e. with a cracked shell and contaminated with droppings was evaluated daily during the duration of the research from the total number of eggs laid, i. e. 92 days in cages, 92 days on deep litter and 92 days in aviaries.

Number of samples for evaluation of energy consumption – energy consumption was evaluated daily during the research period of 92 days in cages, 92 days on deep litter and 92 days in aviaries.

Number of repeated analyses: 1

Number of experiment replication: 1

Design of the experiment: The experiment was carried out in practical conditions on a poultry farm breeding laying hens in the Slovak Republic. Design of the experiment is shown in Table 1.

Table 1 Disign of the experiment.

Hall with laying hen breeding system	Same conditions	Different conditions
Enriched cages	Hybrid combination of Bovans Brown hens Feed mixture HYD 10 – *ad libitum*	initial state 30,892 pcs
Deep litter	Microclimatic conditions – lighting, ventilation, and temperature	initial state 11,130 pcs
Aviaries	Age of laying hens from 34 to 47 weeks The same research indicators	initial state 27,958 pcs

Selected indicators of animal welfare, quality of table eggs and electricity consumption in halls were compared and evaluated during the research period under the same nutritional conditions, microclimatic conditions and with the same hybrid combination of Bovans Brown. The hybrids were reared in three different rearing systems, namely in enriched cages with an initial condition of 30,892 pcs, on deep litter with an initial state of 11,130 pcs and in aviaries with an initial state of 27,958 pcs in the period after reaching the peak of laying. Experiments were carried out at the age of laying hens from 34 to 47 weeks during three calendar months, namely in August, age of laying hens from 34 to 38 weeks, in September, the age of laying hens from 39 to 43 weeks and in October, the age of laying hens from 43 to 47 weeks.

Statistical Analysis

Statistical analyzes of the measured data of the established indicators were performed with the SAS program package, version 8.2. The SAS system program sorted the data, ordering observations according to the values of specific quantities. The SAS program sorted the data in a certain order in the first operation of mathematical-statistical calculations. A SAS file and a sorting procedure were created from the data. From the data sorted by the SAS program, mathematical-statistical calculations of descriptive characteristics of the indicators according to the breeding systems of laying hens were performed based on the values of certain quantities such as arithmetic mean, standard deviation, and coefficient of variation. The Scheffe's test was used to test the statistical significance of differences in the indicator between breeding systems of laying hens at the level of significance $p \leq 0.05$. The basic set in each compared statistical set represented statistical units in the research on the farm n = 92, i. e. n >30 as a large statistical set and n = 14, i. e. n ≤30 (only body weight of laying hens) as a small statistical set. The statistical method Scheffe's test is a commonly used method for evaluating the difference in the means of more than two groups. The p-value, which was set in the SAS program for the Scheffe's test, represents the probability of an error caused by accepting the researcher's hypothesis about the existence of a difference between the researched laying hen systems. It is the probability of error that the null hypothesis of no difference between the groups was rejected if this was indeed true. Within individual systems of laying hens, the correlation between the determined indicators was determined according to the Pearson coefficient (r in the range of 1 to -1), which expresses the degree of tightness in linear regression, if they are determined on an interval scale. The correlation

coefficient results were verified by statistical evidence at the level of significance $p \leq 0.001$, $p \leq 0.01$, $p \leq 0.05$. According to the scale **[18]** to interpret the correlation coefficient result, a linear relation of 0.9 – 1 means almost perfect, 0.7 – 0.9 very strong, above 0.5 strong, 0.3 – 0.5 medium, 0.1 – 0.3 weak and below 0.1 trivial, simple, light.

RESULTS AND DISCUSSION

Historically, animal welfare has been defined by the absence of negative experiences such as disease, hunger, thirst, stress, or reduced fitness **[19]**. Most animal welfare research over the past 40 years has focused on avoiding negative states. However, there is increasing interest and research in experiences with positive animal welfare states **[20].** This shift in animal welfare science has led to the understanding that animal welfare cannot be achieved without experiencing positive affective states such as comfort, pleasure, and a sense of control **[21]**. Public attention to the welfare of laying hens in the last two decades has been stimulated by the gradual transition from conventional cage systems with an increase in the proportion of enriched cages that provide them with more space for movement, nest, perch and bedding substrate and cage-free systems in some countries, initially in the European Union **[1]**, and subsequently outside Europe. For example, as of March 2020, nearly 24% of all layers in the United States were housed in cage-free systems, up from 12% in 2016 to 4% in 2010 **[22]**. Considering the ethical dimension in this sector has led to many examples of major changes in how eggs are produced to respond to society's demands. The main changes in the breeding system relate to the gradual retreat from cage systems **[23].** A breeding system can cause social problems when it causes frustration. The opportunity for hens to exhibit the behaviours they are motivated to engage in is key to achieving positive welfare states **[24]**.

Mortality of the laying hens: The average value of the proportion of the laying hen mortality from the total number in the cage breeding system, on the deep litter and in the aviaries, and the statistical evaluation of the proportion of the laying hen mortality are shown in Table 2.

Table 2 The average value of the proportion of laying hen mortality from the total number in the cage system, on the deep litter and in aviaries, % and statistical evaluation of the proportion of laying hens dying.

Breeding system	n	% ± SD	c_v, %
Cages	92	$0.02^{a} \pm 0.010$	36.11
Deep litter	92	$0.02^{a} \pm 0.010$	48.04
Aviaries	92	$0.01^{b} \pm 0.003$	31.77
F-test		(7.72^{++}, $p \leq 0.01$)	

Note: n – multiciplity, % – mean in percentage, SD – standard deviation, c_v – coefficient of variation, ++ or $p \leq 0.01$ means a statistically highly significant difference, different letters in the superscript mean a statistically significant difference $p \leq 0.05$, the same letters in the superscript mean a statistically non-significant difference $p > 0.05$.

The variances of the proportion of the mortality of laying hens in the three monitored different breeding systems differed statistically significantly ($F = 7.72^{++}$, $p \leq 0.01$), which was confirmed by the result of the F-test, which verified the assumption of the equality of the variances. The null hypothesis H0 of no difference between the laying systems was rejected.

The average mortality of laying hens was 0.02% in the cage system and 0.02% in the deep litter system and 0.01% in the aviaries. The difference in the proportion of the mortality of laying hens was statistically significant ($p \leq 0.05$) by comparing the cage system of breeding with aviaries and deep litter breeding with aviaries. Comparing the cage breeding system with the deep litter breeding system, no statistically significant difference was found ($p > 0.05$). The statistical evaluation of the proportion of laying hen mortality expressed by the standard deviation revealed the same fluctuation of values in cages and on deep litter and smaller in aviaries (SD = 0.01, 0.01 and 0.003) and by the coefficient of variation a larger fluctuation of values in order on deep litter, in cages and in aviaries (c_v = 48.04, 36.11 and 31.77).

Enriched cage and cage-free systems allow laying hens to move around and exhibit natural behaviour, but concerns have been raised regarding the observation of higher mortality rates in cage-free systems **[25]**, **[26]**. The mortality of laying hens is considered one of the most important indicators of health **[27]** since a higher mortality would indicate a deteriorated health status. However, a thorough understanding of the causes of mortality in different breeding systems is needed to substantiate such a claim. If confirmed, it would mean that the health and welfare of laying hens could be partially compromised after the transition to cage-free systems. However, it is not yet clear whether the death rate is higher in cage-free systems. Information on mortality is not systematically

collected in laying hen farms producing table eggs and few reviews have focused on this topic **[28]**. Therefore, knowledge is considered inconsistent. Where mortality differences were found between breeding systems **[25]**, they were non-significant when the confounding effect of beak trimming status was controlled (although beak trimming is a painful procedure with a significant negative impact on hen welfare) **[29]**, its effect on reducing mortality due to harmful feather pecking is well known **[22]**. In study **[30]** is reported, based on the results of the research project, that the mortality rate of laying hens was lower in enriched cages than in litter stalls. The observation of a strong association between laying hen mortality and breeding system was replicated with different meta-analytic models and in different sensitivity analyses. Using two independent data sets, a strong and significant decrease in mortality in cage-free aviaries was observed over time. These results also find support in various previous observations **[31]**. Leenstra et al. **[32]**. also report data showing a decrease in the mortality of laying hens reared in aviaries. Our research results also confirm a statistically significant reduction in the mortality of laying hens in aviaries compared to the mortality of laying hens in the cage system and on deep litter. In a study by Saldaña et al. **[33]** mortality during the laying phase is reported to be 1.8% in hybrid Lohmann Brown Classic and unrelated to treatment (data not shown).

Feed consumption per laying hen and day: The average feed consumption per laying hen and day in the cage breeding system, on deep litter and in aviaries and the statistical evaluation of feed consumption per laying hen and day are shown in Table 3.

Table 3 The average feed consumption per laying hen and day in the cage breeding system, on the deep litter and in aviaries, g and statistical evaluation of feed consumption per laying hen and day.

Breeding system	n	g ±SD	c_v, %
Cages	92	111.51^{a} ±4.18	3.75
Deep litter	92	116.75^{b} ±2.55	2.18
Aviaries	92	108.31^{a} ±7.81	7.21
F-test		(9.59^{+++}, $p \leq 0.001$)	

Note: n – multiciplity, % – mean in percentage, SD – standard deviation, c_v – coefficient of variation, +++ or $p \leq 0.001$ means a statistically very highly significant difference, different letters in the superscript mean a statistically significant difference $p \leq 0.05$, the same letters in the superscript mean a statistically non-significant difference $p > 0.05$.

The variances of feed consumption per laying hen and day in the three different breeding systems were statistically very significantly different ($F = 9.59^{+++}$, $p \leq 0.001$), which was confirmed by the F-test result, which verified the assumption of the equality of variances. The null hypothesis H0 of no difference between the laying systems was rejected.

The average feed consumption per laying hen per day was 111.51 g in the cage system, 116.75 g in the deep litter system and 108.31 g in the aviaries. The difference in feed consumption per laying hen and day was statistically significant ($p \leq 0.05$), comparing the cage system with the litter system and the litter system with aviaries. No statistically significant difference was found by comparing the breeding cage system with aviaries ($p > 0.05$). Statistical evaluation of feed consumption per laying hen and day, expressed by standard deviation and coefficient of variation, revealed fluctuations in values from the largest in order in aviaries, cages and on deep litter (SD = 7.81, 4.18 and 2.55, c_v = 7.21, 3.75 and 2.18).

Feed consumption per egg: The average feed consumption per egg in the cage breeding system, on the deep litter and in the aviaries, and the statistical evaluation of the feed consumption per egg are shown in Table 4.

Table 4 The average feed consumption per egg in the cage breeding system, on deep litter and in aviaries, g and statistical evaluation of feed consumption per egg.

Breeding system	n	g ±SD	c_v, %
Cages	92	130.01^{a} ±4.18	12.76
Deep litter	92	165.32^{b} ±2.55	9.79
Aviaries	92	142.47^{a} ±21.32	14.97
F-test		(15.01^{+++}, $p \leq 0.001$)	

Note: n – multiciplity, % – mean in percentage, SD – standard deviation, c_v – coefficient of variation, +++ or $p \leq 0.001$ means a statistically very highly significant difference, different letters in the superscript mean a statistically significant difference $p \leq 0.05$, the same letters in the superscript mean a statistically non-significant difference $p > 0.05$.

The variances of feed consumption per egg in the three different breeding systems were statistically very significantly different ($F = 15.01^{+++}$, $p \leq 0.001$), which was confirmed by the F-test result, which verified the assumption of the equality of variances. The null hypothesis H0 of no difference between the laying systems was rejected.

The average feed consumption per egg was 130.01 g in the cage breeding system, 165.32 g in the deep litter system and 142.47 g in the aviaries. The difference in feed consumption per egg was statistically significant ($p \leq 0.05$) comparing the cage system with the litter system and the litter system with aviaries. No statistically significant difference was found by comparing the breeding cage system with aviaries ($p > 0.05$). Statistical evaluation of feed consumption per egg, expressed by standard deviation and coefficient of variation, revealed a fluctuation of values from the largest in order in aviaries, cages and on deep litter (SD = 21.32, 16.59 and 16.19, c_v = 14.97, 12.76 and 9.79).

Water consumption per laying hen and day: The average water consumption per laying hen and day in the cage breeding system, on the deep litter and in the aviaries, and the statistical evaluation of feed consumption per laying hen and day are shown in Table 5.

Table 5 The average water consumption per laying hen and day in the cage breeding system, on deep litter and in aviaries, ml and statistical evaluation of water consumption per laying hen and day.

Breeding system	n	ml ± SD	cv, %
Cages	92	$226.95^{a} \pm 31.45$	13.86
Deep litter	92	$209.20^{a} \pm 27.84$	13.31
Aviaries	92	$194.78^{a} \pm 23.32$	11.97
F-test		$(2.36^{-}, p > 0.05)$	

Note: n – multiciplity, ml – mean in percentage, SD – standard deviation, cv – coefficient of variation, - or $p > 0.05$ means a statistically non-significant difference, the same letters in the superscript mean a statistically not significant difference $p > 0.05$.

The variances of water consumption per laying hen and day in three different breeding systems differed statistically non-significantly (2.36^{-}, $p > 0.05$), which was confirmed by the result of the F-test, which verified the assumption of the equality of variances. The null hypothesis H0 of no difference between the laying systems was rejected.

The average value of water consumption per laying hen per day was 226.95 ml in the cage system, 209.20 ml in the deep litter system and 194.78 ml in the aviaries. The difference in water consumption per laying hen and day was not statistically significant ($p > 0.05$) by comparing the monitored different laying hen breeding systems. Statistical evaluation of water consumption per laying hen and day expressed by standard deviation and coefficient of variation revealed a fluctuation of values from the largest in order in cages, on deep litter and in aviaries (SD = 31.45, 27.84 and 23.32, c_v = 13,86, 13.31 and 11.97).

In the study **[34]**, a laying hen's average water consumption is 215 ml.day^{-1} for hybrids Lohmann Selected Leghorn and Lohmann Brown. This is in close agreement with the measured drinking water intake of adult brown and white leghorn laying hens (from 214 to 228 ml.day^{-1}) **[35]**. Our results of the average water intake of laying hens in the cage system agree with those mentioned. We have noted lower water consumption in the system of rearing laying hens on deep litter or in aviaries compared to the literature sources mentioned above. This fact may also be related to the laying hen hybrid; in our case, it was Bovans Brown.

Body weight of laying hens: The average body weight of laying hens in the cage breeding system, on the deep litter and in the aviaries, and the statistical evaluation of the body weight of the hens are shown in Table 6.

Table 6 The average weight of laying hens in the cage breeding system, on the deep litter and in aviaries, g, and statistical evaluation of the weight of hens.

Breeding system	n	g ±SD	c_v, %
Cages	14	1790.21^{a} ±31.87	1.78
Deep litter	14	1789.21^{a} ±104.58	5.84
Aviaries	14	1786.43^{a} ±57.99	3.25
F-test		(0.59^{-}, p >0.05)	

Note: n – multiciplity, ml – mean in percentage, SD – standard deviation, cv – coefficient of variation, - or p >0.05 means a statistically non-significant difference, the same letters in the superscript mean a statistically non-significant difference p >0.05.

The variances of laying hen weight in three different rearing systems differed statistically non-significantly (0.59^{-}, p >0.05), which was confirmed by the F-test result, which verified the assumption of equality of variances. The null hypothesis H0 of no difference between the laying systems was rejected.

The average weight of laying hens was 1790.21 g in the cage breeding system, 1789.21 g in the deep litter breeding system and 1786.43 g in the aviaries. The difference in the weight of laying hens was not statistically significant (p >0.05) by comparing the observed different breeding systems. The statistical evaluation of the weight of laying hens expressed by the standard deviation and the coefficient of variation revealed a fluctuation of the values from the largest in order on deep litter, in aviaries and cage (SD = 104.58, 57.99 and 31.87, c_v = 5.84, 3.25 and 1.78).

In study **[36]**, the body weight of laying hens in a floor system is higher than hens in cage systems. The study **[37]** reported that laying hens in a free-range system achieves a higher final body weight than hens in enriched cages.

Egg weight: The average egg weight in the cage breeding system, on the deep litter and in the aviaries, and the statistical evaluation of the egg weight are shown in Table 7.

Table 7 The average weight of eggs in the cage breeding system, on deep litter and in aviaries, g and statistical evaluation of egg weight.

Breeding system	n	g ±SD	c_v, %
Cages	92	130.01^{a} ±0.20	0.33
Deep litter	92	165.32^{b} ±0.39	0.62
Aviaries	92	142.47^{c} ±2.24	3.90
F-test		(15.01^{+++}, $p \leq 0.001$)	

Note: n – multiciplity, % – mean in percentage, SD – standard deviation, c_v – coefficient of variation, +++ or $p \leq 0.001$ means a statistically very highly significant difference, different letters in the superscript mean a statistically significant difference p≤0.05, the same letters in the superscript mean a statistically non-significant difference p >0.05.

The variances of feed consumption per egg in the three different rearing systems were statistically very significantly different (109.77^{+++}, $p \leq 0.001$), which was confirmed by the F-test result, which verified the assumption of the equality of variances. The null hypothesis H0 of no difference between the laying systems was rejected.

The average egg weight was 60.88 g in the cage-rearing system, 63.22 g in the deep litter breeding system and 57.44 g in the aviaries. The difference in egg weight was statistically significant ($p \leq 0.05$) by comparing the monitored different laying systems. A statistical evaluation of the weight of eggs expressed by the standard deviation and the coefficient of variation revealed a fluctuation of values from the *l*argest in order in aviaries, on deep litter and in cages (SD = 2.24, 0.39 and 0.20, c_v = 3.90, 0.62 and 0.33).

In published studies, it is stated that egg weight is influenced by the breeding system of laying hens **[38]**, **[39]**, **[40]**.

Damaged eggs with cracked shells: The average value of the proportion of damaged eggs with a cracked shell in the cage breeding system, on deep litter and in aviaries, and the statistical evaluation of the proportion of damaged eggs with a cracked shell are shown in Table 8.

Table 8 The average proportion of damaged eggs with a cracked shell in the cage breeding system, on deep litter and in aviaries, % and statistical evaluation of the proportion of damaged eggs with a cracked shell.

Breeding system	n	% ±SD	c_v, %
Cages	92	1.96^{a} ±0.54	27.78
Deep litter	92	3.20^{b} ±0.74	23.09
Aviaries	92	1.16^{a} ±0.25	21.52
F-test		(37.57^{+++}, $p \leq 0.001$)	

Note: n – multiciplity, % – mean in percentage, SD – standard deviation, c_v – coefficient of variation, +++ or $p \leq 0.001$ means a statistically very highly significant difference, different letters in the superscript mean a statistically significant difference $p \leq 0.05$, the same letters in the superscript mean a statistically non-significant difference $p > 0.05$.

The variances of damaged eggs with a cracked shell in the three different breeding systems were statistically very significantly different (37.57^{+++}, $p \leq 0.001$), which was confirmed by the F-test result, which verified the assumption of the equality of variances. The null hypothesis H0 of no difference between the laying systems was rejected. The average value of the proportion of damaged eggs with a cracked shell was 1.96% in the cage breeding system, 3.20% in the deep litter breeding system and 1.16% in the aviaries. The difference in the proportion of damaged eggs with a cracked shell was statistically significant ($p \leq 0.05$) by comparing the observed different laying systems. A statistical evaluation of the proportion of damaged eggs with a cracked shell expressed as a standard deviation, revealed a fluctuation of values from the largest in order on deep litter, in cages and in aviaries (SD = 0.74, 0.54 and 0.25) and the coefficient of variation from the largest in order in cages, on deep litter and in aviaries (c_v = 27.78, 23.09 and 21.52).

The study [38] found that the proportion of damaged eggs with cracked shells did not differ in cage, free-range, barn and organic systems. The proportion of damaged eggs with cracked shells out of all eggs laid was higher in an enriched cage system compared to free range. This may be since egg collection occurred once a day, and the distance between the nest and the egg belt may have increased the risk of egg damage **[37]**. In the study **[41]** is found a higher percentage of cracked eggs ($p \leq 0.01$) in a breeding system with enriched cages (7.8%) compared to the alternative system (4.1%).

Damaged eggs contaminated with dropping: The average value of the proportion of damaged eggs contaminated with dropping in the cage breeding system, on bedding and in aviaries, and the statistical evaluation of the proportion of damaged eggs contaminated with dropping are shown in Table 9.

Table 9 The average proportion of damaged eggs contaminated with dropping in the cage breeding system, on deep litter and in aviaries, % and statistical evaluation of the proportion of damaged eggs contaminated with dropping.

Breeding system	n	% ±SD	c_v, %
Cages	92	1.56^{a} ±0.36	22.87
Deep litter	92	3.41^{b} ±0.88	25.72
Aviaries	92	0.12^{c} ±0.06	48.03
F-test		(90.31^{+++}, $p \leq 0.001$)	

Note: n – multiciplity, % – mean in percentage, SD – standard deviation, c_v – coefficient of variation, +++ or $p \leq 0.001$ means a statistically very highly significant difference, different letters in the superscript mean a statistically significant difference $p \leq 0.05$.

The variances of the damaged eggs contaminated with dropping in the three different breeding systems were statistically very significantly different (90.31^{+++}, $p \leq 0.001$), which was confirmed by the F-test result, which verified the assumption of the similarity of the variances. The null hypothesis H0 of no difference between the laying systems was rejected. The average proportion of damaged eggs contaminated with dropping was 1.56% in the cage system, 3.41% in the deep litter system and 0.12% in the aviaries.

The difference in the proportion of damaged eggs contaminated with dropping was statistically significant ($p \leq 0.05$) by comparing the monitored different laying hen breeding systems. The statistical evaluation of the damaged eggs contaminated with dropping expressed by the standard deviation revealed a fluctuation of the values from the largest in order on deep litter, in cages and in aviaries (SD = 0.88, 0.36 and 0.06) and the coefficient of variation from the largest in order in aviaries, on deep litter and in cages (c_v = 48.03, 25.72 and 22.87).

Contaminated eggs have been a problem in free-range systems. The main factors affecting these results are nest contamination or egg laying on litter **[42]**, **[43]**. The study **[37]** reported that the proportion of dirty eggs higher in the free-range system compared to the cage system, especially in the hens' last phase of the production period, could be related to unfavorable rainy weather conditions. In another study **[41]**, no statistically significant differences in the proportion of dirty eggs between enriched cages and cage-free systems were observed.
Also, other studies **[44]**, **[45]** is found contaminated eggs eggshells were more contaminated with aerobic bacteria in an aviary compared to an enriched cage system and is found significantly lower bacterial counts in eggshells from enriched cages compared to an alternative laying hen rearing system **[41]**.

Energy consumption per laying hen and day: The average energy consumption per laying hen and day in the cage breeding system, on the deep litter and in the aviaries, and the statistical evaluation of the energy consumption per laying hen and day are shown in Table 10.

Table 10 The average energy consumption per laying hen and day in the cage breeding system, on deep litter and in aviaries, kW and statistical evaluation of energy consumption per laying hen and day.

Breeding system	n	g ±SD	c_v, %
Cages	92	0.01^{a} ±0.004	38.62
Deep litter	92	0.01^{b} ±0.003	29.66
Aviaries	92	0.01^{a} ±0.002	23.40
F-test		(9.05^{+++}, $p \leq 0.001$)	

Note: n – multiciplity, % – mean in percentage, SD – standard deviation, c_v – coefficient of variation, +++ or $p \leq 0.001$ means a statistically very highly significant difference, different letters in the superscript mean a statistically significant difference $p \leq 0.05$, the same letters in the superscript mean a statistically non-significant difference $p > 0.05$.

The variances of energy consumption per laying hen and day in the three different breeding systems were statistically very significantly different ($F = 9.05^{+++}$, $p \leq 0.001$), which was confirmed by the F-test result, which verified the assumption of the equality of variances. The null hypothesis H0 of no difference between the laying systems was rejected.

The average value of energy consumption per laying hen and day was the same 0.01 kW in all three observed different laying hen breeding systems. Statistical significance ($p \leq 0.05$) in energy consumption per hen per day was recorded by comparing the cage system with the litter system and the litter system with aviaries. No statistical significance was found by comparing the cage breeding system with aviaries ($p > 0.05$). Statistical evaluation of energy consumption per laying hen and day expressed by standard deviation and coefficient of variation revealed a fluctuation of values from the largest in order in cages, on deep litter and in aviaries (SD = 0.004, 0.003 and 0.002, c_v = 38.62, 29.66 and 23.40).

At European level, significant data are available on production levels, production patterns **[46]**, **[47]** and livestock financial accounts [**48**] maintained in the EU. However, relatively little information is available on the energy consumption associated with animal products in the EU and for specific livestock categories. For the development and implementation of the goals and to achieve the goals set in the Green Deal and the Farm to Fork Strategy, a clear understanding of the energy concentrations used in the livestock sector in production systems and production phases is a necessary condition, especially in this period **[49]**.

There is also relatively little EU information on table egg production concerning energy consumption, making it difficult to conclude. A study **[48]** is investigated four egg production systems in the Netherlands, although geographically limited, covered the main production systems in the EU. This study stated that from 20.5 to 23.5 MJ of energy inputs were required to produce 1 kg of eggs and that in all cases, at least 50% of all energy inputs were associated with feed.

Correlation relations between variables studied on the laying hen farm

When evaluating the dependence between variables in the system of breeding hens in cage and cage-free breeding systems, we focused on the strength of dependence r = above 0.5 and -0.5 with statistical significance ($p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$).

Correlation relations between variables studied on the laying hen farm in the caged system:

Correlation relations between variables studied on the laying hen farm in the caged system are shown in Table 11.

Table 11 Correlation relations between variables studied on the laying hen farm in the cage system.

Variable	Feed, hen.day^{-1}	Feed, pc.egg^{-1}	Water hen.day^{-1}	Body weight, g	Egg weight, g	Cracked egg, %	Droping egg, %	Energy in kW, hen.day^{-1}
Mortality	0.73^{++}	0.60^{+}	-0.41^{-}	0.0004^{-}	-0.28^{-}	-0.59^{+}	0.58^{+}	-0.44^{-}
Feed, hen.day^{-1}		0.69^{++}	-0.47^{-}	-0.10^{-}	-0.40^{-}	-0.74^{++}	0.40^{-}	-0.71^{++}
Feed, pc.egg^{-1}			-0.70^{++}	-0.54^{+}	-0.84^{+++}	-0.68^{++}	0.005^{-}	-0.76^{+++}
Water hen.day^{-1}				0.68^{++}	0.76^{+++}	0.66^{++}	0.25^{-}	0.83^{+++}
Body weight, g					0.72^{++}	0.40^{-}	0.55^{+}	0.56^{+}
Egg weight, g						0.61^{+}	0.25^{-}	0.72^{++}
Cracked egg, %							0.02^{-}	0.78^{+++}
Droping egg, %								0.08^{-}

Note: the numerical data means the result of the correlation coefficient (r), - means a statistically non-significant linear relation between two compared variables ($p > 0.05$), + means a statistically significant linear relation between two compared variables ($p \leq 0.05$), ++ means statistically highly significant linear relation between two compared variables ($p \leq 0.01$), +++ means statistically very highly significant linear relation between two compared variables ($p \leq 0.001$).

By evaluating the correlation relationship between two variables for monitored, selected indicators on the farm in the system of breeding laying hens in cages, the power was found from linear positive trivial (r = below 0.1) to linear positive and negative weak (r = 0.1 to 0.3 and -0.1 to -0.3) or linear positive and negative medium (r = 0.3 to 0.5 and -0.03 to -0.05) to linear positive and negative strong (r = above 0, 5 and -0.5), but with a different level of statistical significance or without statistical significance.

A strong linear positive correlation, statistically highly significant $p \leq 0.001$, was noted between water consumption per hen and day and egg weight, also between water consumption per hen and day and energy consumption per hen and day, but also between the proportion of eggs with a cracked shell and energy consumption per laying hen per day.

A strong linear positive correlation, statistically highly significant $p \leq 0.01$, was found between the mortality rate of laying hens and feed consumption per hen, also between feed consumption per hen and day and feed consumption per egg, between water consumption per hen and day and weight laying hens, between water consumption per hen and day and the proportion of eggs with a cracked shell, further between hen weight and egg weight, but also between egg weight and energy consumption per hen and day.

A strong linear positive correlation, statistically significant $p \leq 0.05$, was noted between the proportion of laying hens mortality and feed consumption per egg, also between the proportion of laying hens mortality and the proportion of eggs contaminated with dropping, but also between the weight of laying hens and the proportion of eggs contaminated with dropping, between the weight hens and energy consumption per hen per day and also between egg weight and the proportion of eggs with cracked shells.

A strong linear negative correlation, statistically very highly significant $p \leq 0.001$, was found between feed consumption per egg and egg weight and between feed consumption per egg and energy consumption per hen per day.

A strong linear negative correlation, statistically highly significant $p \leq 0.01$, was found between feed consumption per hen and day and the proportion of eggs with a cracked shell, also between feed consumption per hen and day and energy consumption per hen and day, further between consumption feed per egg and water consumption per hen and day, but also between feed consumption per egg and the proportion of eggs with a cracked shell.

A strong linear negative correlation, statistically significant $p \leq 0.05$, was noted between the proportion of laying hens' mortality and eggs with cracked shells and between feed consumption per egg and laying hen weight.

Correlation relations between variables studied on the laying hen farm in the deep litter system: Correlation relations between variables studied on the laying hen farm in the deep litter system are shown in Table 12.

Table 12 Correlation relations between variables studied on the laying hen farm in the deep litter system.

Variable	Feed, hen.day^{-1}	Feed, pc.egg^{-1}	Water hen.day^{-1}	Body weight, g	Egg weight, g	Cracked egg, %	Dropping egg, %	Energy in kW, hen.day^{-1}
Mortality	0.19^{-}	0.06^{-}	0.34^{-}	0.31^{-}	-0.37^{-}	-0.08^{-}	-0.20^{-}	-0.51^{-}
Feed, hen.day^{-1}		0.05^{-}	0.13^{-}	0.06^{-}	-0.32^{-}	0.06^{-}	-0.45^{-}	0.21^{-}
Feed, pc.egg^{-1}			-0.54^{+}	-0.004^{-}	0.54^{+}	0.67^{++}	0.63^{+}	-0.46^{-}
Water hen.day^{-1}				0.68^{++}	-0.81^{+++}	-0.70^{++}	-0.55+	0.82^{+++}
Body weight, g					-0.42^{-}	-0.20^{-}	-0.24^{-}	0.63^{+}
Egg weight, g						0.74^{++}	0.73^{++}	-0.94^{+++}
Cracked egg, %							-0.43^{-}	-0.61^{+}
Droping egg, %								-0.68^{++}

Note: the numerical data means the result of the correlation coefficient (r), - means a statistically non-significant linear relation between two compared variables ($p > 0.05$), + means a statistically significant linear relation between two compared variables ($p \leq 0.05$), ++ means statistically highly significant linear relation between two compared variables ($p \leq 0.01$), +++ means statistically very highly significant linear relation between two compared variables ($p \leq 0.001$).

By evaluating the correlation relation between the two variables for the monitored, selected indicators on the farm in the system of breeding hens on the deep litter, the power was found from linear positive and negative trivial (r = under 0.1a -0.1) through linear positive and negative weak (r = 0 .1 to 0.3 and -0.1 to -0.3) or linear positive and negative moderate (r = 0.3 to 0.5 and -0.03 to -0.05) to linear positive and negative strong (r = above 0.5 and -0.5), but with a different level of statistical significance or without statistical significance.

A strong linear positive correlation, statistically very highly significant $p \leq 0.001$, was noted between water consumption per layer and energy consumption per layer per day.

A strong linear positive correlation, statistically highly significant $p \leq 0.01$, was found between feed consumption per egg and the proportion of eggs with a cracked shell, further between water consumption per hen and day and weight of laying hens, also between egg weight and proportion of eggs with cracked shells by the shell, but also between the weight of the eggs and the proportion of eggs contaminated with dropping.

A strong linear positive correlation, statistically significant $p \leq 0.05$, was noted between feed consumption per egg and egg weight, also between feed consumption per egg and the proportion of eggs contaminated with dropping.

A strong linear negative correlation, statistically highly significant $p \leq 0.001$, was found between water consumption per hen and day and egg weight and between egg weight and energy consumption per hen and day.

A strong linear negative correlation, statistically highly significant $p \leq 0.01$, was found between water consumption per hen and day and the proportion of eggs with cracked shells and between the proportion of eggs contaminated with dropping and energy consumption per hen and day.

A strong linear negative correlation, statistically significant $p \leq 0.05$, was recorded between feed consumption per egg and water consumption per hen and day, also between water consumption per hen and day and the proportion of eggs contaminated with dropping, further between the proportion of eggs with a cracked shell and energy consumption per laying hen per day.

Correlation relations between variables studied on the laying hen farm in the system aviaries:

Correlation relations between variables studied on the laying hen farm in the system aviaries are shown in Table 13.

Table 13 Correlation relations between variables studied on the laying hen farm in the system aviaries.

Variable	Feed, hen.day^{-1}	Feed, pc.egg^{-1}	Water hen.day^{-1}	Body weight, g	Egg weight, g	Cracked egg, %	Droping egg, %	Energy in kW, hen.day^{-1}
Mortality	-0.09^{-}	0.42^{-}	-0.47^{-}	-0.63^{++}	-0.45^{-}	-0.38^{-}	0.00	-0.25^{-}
Feed, hen.day^{-1}		0.13^{-}	0.53^{+}	0.34^{-}	0.42^{-}	-0.15^{-}	0.05^{-}	-0.08^{-}
Feed, pc.egg^{-1}			-0.54^{+}	-0.55^{+}	-0.45^{-}	-0.34^{-}	-0.71^{+}	-0.66^{++}
Water hen.day^{-1}				0.77^{++}	0.57^{+}	0.36^{-}	0.38^{-}	0.55^{+}
Body weight, g					0.83^{+++}	0.20^{-}	0.51^{-}	0.53^{+}
Egg weight, g						0.17^{-}	0.35^{-}	0.22^{-}
Cracked egg, %							-0.04^{-}	0.03^{-}
Droping egg, %								0.57^{-}

Note: the numerical data means the result of the correlation coefficient (r), - means a statistically non-significant linear relation between two compared variables ($p > 0.05$), + means a statistically significant linear relation between two compared variables ($p \leq 0.05$), ++ means statistically highly significant linear relation between two compared variables ($p \leq 0.01$), +++ means statistically very highly significant linear relation between two compared variables ($p \leq 0.001$).

By evaluating the correlation relationship between two variables for monitored, selected indicators on the farm in the system of breeding laying hens in aviaries, the power from no dependence (r = 0) to linear positive and negative trivial (r = under 0.1 and -0.1) or linear positive and negative weak (r = 0.1 to 0.3 and -0.1 to -0.3) or linear positive and negative medium (r = 0.3 to 0.5 and -0.03 to -0 .05) to linear positive and negative strong (r = above 0.5 and -0.5), but with a different level of statistical significance or without statistical significance.

A strong linear positive correlation, statistically very highly significant $p \leq 0.001$, was noted between hen weight and egg weight.

A strong linear positive correlation, statistically significant $p \leq 0.01$, was found between water consumption per hen per day and hen weight.

A strong linear positive correlation, statistically significant $p \leq 0.05$, was recorded between water consumption per hen and day and egg weight, also between water consumption per hen and day and energy consumption per hen and day, further between hen weight and energy consumption per laying hen and day.

A strong linear negative correlation, statistically significant $p \leq 0.01$, was found between laying hen mortality and hen weight, also between feed consumption per egg and energy consumption per hen per day.

A strong linear negative correlation, statistically significant $p \leq 0.05$, was noted between feed consumption per egg and hen weight and between feed consumption per egg and the proportion of eggs contaminated with dropping.

In the study **[50]** it is stated that the mortality of laying hens and the production of eggs per housed hen are negatively correlated; as a result, the reduction of mortality will increase the productivity per housed hen, even without increasing the production of eggs per present hen. In this research, a linear negative relation was confirmed, but with medium strength without statistical significance ($p > 0.05$) in the system of breeding hens in cages and aviaries (respectively r = -0.45, r = -0.42). A linear positive trivial dependence (r = 0.02) was noted for the laying hen system on deep litter.

A positive correlation exists between the body weight of laying hens and egg weight **[51]**. The results of our research confirmed a positive linear relationship with a strong dependence r = 0.72 statistically highly significant ($p \leq 0.01$) the opinion of the study mentioned above in the system of breeding laying hens in cages and r = 0.83 statistically very highly significant ($p \leq 0.001$) in the system breeding of laying hens in aviaries.
A linear negative medium dependence (r = -0.42) was recorded in the laying hen breeding system on deep litter without statistical significance.

CONCLUSION

In conclusion, we can state that the issue of welfare and its improvement in cage-free systems requires further research to adopt best practices in terms of resource-based, management- and animal-based parameters.

Investigating the effect of microbial indicators on the hygiene of the production of table eggs is an open question for further research both from the aspect of the health of egg consumers and the quality of eggshells.

On the basis of the ongoing evaluated results in practical conditions on the farm, we can state that after the peak of laying at the age of hens 34 to 47 weeks in the months of August, September and October 2022:

- the share of the death of laying hens from the total number of laying hens monitored daily was statistically demonstrably lower in aviaries compared to cages and on deep bedding; in cages and on deep litter was comparable without statistical evidence,
- the intensity of laying was statistically unprovably highest in cages compared to lower in aviaries and statistically lowest compared to deep bedding; in aviaries compared to deep litter it was statistically demonstrably higher,
- feed consumption per laying hen and day was statistically significantly higher on deep bedding than lower in cages and statistically lowest in aviaries; in cages compared to aviaries it was statistically unprovably higher,
- feed consumption per egg was statistically significantly higher on deep litter than lower in aviaries and lowest in cages; in aviaries compared to cages it was statistically unprovably higher,
- water consumption per laying hen and day was statistically unprovably highest in cages, lower on deep bedding and lowest in aviaries,
- the weight of laying hens was comparable in all three monitored breeding systems without statistical evidence,
- the weight of the eggs was statistically significantly higher on litter than lower in cages and lowest in aviaries; in cages compared to aviaries it was statistically demonstrably higher,
- the share of eggs with a cracked shell out of the total number of eggs laid was statistically significantly higher on litter than lower in cages and lowest in aviaries; in cages compared to aviaries it was statistically demonstrably higher,
- the proportion of eggs contaminated with droppings from the total number of eggs laid was statistically significantly higher on bedding than lower in cages and lowest in aviaries; in cages compared to aviaries it was statistically demonstrably higher,
- energy consumption per laying hen per day was statistically demonstrably comparable on deep litter versus cages and aviaries; in cages versus aviaries was statistically comparable without being proven.

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The farm on which we carried out the research is registered by the State Veterinary and Food Administration of the Slovak Republic and is regularly supervised by the fertility control authorities.

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Green resolution and resilience of palm oil exports in Indonesia: Strengthening local value chains

Randi Mamola, Herdis Herdiansyah

ABSTRACT

This study examines the scarcity of palm oil in Indonesia's CPO oil food commodity and the government's conservative steps through green resolution policies and strengthening local value chains. The validation of green economy resolution variable indicators in this study is green financing and local value chains in CPO exports as measured by product prices and production values. In addition to these variables, household consumption expenditure is the control variable used as a determining variable for CPO export levels. The research data uses data from the 2013Q1 to 2022Q4 time series. The research methodology describes the ARDL model for testing long-run effects and the ECM method for observing the economy's acceleration towards equilibrium during short-term shocks. The results showed that the long-term correlation between green financing resolution, product prices, and production value significantly affected the level of CPO exports at a significance level of 5% (p <0.05). However, the variable household consumption expenditure is not significant to the level of CPO exports in the long run at a significance of 5% (p <0.05). Then the short-term correlation shows that the green financing resolution variables, product prices, production values, and household consumption expenditures significantly affect the level of CPO exports at a significance of 5% (p <0.05).

Keywords: green resolution, export CPO, local value chain, local food security, ARDL-ECM

INTRODUCTION

Technological advances and world trade liberalization gave rise to a paradigm of global society in the structure of the international economy **[4]**. The revealed meaning of global society in the world economy has been formed from the events of the global economic crisis in several countries, thus creating the effects of negative and positive conditions on economic development in developed and developing countries **[6]**. Overcoming the future crisis of an increasingly deteriorating economy, world leaders began forming alliances for G20 policy governance to boost the world economy through global trade **[8]**. The current news is that the G20 member countries are jointly promoting prioritized structural policies to promote comprehensive and sustainable economic growth. Economic development planning agencies towards a sustainable system are carried out through conferences and diplomacy in several countries that focus on leading to mutually beneficial trade engagement **[11]**. If reflecting on these regulations, now the whole world is starting to establish a green economy approach as a framework for international trade cooperation **[26]**.

The green resolution application model is a policy strategy step that does not rely on environmental technology to meet needs but reduces scarcity to create a conventional and socially just economy **[7]**. Indonesia, which is a member country of the G20, has begun enforcing green resolutions as an agency for increasing inclusive and sustainable economic growth. Various economic recovery efforts have been prioritized in building green economy resolution consisting of green trading markets, green financing, and green entrepreneurship **[27]**. On the other hand, Indonesia's economic approach is still classified as an extractive and short-term economy **[34]**. That is,

economic activity does not pay attention to the quality of natural resources and the environment, so this has an impact on the scarcity of natural resources.

The case of the phenomenon of production flows and international trade law does not apply to the system in Indonesia, which is currently involved by the scarcity of palm oil, which is a food need in society. Statistical centre data reports Indonesia is listed as the highest palm oil supplying country in ASEAN as evidenced by the level of CPO export in Indonesia worth US$73 billion with total export reaching 9.88% in export destination countries, namely India, China, the European Union, and the United States (US). The interpretation of CPO export growth in Indonesia for three export destination countries from 2013Q1 to 2022Q4, except for 2013 to 2016, CPO export experienced a significant rate (Figure 1). During this period, Indonesia applied additional quotas for CPO production in the US and the European Union. Thus the movement in the rate of CPO export was caused by the high level of demand in the two export countries. Then, since the 2017 period, Indonesia has been faced with intense competition in distributing CPO commodities and changing distribution data to fluctuations. Moreover, the existence of CPOs in the realm of competition, which is quite strong between Malaysia and Thailand, Indonesia must decline due to negligence caused by the scarcity of domestic palm oil (Figure 2). Given this deficit, the Indonesian government must continuously strengthen the palm oil plantation sector by strengthening local value output as a priority for food production.

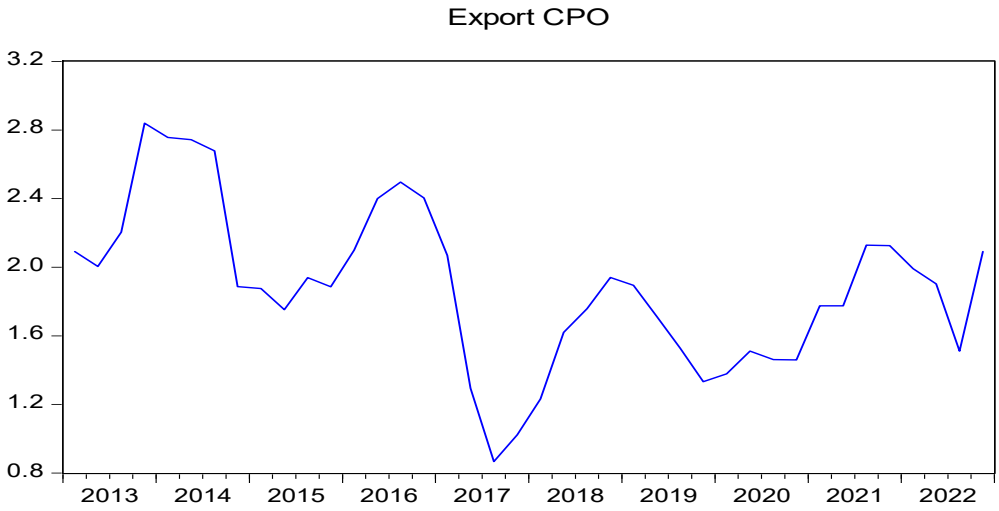

Figure 1 Indonesia CPO export growth rate (2013Q1-2022Q4).

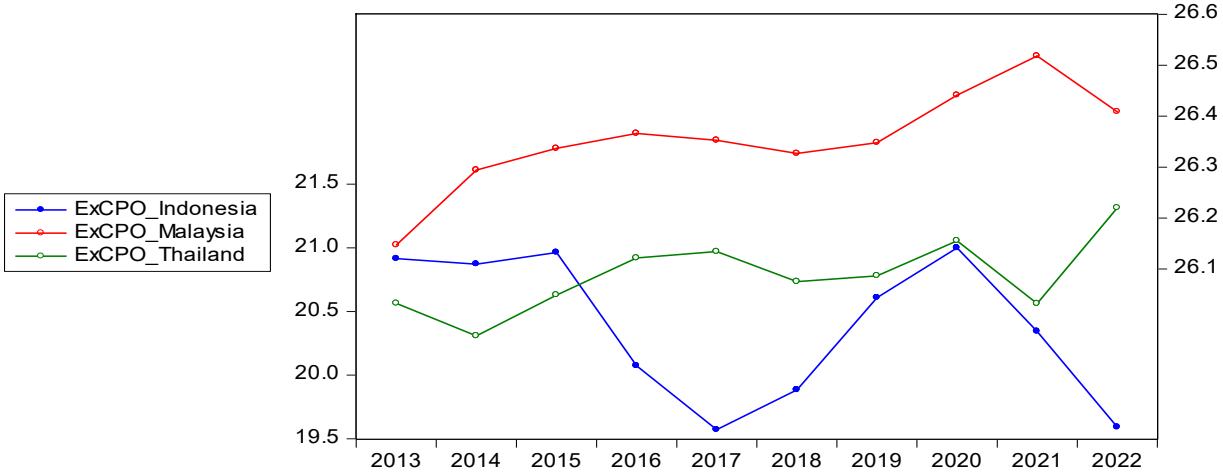

Figure 2 Comparison export of CPO in Indonesia, Malaysia, & Thailand (Current US$).

As the largest palm oil food-producing country in ASEAN after Thailand and Malaysia, Indonesia prioritizes meeting consumption levels and production values, which are continuously increasing, so that palm oil export opportunities in Indonesia can potentially increase the growth rate of the economic sector. Even though CPO production in Indonesia has increased annually, CPO export in 2022 has decreased by US$4.03 billion or 11.4% **[15]**. This condition is caused by a scarcity that causes soaring palm oil prices in society, so the government

requires intervention policies and regulations to maintain the availability of palm oil food by stopping export to several destination countries. The cessation of export activities in Indonesia does not allow the distribution of palm oil food consumption in society to increase as well. Prices soared because of the high world market demand is a view of supremacy in the eyes of Indonesia, especially the government, to stabilize domestic palm oil food prices.

There are various discussions of studies conducted by other researchers to reveal the determinants that affect the export of palm oil (CPO). The research hypothesis **[23]** states that the development of financing to support the integration of environmental resources affects the increase in the contribution of CPO food export in Indonesia. Then, the relationship to the hypothesis of the research experiment by **[32]** explains that price and production value determine changes in the CPO export rate structure. The country will export its products to add value to the product's price towards trade openness and meet domestic needs for the goods it produces. As with the study of absolute or absolute advantage, trade theory is related to state profits as a producer of goods or services in the specialization of fulfilling domestic needs and the trade value chain.

Based on the phenomena described, this study explores how strong green financing resolutions are in facilitating the reinforcement local value chain of palm oil food export in Indonesia. The urgency of this research provides an interesting discussion because there are two topics. First, the existence of CPO production tends to be exploitative, so the efficiency of palm oil production has not considered environmental elements and the empowerment of local production. Second, Indonesia is the largest CPO exporting country in ASEAN, experiencing a scarcity of the food commodity palm oil, which causes the price of palm oil to soar.

Scientific Hypothesis

The scarcity of priority food sectors such as palm oil is a crucial issue for policymakers, given their significant contribution to increasing economic growth in Indonesia. International cooperation ties motivated Indonesia to join in sparking green resolution talks by focusing on principles of the local value chain in domestic food production. Strong suspicions regarding the theory of international trade law and the empirical study of **[9]**, researchers formulate a hypothetical framework for this research in long-term and short-term analysis with the following variable criteria:

H1: Increasing the resolution of green financing will have a negative impact on CPO export in the long term and the short term.

H2: An increase in product prices will positively impact CPO export in the long term and the short term.

H3: An increase in production value will positively impact CPO export in the long term and the short term.

H4: An increase in household consumption expenditure will positively impact CPO export in the long term and the short term.

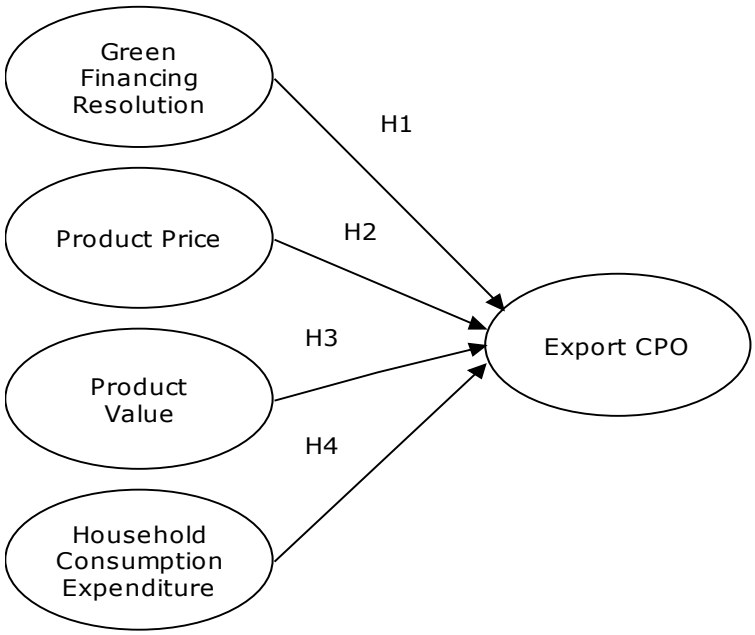

Figure 3 Research hypothesis.

MATERIAL AND METHODOLOGY

Description of the Experiment

The focus or scope that becomes the research study variable raised is the CPO export level variable in Indonesia as the dependent variable to the independent variable, namely green financing resolution, product prices, and production value. While the control variable in this study, namely household consumption expenditure. This type of research method is a quantitative method that is processed using time series analysis, which is a type of data analysis by predicting annual data from 2013:Q1 to 2022:Q4 in Indonesia.

In addition, this research defines data operationalization as an additional econometric estimate that captures the structure of various empirical studies descriptively (Table 1). By understanding the characteristics of the status variable, the researcher obtains accurate information from the type of data source to be processed and processes the output of the linear regression estimation, which will be discussed according to the hypothesis stated in the researcher's frame of mind.

Table 1 Data operationalization.

Variable Determinants	Symbol	Description	Empirical study
Export CPO	EXCP	Export transfers commodity products produced by service providers or state agencies to consumers and competitors in other companies. The level of commodity export volume on the world market has factored in increasing export performance, namely domestic production, international product prices, and consumption value.	**[24]**, **[31]**
Green financing resolution	GF	Green financing resolution refers to financial investment to finance green development projects, environmental preservation, and policies that drive the economy toward a sustainable system. The definition of green financing resolution in Indonesia is defined as government support in carrying out sustainable growth in financial services and is a combination of economic, social, and environmental actors.	**[3]**, **[5]**
Product price	PC	Product prices in international trade mean the transaction value of the exchange of goods and services between exporters and importers based on agreements between countries. The high and low value of the price of export commodity products depends on the quantity and quality of goods produced by looking at competition factors and price stability.	**[19]**, **[28]**
Production value	PV	Production value is a series of formations of output value through the stages of a combination of input sources that aim to increase the value of production benefits for the use of goods and services. The physical characteristics of production value must rely on resources such as the labour force, capital, and natural elements (soil, minerals, and natural materials).	**[21]**, **[29]**

Table 1 Cont.

Household consumption expenditure	HCE	The measure of household consumption expenditure is a measure of the value of community welfare indicators, both individually and socially, which show the stages of aggregate economic growth. A stable consumption expenditure ratio indicates that the level of income earned by household actors is higher, and vice versa; if the income disparity increases, it will have consequences for low purchasing power in household consumption expenditures.	**[17]**, **[18]**

Data

The data compilation method in this research uses a series of secondary report sources. Secondary data is collected by searching the legal basis, writings, news, and research related to the selected topic **[14]**. In addition, data collected in this research was obtained from the Central Bureau of Statistics, the Indonesian Family Life Survey (IFLS), and the Ministry of Environment, as well as other supporting data cited from literature studies, books, and previous research. The stages of data analysis in this research used ARDL and ECM regression through a series of stationarity tests (Unit Root).

Statistical Analysis

ARDL is an econometric testing tool that assumes that the variables studied will affect the variables themselves in the previous year **[2]**. The stages of testing the ARDL are the same as the ECM model test, which lies in analysing the data stationarity test, the optimal lag test, the cointegration test, and ARDL linear regression **[10]**. The optimal lag length used for regression estimation is based on the stationarity level criteria **[2]**. According to **[10]**, if the estimated lag length displays a level different value for the regression model, this lag length result will be chosen to determine the ARDL regression model. The specifications for the results of ARDL and ECM processing in this study, our analysis uses the input application of the Eviews 9 statistical software.

The formulation of the time series in this study is to examine the influence of green financing resolution variables, product prices, and production values, as well as control variables that affect the level of CPO export in Indonesia, namely household consumption expenditure. Based on the variables to be studied, the model or regression equation can be written as follows:

$$\mathrm{EXCP_t} = \beta_0 + \beta_1 \mathrm{GF_t} + \beta_2 \mathrm{PC_t} + \beta_3 \mathrm{PV_t} + \beta_4 \mathrm{HCE_t} + \mu_t \quad (1)$$

After testing several series of stationary and optimal lag tests, the above model can be converted into an equation analysis of the ARDL model as follows **[6]**:

$$\Delta \mathrm{LnEXCP_t} = \alpha_0 + \sum_{i=1}^{p} \alpha_1 \, \Delta \mathrm{LnEXCP_{t-i}} + \sum_{i=1}^{p} \alpha_2 \, \Delta \mathrm{LnGF_{t-i}} + \sum_{i=1}^{p} \alpha_3 \, \Delta \mathrm{LnPC_{t-i}} + \sum_{i=1}^{p} \alpha_4 \, \Delta \mathrm{LnPV_{t-i}} + \sum_{i=1}^{p} \alpha_5 \, \Delta \mathrm{LnHCE_{t-i}} + \beta_1 \mathrm{LnEXCP_{t-i}} + \beta_2 \mathrm{LnGF_{t-i}} + \beta_3 \mathrm{LnPC_{t-i}} + \beta_4 \mathrm{LnPV_{t-i}} + \beta_5 \mathrm{LnHCE_{t-i}} + \mu_t \quad (2)$$

Where:
$LnEXCP$ = CPO export; $LnGF$ = Green financing resolution; $LnPC$ = Price product; $LnPV$ = Production value; $LnHCE$ = Household consumption expenditure; $\alpha_1 - \alpha_5$ = Short-term estimation coefficient; $\beta_1 - \beta_5$ = Coefficient of long-run estimation.

Test the initial stages of the (ARDL) analysis processing procedure starting from the bound cointegration test, namely carrying out an equation model test using the ordinary least square method, which aims to simultaneously provide short-term and long-term results **[2]**. Statistical F-test test is a test to analyze long-term correlation regarding the estimator variable being tested **[2]**.

Results of regression testing of model equations that have a long-term relationship, there is an explanation for verification of the error rate of the results listed in the short-term model **[16]**. The purpose of testing the ECM model analysis in a model is to see whether the model that has been analyzed in the long-term model has an effect or not on the variable **[16]**. In other words, the ECM test is an analysis stage to check for error correction from the previous variable **[20]**. Furthermore, the ECM test is carried out after testing the long-term model to determine the cointegration and influence between variables in the short term with the following model equation **[13]**:

$$\Delta \mathrm{LnEXCP}_t = \alpha_0 + \sum_{i=1}^{p} \alpha_1 \, \Delta \mathrm{LnEXCPO}_{t-i} + \sum_{i=1}^{p} \alpha_2 \, \Delta \mathrm{LnGF}_{t-i} + \sum_{i=1}^{p} \alpha_3 \, \Delta \mathrm{LnPC}_{t-i} + \sum_{i=1}^{p} \alpha_4 \, \Delta \mathrm{LnPV}_{t-i} + \sum_{i=1}^{p} \alpha_5 \, \Delta \mathrm{LnHCE}_{t-i} + \theta \mathrm{ECT}_{t-i} + \mu_t \quad (3)$$

Where:
θECT_{t-i} = Variable Error Correction (residual) of the previous period.

The coefficient value of the ECM model displays the degree of quick suitability for the balance between long-term and short-term economies constrained by shocks **[20]**. The variable values of the ARDL and ECM models have valid criteria for seeing the level of significance of a variable equation and the correlation of cointegration values between the dependent and independent variables **[16]**.

RESULTS AND DISCUSSION

Selection of the ARDL and ECM Models: The selection of model variables analyzed by this study were independent variables, namely green financing resolution, product prices, and production value, as well as household consumption expenditure as a control variable. The dependent variable data in this research is the level of CPO export. Testing the estimation of the ARDL and ECM models analyzes the long-term and short-term equations between the relationship between the independent and dependent variables **[2]**, **[10]**, **[16]**. Before determining the selection of ARDL and ECM models, a step testing process is needed, namely the unit root test (stationarity) **[11]**. The stationarity test is a test on a time series model that is useful for knowing whether or not the estimated data is affected by the problem of unit roots. If the results of the estimation of the data obtained contain unit roots, it is stated that the data is not stationary, so the estimated data is spurious.

The spurious regression model in the time series analysis equation is a type of regression that looks at the relationship and influence between the dependent and independent variables, which shows significant results in terms of probability, but the magnitude value diagnosis does not display a regression coefficient that matches the residual value **[2]**, **[10]**, **[11]**. The step to avoid the problem of coaxing correlation in estimating the variables to be studied is through a stationarity test (unit root test) which is used to estimate whether the regression is stationary or not. The absolute completeness requirements for stationarity testing using the Augmented Dickey-Fuller test method are explained in the following table:

Table 2 The results of the level stationarity test.

No.	Variable	Level		Description
		ADF	MacKinnon Critical Limit (5%)	
1.	LnEXCP	-2.771384	-2.941145	Not stationary
2.	LnGF	-5.040588	-2.938987	Stationary
3.	LnPC	-1.775495	-2.938987	Not stationary
4.	LnPV	-1.978861	-2.941145	Not stationary
5.	LnHCE	-1.511987	-2.943427	Not stationary

Note: Source – Eviews 9.0 data processing.

Based on the results of the stationarity test (unit root) (Table 1), all variables are at levels. Variables that do not pass the stationary test at the level are CPO export, product prices, production values, and household consumption expenditures. These three variables can be seen from the statistical values, which show the absolute value of ADF is lower than the critical value (MacKinnon = 5%). The difference in the stationarity value of the green financing resolution variable at the level has the largest ADF absolute statistical degree compared to the

critical scale (MacKinnon = 5%); it is concluded that the level of observation of the green financing resolution variable data indicates stationary. Stationary results that vary across the test factors require further stationary experiments at the 1st difference with the details below:

Table 3 Stationarity test results at the 1st difference level.

No.	Variable	Level		Description
		ADF	MacKinnon Critical Limit (5%)	
1.	LnEXCP	-4.322076	-2.941145	Stationary
2.	LnGF	-3.454610	-2.941145	Stationary
3.	LnPC	-6.650760	-2.941145	Stationary
4.	LnPV	-10.74242	-2.941145	Stationary
5.	LnHCE	-7.473374	-2.943427	Stationary

Note: Source – Eviews 9.0 data processing.

After verifying the stationarity test values at the 1st difference on all research variables (Table 2), it is known that they meet the standard stationarity. The variables as a whole have estimated regression values which show that the ADF stationary test has a large difference from its critical limit (MacKinnon = 5%). This means that there are no unit root tests, and all research equations pass the stationarity test.

Estimation of the Long-Term Model (ARDL) and Short-Term Model (ECM): Since the value has been generated by the unit root test (stationarity), the estimation equation can be continued through long-term analysis. The method that must be considered to determine the best estimation criteria for the ARDL and ECM models is stationarity at the level. The ARDL model automatically determines its model by weighting the best stationary test based on the level 1st difference value. The long-term estimation results of ARDL are as follows:

Table 4 ARDL long-term model estimation with 1st difference value.

Regression coefficient				
Variables	**Coefficient**	**Std. Error**	**T-statistics**	**Prob.**
D(LnEXCP(-1),-2)	0.779773	0.189834	4.107655	0.0007**
D(LnGF(-1)	13.305137	4.427599	3.005046	0.0076**
D(LnPC(-2)	2.219864	1.493220	1.486629	0.0040**
D(LnPV(-1),-3)	-0.039756	0.019273	-2.062728	0.0054**
D(LnHCE)	-0.152002	0.305549	-0.497471	0.6249**
CointEq(-1)	-1.263935	0.224449	-5.631271	0.0000**
Cointeq = LnEXCP - (-4.5049*LnGF + 5.3742*LnPC + 0.0775*LnP – 0.1203*LnHCE -0.6583)				

Note: Source – Eviews 9.0 data processing. *** p <0.01, ** p <0.05, * p <0.1.

Table 5 Long-Term model coefficients.

Variable regression	Coefficient	Std. Error	T-statistics	Prob.
LnGF	-4.504930	0.751797	-5.992214	0.0000**
LnPC	5.374187	0.906346	5.929510	0.0000**
LnPV	0.077517	0.025279	3.066459	0.0066**
LnHCE	-0.120261	0.245727	-0.489409	0.6305**
C	-0.658267	0.980034	-0.671678	0.5103**
R^2	0.907573			
F-stat	10.39691			
Prob (F-stat)	0.000004**			

Note: Source – Eviews 9.0 data processing. *** p <0.01, ** p <0.05, * p <0.1.

Data processing above (Table 4), the estimation of the ARDL model uses level 1st difference value. The endogenous variable studied was the level of CPO export, while the exogenous variables consisted of green financing resolution, product prices, production value, and household consumption expenditure. This model uses a significance level of 5% (p <0.05) with the long-term ARDL method, which analyses that the t-statistic test for green financing resolution, product prices, and production value has a higher t-statistic than the t-table (1.6883). However, the household consumption expenditure has a lower t-statistic than the t-table (1.6883). A simultaneous test of 0.00004 means that the simultaneous impact of exogenous variables can affect the level of CPO export. These results concluded that the variable green financing resolution, product prices, and production value had a relevant effect on the level of CPO export in the long term.

The balance of the economy is not only observed by long-term relationships but must explore the impact on the economic side in the short term. Short-term relationships show fast-growing economic conditions and restore long-term balance if you experience a shock in the short-term **[14]**. The research variables in the estimation of the ECM short-term model through unit root testing (stationarity) are as follows:

Table 6 ECM short-term model estimation with 1st difference.

Variable regression	Coefficient	Std. Error	T-statistics	Prob.
LnGF	-1.638781	2.762418	-2.593241	0.0078**
LnPC	0.681698	1.836647	2.371164	0.0133**
LnPV	0.310150	1.320553	2.493850	0.0253**
LnHCE	0.503407	1.243436	2.013993	0.0089**
ECT(-1)	-0.248661	1.205716	-2.208756	0.0368**
R^2	0.973673			
F-stat	48.74385			
Prob (F-stat)	0.000000**			

Note: Source – Eviews 9.0 data processing. ***p <0.01, ** p <0.05, * p <0.1

The regression model error correction model (ECM) of -0.248661. The ECT probability is 0.0368 at a significance limit of 5% (p <0.05), meaning that the absolute value for obtaining economic balance in the short-term model is valid or meets the residual standard. The development of variables in the short-term estimation (ECM) proves individually to have a significant coefficient at 5%, which states that the t-statistical test for all independent variables is very high from the t-table (1.68830). The simultaneous test analysis also meets the criteria because of the Prob. (F-statistic) is 0.00000, meaning that the simultaneous equation in all independent variables influences the dependent. Based on these results, it was concluded that green financing resolution variables product prices, production values, and household consumption expenditures significantly influence the level of CPO export in the short-term.

The Contribution Diagnosis of Green Financing Resolution and CPO Export in the Long-Term and Short Term: The contribution impact to the resolution of green financing has the consistency to reach equilibrium in the long term, which is estimated to be around 2.4 quarters or around 4.3 months (Figure 3). So far, the contribution of the long-term response to the resolution of green financing in Indonesia has met the requirements for reducing the CPO export quota, which encourages food commodity resilience, anti-exploitation, and is environmentally friendly **[27]**. On the other hand, a review of green financing resolution can adapt to instruments for strengthening local food production or local value chain which provide priority food security supplies such as palm oil in the short-term **[9]**.

The short-term estimation findings also adjust for the same results as the long-term and show that green financing resolution produces a negative and significant effect. The results specifically explain that a 1% increase in green financing resolution policies impacts reducing the standard deviation of around 0.7517 and 2.7624 to the ratio of the average CPO export level in Indonesia. This finding is in line with previous empirical results, namely, among others, **[9]**, **[12]**, and **[25]**.

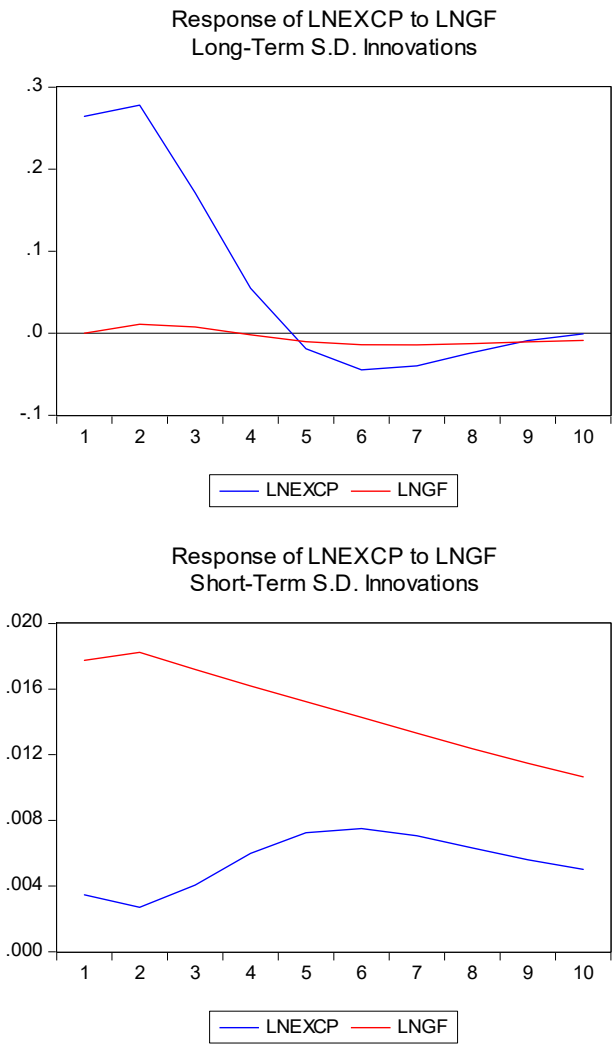

Figure 4 Diagnosis of green financing resolution responses to CPO export in Indonesia period 2013Q1-2022Q4. Source: Processed data.

In the long term, green financing resolutions have a support obligation for economic sustainability, especially international trade **[35]**. Export marketing activities that are not supported by the reconstruction of the use of natural resources and are only limited to economic benefits, on the contrary, cause scarcity due to exploitation without thinking about resilience and sustainability in the future **[34]**. The relationship between green financing resolution in the short-term can control green investment to reduce unhealthy export. This means that export activities are limited according to the capacity of the local production chain, which aims to minimize dependence on imported oil by tightening the resilience of palm oil production to reduce the scarcity of domestic demand **[25]**. The level of CPO export is high and is not matched by stock availability and production balance, so in the short term, polemics will emerge regarding the disparity in consumption output **[23]**. Green financing resolution from a collective perspective of the production chain can finance the acceleration of local palm oil food production security that is environmentally friendly and sustainable **[3]**.

The Contributions Diagnosis of Local Value Chain and CPO Export in the Long-Term and Short-Term: The quality precision of strengthening the local value chain is determined by how much domestic production is produced in meeting the availability of distribution of goods and services in the producing areas **[4]**. For example, the food production capacity of crude palm oil is converted into finished or ready-to-use goods, which will be distributed in the form of CPO marketing to various countries. The high demand for palm oil food supply indicates that the contribution of CPO export in Indonesia has significantly brought the dynamics of the production line into the circulation of the temporal trade subsystem **[29]**. In line with the comparative trade theory, the local value chain is an interaction factor influenced by product prices and production values to achieve an equilibrium level of the trade balance [6]. Household consumption expenditure also plays an important role in moderating local value chain interactions on the resilience of CPO export **[30]**.

Based on the aggregate level, the diagnosis of the contribution of green financing resolution and strengthening of local value chains to the value chain indicators, namely product price and production value, shows a significant contribution both in the short and long-term, except for household consumption expenditure in the short term which does not show a significant diagnosis. In short, the implications of green financing resolutions facilitate sustainability activities to reduce imported oil food production and finance responses to developing local value chain indicators in encouraging CPO export resilience, as shown in the following graph in Figure 4.

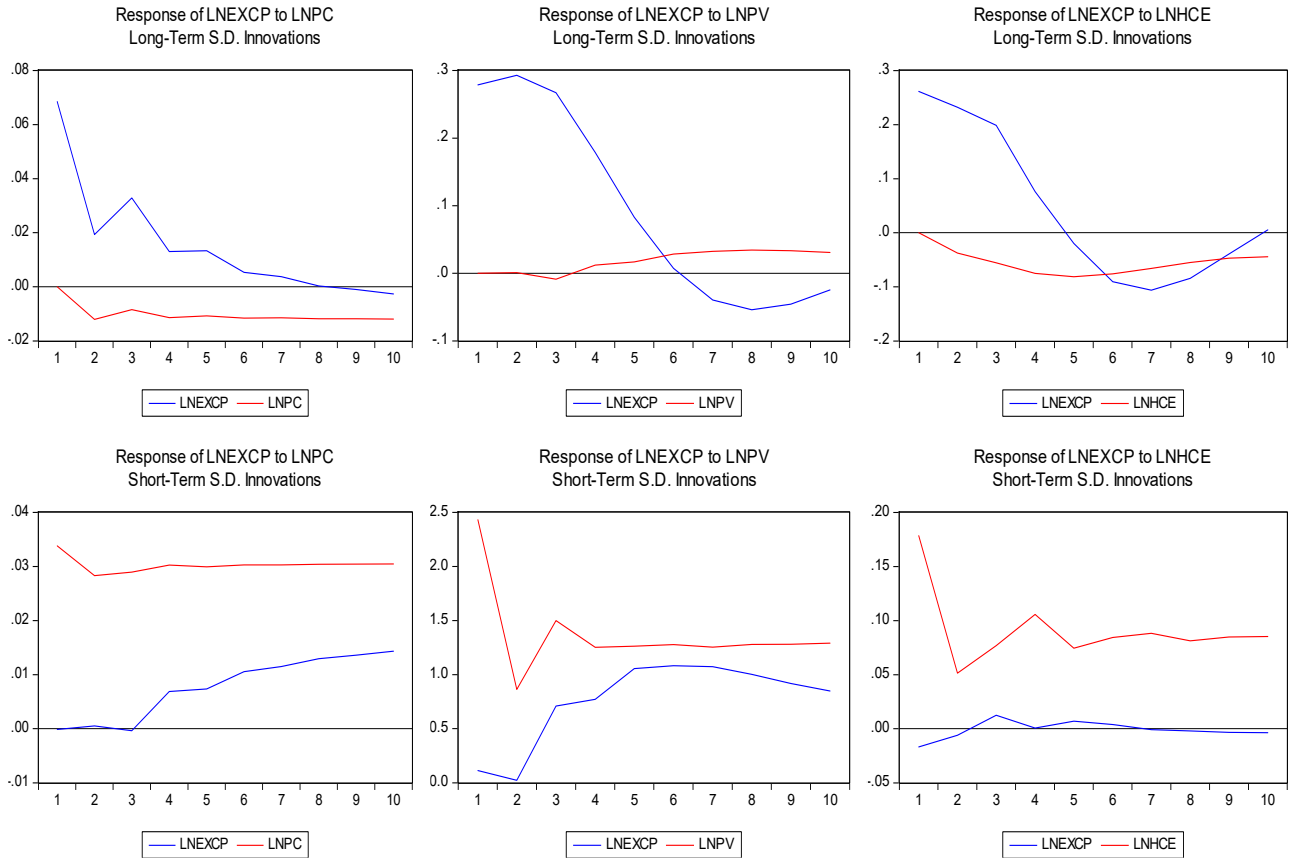

Figure 5 Diagnosis of local value chain response to CPO export in Indonesia period 2013Q1-2022Q4. Note: Source – Processed data.

Product prices were found to have a positive effect in the long-term and short-term on CPO export, and this means that an increase in this variable causes an increase in the local value chain system to support the resilience of palm oil export in Indonesia. Therefore, a 1% increase in product price has the power to change the response of local value chain indicators by 0.9064 to 1.8367%, which corresponds to research **[1]**, **[22]**, and **[28]**. Importing countries will consume palm oil food production from exporting countries if product price stability decreases, but the increasing consumption needs of other countries to consume CPO oil food make countries have to buy imported CPO oil products at a fixed price **[24]**. This advantage will be achieved if the exporting country can increase the value chain of local production and set a high price for exporting palm oil food commodities to importing countries.

The positive relationship between production value and export CPO in Indonesia is due to the demand shock effect, both in the long term and short term. Once the level of trade reaches balance, a higher productivity value of 1% encourages producing countries to increase output, or in other words, production values contribute to the export resilience response of 0.0253 to 1.3206%. The implication of strengthening the value of palm oil food production is a factor in forming the resilience of CPO export in Indonesia **[1]**. When the production value increases, the number of CPO export offers also increases and conversely **[28]**. Achieving local production values is no less important than increasing the production chain's capacity to market the palm oil industry. The success of the surplus to the stability of production value can be identified by strengthening local value chain innovation so that indicators of production value and the CPO export trade balance in Indonesia can reach the maximum balance point **[22]**.

The focus on long-term coefficient estimation shows that household consumption expenditure does not significantly affect CPO export in Indonesia with a negative significant. In contrast, the results of short-term

diagnostic specifications are positive and significant. The discussion stated that the reduction in household consumption expenditure in strengthening the local value chain resulted in a CPO export coefficient of 0.2457 to 1.2434%. Once observed, this response supports the findings of **[18]**, **[33]** with no significant effect because export trends do not only focus enough on the level of consumption in a country but also view the exchange rate as a measure of volatility in international trade. The fluctuating exchange rate causes the volume of CPO oil export prices to increase drastically, so importing countries experience a deficit due to high import tax rates **[19]**, **[22]**. This disturbance of exchange rate volatility fluctuations makes household consumption expenditures not correlate with CPO export in Indonesia **[18]**.

CONCLUSION

This study defines two parts of the analysis of independent and control variables in the long-term and short-term estimation using the ARDL and ECM approaches. The results of the processing of green financing resolution variables have a negative and significant effect on the level of CPO export, both in the long term and in the short term. This means that every increase in green financing resolution can reduce the limit for CPO export activities to tighten domestic oil demand. Then, the research direction consists of local value chain variables, namely product prices, production values, and household consumption expenditures, as control variables. The long-term and short-term approaches to the product price variable and production value positively affect the CPO export level, where when the product price and production value increase, the CPO export level also increases. These results have been consistent in supporting studies of previous empirical research results. Only household consumption expenditure has a negative and insignificant long-term effect. However, in the short term, household consumption expenditure positively and significantly affects the level of CPO export.

The results of this study resulted in several recommendations: first, regulation of the G20 cooperation in Indonesia, the role of the government needs to create intervention policies that focus on building a green economy structure in reducing the scarcity of natural resources, especially in the palm oil commodity. Apart from looking at export objectives, environmental empowerment of the palm oil production process is expected to change the export order, which is oriented towards excellence and competitiveness and can sustainably process local resources. Second, Indonesia is the highest CPO exporting producer in ASEAN and should be able to meet palm oil production in its own country. Through strengthening local added value, the downstream supply chain for palm oil production must rely on local raw materials and sustainable import substitution to support domestic production. With this strengthening, the government's role can restructure export policies to reduce scarcity, affecting the stability of palm oil commodity prices.

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Conflict of Interest:

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

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The effect of storage temperature on the quality of avocado fruits from different climatic zones

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ABSTRACT

Avocado is one of the most valuable products, as it is characterized by a high content of biologically active substances, including vitamins, mineral elements, fats, and dietary fibers. According to a complex of organoleptic and physicochemical indicators, the consumption properties of avocado fruits from different countries of origin, which are sold in Ukraine, have been investigated. Among the organoleptic indicators, the state of peel and pulp, taste, and smell has been determined according to the developed scoring scale. It has been established that the Haas type (Colombia) fruits have a light green pulp and a deep green peel that does not lag well behind the flesh, they are quite firm, the taste is watery, and there are no significant defects, the stem is not damaged. Haas (Israel) avocados had light green pulp and a brownish-black peel that separated from the flesh very well, with little evidence of pollination, a nice buttery flavour, and a nice texture. There is a slight peel defect (pollination mark) with an area of less than 4 cm^2, which does not affect the fruit's flesh, and the stem is not damaged. The fruit of the Fuerte type (Israel) had a light green pulp and a deep-green peel that did not lag well behind the flesh, a somewhat grassy taste, and a loose flesh texture. The fruit had a defect in the peel (lens) with an area of less than 6 cm^2, which does not affect the fruit's flesh, and the stem is not damaged. It has been found that the researched types of avocado fruits from different countries of origin differ in shape, size, and the ratio of peel, pulp, and stone. From the physicochemical parameters, the mass fraction of moisture, the content of dry soluble substances, active acidity, the content of ascorbic acid, and the fatty acid composition of lipids of avocado fruits have been determined.

Keywords: avocado, quality, consumption properties, fatty acid composition

INTRODUCTION

In most countries, the production of fruit and vegetable products increases annually due to the growing demand for them both on the national and global markets. According to the Healthy Nutrition Plate developed by experts from the Harvard School of Public Health, more than half of the daily diet shall consist of a variety of fruit and vegetable products, and the share of plant products, in general, can increase to 70-75% **[1]**. More than 400 types of avocados are known, among which the most popular for consumers are the fruits of Zutano, Big, Haas, Fuerte, Pinkerton, Ettinger, Bacon, Gwen, Reed, Puebla, and Cocktail varieties. The size, weight, peel color, and nutritional properties of avocados, including the mass fraction of lipids, vary and depend on the avocado type and the fruit's country of origin. The main suppliers of avocado fruits to the world market are RSA, Peru, Israel and Kenya, Mexico, Spain, and Chile. The main feature of avocado, distinguishing it from other fresh fruits, is a high mass fraction of lipids, which can vary depending on the type, country of origin, and harvest season, ranging from 3-30% and is 23.5% on average **[2]**. Unlike lipids of animal origin, avocado lipids are easier to digest and do not contain cholesterol. They are mainly represented by triglycerides (85%); the remaining 15% are mono- and diglycerides, phospholipids, and glycolipids. Free fatty acids are present in small amounts. The main fatty acids that make up glycerides are oleic (depending on the type, its share ranges from 49 to 73%), palmitic (15.7-30.8%), linoleic (0.3-15.8%), and palmitoleic (2.8-11.0%) **[3]**.

Basic information on the quality of avocados is presented in the UNECE standard FFV-42 **[4]**. Avocados' packaging and transportation is carried out per Code of Practice for Packaging and Transport of Fresh Fruit and Vegetables (CAC/RCP 44-1995) **[5]**. Relative humidity should be 90%. The optimal storage temperature depends on live on the variety and ripeness of the fruits. Considering the differences in the chemical composition of avocado fruits of different types and producing countries, suitability for storage, duration, conditions of transportation, and sale, the study of the consumption properties of avocado fruits, which are sold in Ukraine, is relevant.

Scientific hypothesis

The hypothesis of the scientific work is to establish the dependence of the consumption properties of avocado fruits of different types and countries of origin on the temperature conditions of storage to determine the optimal storage terms while preserving the marketable quality.

MATERIAL AND METHODOLOGY

Samples

The object of the research was avocado (*Persea americana*) fruits of three different types: Haas and Fuerte. The researched fruit samples were delivered to the laboratory for experimental research in compliance with the recommended temperature regimes along the entire logistical path.

Chemicals

Acetone, C_3H_6O (Torhovyi Dim Enerhostroiinvest, Ukraine; Sodium hydroxide, NaOH (Khimlaborreaktyv LLC, Ukraine), Ascorbic acid, vitamin C (Khimlaborreaktyv LLC, Ukraine), Metaphosphoric acid, HPO_3 (Khimlaborreaktyv LLC, Ukraine), Pyrocatechin, $C_6H_4(OH)_2$ (Khimlaborreaktyv LLC, Ukraine), Chloroform, $CHCl_3$ (CHEMICO GROUP, Great Britain), Methanol, CH_3OH (CAS, Netherlands), Hexane, C_6H_{14} (Hammerite, Netherlands).

Animals and Biological Materials

The following types of avocados were chosen for the study: Haas type, country of origin - Colombia, importer (Flamingoco); fruits of the Haas type, country of origin - Israel, importer (Nature's Pride); fruits of the Fuerte type, country of origin - Israel, importer (Nature's Pride).

Instruments

Drying cabinet SNOL 67/350 (Thermoengineering LLC, Ukraine), titration device (Labor-Technik LLC, Ukraine), analytical electronic balance KERN ABS 120-4 (Khimtex SE, Ukraine), refractometer IRF-454B2M (KOMZ JSC), pH meter ULAB MP 511 (ULAB, China), gas chromatograph Kristallux-4000M (Meta-Chrom Research and Production Company), refrigerator GGM Gastro (GGM Gastro, Germany).

Laboratory Methods

Experimental studies were carried out using modern standards, generally accepted, and special organoleptic and physicochemical methods. The mass fraction of moisture was determined by drying to a constant mass **[6]**; the content of dry soluble substances – by the refractometric method **[7]**; active acidity – by the potentiometric method **[8]**; the content of ascorbic acid – by the iodometric method **[9]**; fatty acid composition of lipids – by the chromatographic method.

Description of the Experiment

Sample preparation: after evaluating the organoleptic and physicochemical indicators of freshly purchased avocado samples, their quality was studied during 14 days of storage. Avocado samples were stored in a GGM Gastro refrigerator at a temperature of 3-5 °C and a room temperature of 15-18 °C. Fruit from the same batch was taken as a control sample.

Number of samples analyzed: 150.

Number of repeated analyses: 5.

Number of experiment replication: 3.

Design of the experiment: At the first stage of the study, the organoleptic indicators of the quality of avocado fruits were determined: the condition of the peel and pulp, taste, and smell. At the second stage of the study, physicochemical parameters were determined: mass fraction of moisture, the content of dry soluble substances, pH, the content of ascorbic acid, and fatty acid composition. In the last stage, we processed the obtained results, subjected them to statistical analysis, and checked the validity of our hypotheses.

Statistical Analysis

Origin 2021 software (OriginLab Corporation, Massachusetts, USA) was used for data analysis. All assays were repeated at least three times independently, and the experimental data were represented as mean ± standard deviation. The means were compared by Tukey's multiple range test at $p < 0.05$.

RESULTS AND DISCUSSION

The analysis of the scientific works of foreign scientists showed that sufficient attention is paid to the study of avocado quality. In particular, scientists **[10]** investigated changes in the quality of avocado fruits using two storage temperature regimes – T1 (+8 °C) and T2 (+17 °C) for 96 hours with the use of pretreatment of fruits with exogenous ethylene (C_2H_4) to speed up the process of their maturation. The issue of the development process of avocado fruits **[11]**, their ripening after harvesting **[12]**, the influence of different storage conditions on the control of black spot development **[13]**, and in work **[14]** are covered the causes of heterogeneous fruit ripening. The influence of cold shock on the shelf life of naturally ripened avocado fruits is covered in the publication of Jiao Chen, Xixia Liu, Fenfang Li, Yixing Li, and Debao Yuan **[15]**. The research showed that immersing avocado fruits in ice water for 30 minutes effectively slows their biochemical processes. The method proposed by scientists can be used to extend the shelf life of avocados. Alaika Kassim and Tilahun Seyoum Workneh investigated the effect of the combined processing of Hass avocado fruits after their harvest on changes in the fruits' physical, chemical and sensory properties during 28 days of cold storage **[16]**. It was determined that the proposed cold storage conditions (5.5 °C for two days, 5 °C for six days, and 4.5 °C for 20 days at 95% relative humidity) ensure the highest preservation of fruit quality. The combination of a wax coating, packaging made of low-density polyethylene, and the above-mentioned cold storage conditions helps delay avocado fruit ripening by about two weeks. Cold storage has been proven to be important for extending the shelf life and maintaining the quality of avocados during export. The scientists **[17]**, **[18]** substantiated the possibility of using bioactive films to preserve the quality of avocados and extend their shelf life under refrigeration conditions and at room temperature. In research confirmed the feasibility of using packaging with a modified environment to extend the shelf life of Hass avocado fruits **[19]**. In **[20]**, the effect of pre-treatment avocado fruits with 1-Methylcyclopropene (1-MCP) to extend their shelf life after harvest was investigated. Fruits treated with 1–MCP up to 14 days of storage showed similar peel firmness and color values as those treated at harvest. However, when 1–MCP pretreatment was applied in 21 days, the fruits showed signs of ripening similar to those not treated. These research data will help producers to choose the optimal time for applying 1-MCP in Hass avocados and contribute to a deeper understanding of the molecular mechanisms of the avocado ripening process **[21]**. The scientists **[22]**, **[23]** analyzed changes in the structure and composition of the avocado cell wall during softening. Cell wall pectins of Hass avocado fruits were studied during ripening at 20°C after harvest and after cold storage. The scientists **[24]** developed mathematical models that can be used to predict the ripening of a certain batch of Hass avocados in various logistics chains after harvest.

Amado D. with co-authors **[25]** and scientists **[26]** studied the nutritional and biological value of avocado peel and seeds and found that they can be used in the food, cosmetic **[27]**, and pharmaceutical industries **[28]**, **[29]**. It was determined that avocado seeds are rich in polysaccharides, proteins, lipids, vitamins, minerals, and other bioactive substances **[30]**. The work **[31]** summarises and analyses research on the main metabolites of avocado and its antioxidant and pharmacokinetic properties. In addition, the possibility of using avocados when developing new drugs to prevent and treat cancer, microbial, inflammatory diseases, diabetes, and cardiovascular diseases is emphasized. Chinese scientists **[32]** studied the physicochemical, functional, and emulsion properties of food protein made from defatted avocado flour and found that avocado protein contains all essential amino acids.

To develop an effective extraction of avocado oil in industrial processes, Normalina Arp and co-authors **[33]** studied the effect of pre-treated avocado on the properties of fruit pulp and extracted oil using hexane solvent extraction.

Tanweer Ahmad and Mohammed Danish proposed using avocado waste as a raw material to develop an effective adsorbent against various toxicants **[34]**.

It shall be noted that the results of studies on the consumption properties of avocados imported to Ukraine from different countries are practically absent in the domestic scientific literature. Comprehensive studies of avocado quality during storage were not conducted. Therefore, the paper's main purpose is to study the consumption properties of avocado fruits during storage according to a complex of organoleptic and physicochemical quality indicators.

The organoleptic evaluation of the quality of avocado fruits shall be carried out according to the UNECE standard FFV-42 2019 **[34]** and the developed 5-point scale. All examined samples met the requirements: undamaged, good-quality, clean, almost without insect pests or signs of pulp damage, without damage caused by low temperature, and without excessive external humidity and extraneous tastes/odours (Figures 1, 2, 3).

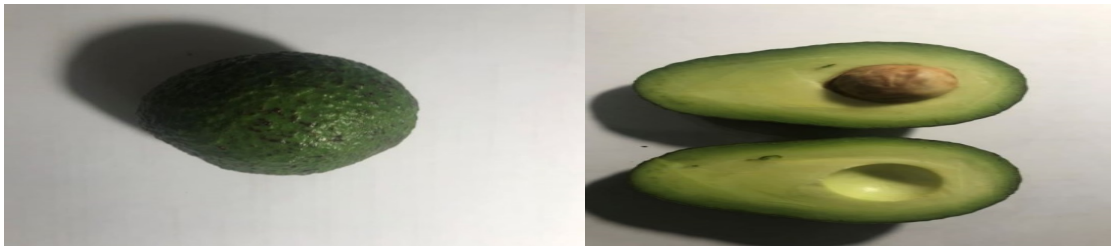

Figure 1 Study sample 1 – fruits of the Haas type, country of origin – Colombia, importer (Flamingoco).

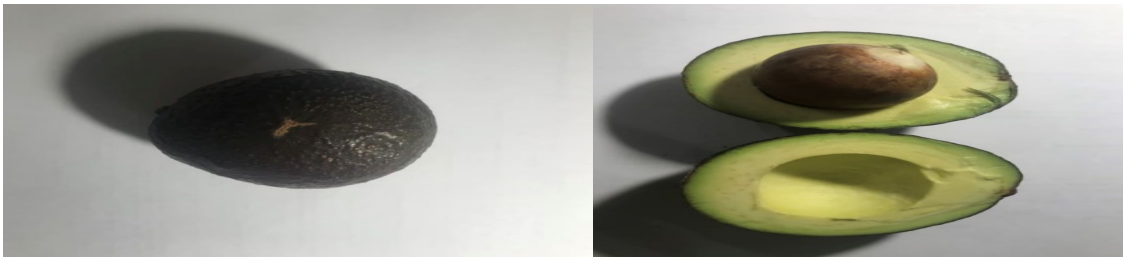

Figure 2 Study sample 2 – fruits of the Haas type, country of origin – Israel, importer (Nature's Pride).

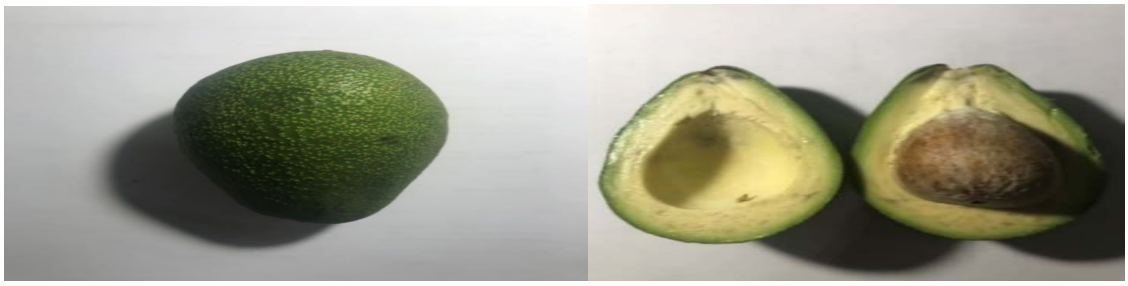

Figure 3 Study sample 3 – fruits of the Fuerte type, country of origin – Israel, importer (Nature's Pride).

Table 1 Organoleptic evaluation of the quality of studied avocado samples.

Indicator	Experts	Researched avocado samples		
		Sample 1	Sample 2	Sample 3
Skin and flesh condition	I	5 ±0.25	4 ±0.20	3 ±0.15
	II	5 ±0.25	4 ±0.20	4 ±0.20
	III	5 ±0.25	4 ±0.20	4 ±0.20
	IV	5 ±0.25	4 ±0.20	3 ±0.15
	V	5 ±0.25	4 ±0.20	3 ±0.15
Aroma	I	3 ±0.15	5 ±0.25	2 ±0.10
	II	4 ±0.20	3 ±0.15	2 ±0.10
	III	3 ±0.15	5 ±0.25	3 ±0.15
	IV	3 ±0.15	4 ±0.20	3 ±0.15
	V	4 ±0.20	4 ±0.20	2 ±0.10
Taste	I	3 ±0.15	5 ±0.25	3 ±0.15
	II	3 ±0.15	5 ±0.25	4 ±0.20
	III	3 ±0.15	5 ±0.25	4 ±0.20
	IV	3 ±0.15	5 ±0.25	3 ±0.15
	V	4 ±0.20	5 ±0.25	4 ±0.20
Secondary ball		3.87	4.40	3.13

The results of the examination by experts (5 experts) of the organoleptic quality indicators of the studied samples of avocado fruits according to the developed 5-point scale are shown in Table 1.

According to the results of the organoleptic evaluation of the quality of the studied samples of avocado fruits, the experts noted that the studied sample 1 is quite firm, has light green flesh and a deep green peel that does not lag behind the flesh, watery taste, without significant defects, the stem is not damaged. Sample 2 has

light green flesh and brownish-black peel that separates very well from the flesh, has a nice buttery flavour and a nice texture, a minor peel defect (pollination mark) of less than 4 cm^2 does not affect the fruit flesh, the stem is not damaged. The studied sample 3 has light green pulp and deep green peel, which separates from the flesh badly, a mild grassy taste, and a loose structure of the fruit flesh. The sample has a defect in the peel (lens) with an area of less than 6 cm^2, which does not affect the fruit's flesh, and the stem is not damaged.

Since among non-oil crops, avocado is considered a fruit with relatively high-fat content. A study was conducted of avocado lipids' fatty acid composition (Table 2).

Table 2 Fatty acid composition of lipids of studied avocado samples.

Acid	Acid concentration in studied avocado samples, %		
	Sample 1	**Sample 2**	**Sample 3**
C 10 caprynova	-	0.0004	-
C 11:0 undecanova	-	0.0029	-
C 12:0 laurinova	0.0011	-	0.0025
C 13:0 triple decan	-	-	0.0025
C 14:0 miristinova	0.0136	0.0420	0.1081
C 14:1 myristoleinova	0.0091	-	0.0035
C 15:0 pentadecanoic	0.0180	0.0110	0.0512
C 15:1 cis-10-pentadecene	0.0136	0.0029	0.0194
C 16:0 palmitic	11.6000	10.5000	4.8390
C 16:1 palmitoleic	8.6870	15.8700	5.4130
C 17:0 heptadecane	0.1057	-	-
C 17:1 cis-10-heptadecene	0.0234	-	-
C 18:0 stearic	0.0832	3.9900	0.0522
C 18:1 oleinov	52.0800	62.4800	52.0000
C 18:2 linoleum	9.9690	2.5810	13.3800
C 18:3p6 gamma linolenic	0.9576	0.0912	0.6688
C 18:3p3 alpha-linolenic	0.0726	0.4177	0.0988
C 20:0 arachinova	0.0869	0.0082	0.0943
C 20:1 gondoinova	0.1296	-	0.1670
C 20:2 eicosadiene	0.0267	0.4440	0.0313
C 20:3p6 cis-8, 11, 14-eicosatriene	-	-	0.0062
C 20:3p3 cis-11,14,17-eicosatriene	0.0047	-	0.4253
C 20:4 arachidonic	0.0785	-	-
C 20:5p3 cis 05,8,11,14,17-eicosapentaenoic	0.0555	0.0183	0.0057
C 22:0 behenov	-	-	0.0100
C 22:1 erukova	0.0761	0.0106	0.4589
C 22:2 cis-13,16 docosadiene	0.0025	0.2639	0.0089
C 23:0 trikozanova	0.0975	0.0385	0.3648
Saturated fatty acids	12.0200	14.5900	5.5250
Unsaturated fatty acids	72.1800	82.1800	72.7500
The sum of saturated and unsaturated fatty acids	84.1919	96.7724	78.2112

The analysis of the fatty acid composition of the lipids of the studied samples of avocado fruits makes it possible to conclude that the main fatty acids are 16:0 palmitic acid, 16:1 palmitoleic, 18:1 oleic (omega-9) and 18:2 linoleic acids are predominant among unsaturated fatty acids. Oleic acid is one of the main useful fatty acids, without which proper metabolism in the human body is impossible. In turn, the linoleic most common omega-6 polyunsaturated fatty acid, which, according to research by the University of Eastern Finland, reduces the risk of premature death when the concentration in the human body increases **[35]**. Unlike oleic and linoleic acids, which have a positive effect on the human body, palmitic acid is not able to be fully metabolized and accumulates in the body causing fatty transformation of whole organs **[36]**, **[37]**.

The next stage of the complex study of avocado quality was to determine physicochemical parameters, the results of the studies are shown in Table 3. According to the results of the studies, it was found that the most

significantly studied avocado (6.6-13.4 mg/100 g) samples differ in ascorbic acid content. The lowest content was observed in sample 1 (6.6 mg/100 g).

Table 3 Physico-chemical indicators of the quality of the studied avocado samples.

Indicator	Researched avocado samples		
	Sample 1	Sample 2	Sample 3
Mass fraction of moisture, %	78.80 ±3.94	73.40 ±3.67	75.50 ±3.77
Content of dry soluble substances, %	7.5 ±0.37	7.8 ±0.39	8,2 ±0.41
pH, units	6.1 ±0.30	6.2 ±0.31	6.8 ±0.34
Ascorbic acid content, mg/100 g	6.6 ±0.33	13.4 ±0.67	11.45 ±0.57

Pomological types of avocado fruits differ in shape, size, and ratio of peel, pulp, and stone. According to the results of the conducted research, it was found that the largest mass of pulp had the avocado of sample 1 and was 85.42% of the total mass of the fruit. The smallest one had sample 3 (75.6%).

The next stage of the comprehensive evaluation of the quality of avocado fruits was the study of changes in their quality during 14 days of storage. The studied avocado samples had two storage modes: in a refrigerator at a temperature of 3-5 C and a room temperature of 15-18 C. Avocado fruits from the same batch, which were cut before the beginning of the study, were taken as control samples. The results of the studied samples are presented in Figures 4, 5, and 6.

Figure 4 Changes in the appearance of avocado fruits of sample 1 during 13 days of storage at temperatures of 3-5 °C (1-4), 15-18 °C (5-8). Note: 1 – at the beginning of storage; 2 – after 5 days of storage; 3 – after 9 days of storage; 4 – after 13 days of storage; 5 – the first day of storage; 6 – after 5 days of storage; 7 – after 9 days of storage; 8 – after 13 days of storage; 9 – section of the fruit after 14 days of storage.

At the beginning of storage, the studied samples of avocado fruits 1 (Figure 4) had a fresh appearance, dry peel, and dense flesh. During storage, the fruit, subjected to cold storage, almost did not change its colour but darkened on one side, and the peel became moistened. The fruit, which was stored at room temperature, became slightly moistened and darkened at the point of contact between the planes of the fruit and the cardboard substrate already on the third day, on the ninth day the fruit became very dark and softened almost over the entire area. It was found that the fruit pulp became loose and had an unpleasant smell, was inedible, and the process of rotting began. The color of the avocado also changed from dark green to brown. Compared to this, the fruit stored in the refrigerator retained all its organoleptic properties, taste, and smell without extraneous flavours and aromas. The fruit is hard; the peel is almost unchanged except for a spot on one side.

1 2 3 4 5

Figure 5 Changes in the appearance of avocado fruits of sample 2 during 13 days of storage at temperatures of 3-5 ºC (1-4). Note: 1 – at the beginning of storage; 2 – after 5 days of storage; 3 – after 9 days of storage; 4 – after 13 days of storage; 5 – section of the fruit after 14 days.

At the beginning of storage (Figure 5), the avocado fruit had a dry peel, was hard over the entire area, fresh in appearance with a minor defect that did not affect the appearance of the sample. On the seventh day, the fruit's peel became slightly moistened and soft near the stem. At the end of the study, the fruit became soft, the peel was wrinkled, near the stem and pollination mark, and the peel secreted juice when touched. The section shows that the fruit has started to rot near the stem and the pollination mark. This explains the softness in these areas that were observed earlier. The pulp has become looser than the control sample, and the peel comes off almost effortlessly. The smell and taste are unchanged, and signs of rotting make this fruit inedible.

1 2 3 4 5

Figure 6 Changes in the appearance of avocado fruits of sample 3 during 13 days of storage at temperatures of 3-5 º C (1-4). Note: 1 – at the beginning of storage; 2 – after 5 days of storage; 3 – after 9 days of storage; 4 – after 13 days of storage; 5 – section of the fruit after 14 days.

The fruit of the studied sample 3 (Figure 6) was firm and fresh at the beginning of storage, the defects (spots) did not affect the appearance of the sample, and the peel was dry. On the seventh day, the existing defects became more pronounced (the spots darkened), the peel became somewhat moistened, and the side on which the fruit lay became soft. At the end of the study, the fruit became soft, and the peel wrinkled. The section shows that the fruit has a physiological defect without signs of rotting. Compared to the control sample, the pulp became looser, and the peel became easier to separate, the smell and taste of the fruit did not change.

It was found that after 14 days of refrigerated storage, the studied sample 1 best preserved its quality, the worst – sample 2 after 14 days of refrigerated storage, and sample 1 after 14 days of storage without a refrigerator.

The main factors affecting the shelf life of fruits are the mass fraction of fruit moisture and storage conditions. Therefore, at the last stage of the comprehensive assessment of avocado quality, the dynamics of changes in the weight of the studied fruit samples during 14 days of storage were studied (Table 4).

The given research results make it possible to conclude that the preserved quality of avocados during storage is significantly influenced by the characteristics of types, the region of fruit cultivation, and their storage conditions. It was found that an increase in the storage temperature of the studied sample by 12 ºC is accompanied by an increase in the loss of fruit moisture by 3.78 times or by 378%.

Table 4 The dynamics of changes in the weight of the studied avocado samples during storage.

Storage period (day)	Investigated avocado samples/storage temperature			
	Sample 1.1 / t 3-5 °C	Sample 1.2 / t 15-18 °C	Sample 2 / t 3-5 °C	Sample 3 / t 3-5 °C
1	116.87 ±5.84	106.31 ±5.31	201.62 ±10.08	212.1 ±10.60
2	116.51 ±5.82	105.17 ±5.25	200.98 ±10.04	211.35 ±10.56
3	116.22 ±5.81	103,98 ±5.19	200.73 ±10.03	210.96 ±10.54
4	115.95 ±5.78	102.77 ±5.13	200.38 ±10.01	210.54 ±10.52
5	115.62 ±5.78	101.66 ±5.08	199.96 ±9.99	210.02 ±10.50
6	115.18 ±5.75	100.39 ±5.01	199.34 ±9.96	209.39 ±10.46
7	114.92 ±5.74	99.28 ±4.96	198.98 ±9.94	209.02 ±10.45
8	114.67 ±5.73	98.16 ±4.90	198.62 ±9.93	208.65 ±10.43
9	114.38 ±5.71	97.04 ±4.85	198.14 ±9.90	208.17 ±10.40
10	114.06 ±5.70	95.93 ±4.79	197.75 ±9.88	207.76 ±10.38
11	113.71 ±5.68	94.67 ±4.73	197.34 ±9.87	207.31 ±10.36
12	113.42 ±5.67	93.52 ±4.67	197.29 ±9.86	206.91 ±10.34
13	113.15 ±5.65	92.53 ±4.62	196.54 ±9.82	206.38 ±10.31
14	112.94 ±5.64	91.45 ±4.57	196.06 ±9.80	205.94 ±10.29
Loss of moisture, %	3.4 ±0.17	13.97 ±0.70	2.75 ±0.14	2.9 ±0.14

CONCLUSION

The nutritional properties of avocados vary depending on the type, country of origin, and other factors and may change significantly during storage. Pomological types of avocado fruits differ in shape, size, and ratio of peel, pulp, and stone. The analysis of the fatty acid composition of the lipids of the studied samples of avocado fruits makes it possible to conclude that the main fatty acids are 16:0 palmitic acid, 16:1 palmitoleic, 18:1 oleic (omega-9) and 18:2 linoleic acids are predominant among unsaturated fatty acids. Summarizing the findings of a study that examined how the quality of avocado fruits changed over 14 days of storage in two different environments (a refrigerator at 3-5 °C and a room temperature of 15-18 °C) it was determined that the consumption properties of avocado fruits changed during storage. It was found that sample 1 had the best quality preservation after 14 days of refrigeration, sample 2 had the worst quality preservation after 14 days of refrigeration, and sample 1 had the poorest quality preservation after 14 days of storage without a refrigerator. The main factors affecting the shelf life of fruits are the mass fraction of fruit moisture and storage conditions. We have found that a 12 °C rise in storage temperature was followed by a 3.78x or a 378% rise in fruit moisture loss. So, it can be concluded that when choosing storage conditions and regimes, the variety, country of origin and other factors of avocado fruits should be considered to preserve their quality as much as possible.

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The efficacy of Bloso fish (*Glossogobius giuris* sp.) in improving hemoglobin, hematocrit, platelet, and albumin levels of Wistar rats with hypoalbuminemia

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ABSTRACT

Tuberculosis (TB) is an infectious disease worldwide that causes death. Common clinical manifestations of patients with TB include anemia, hypoalbuminemia, and malnutrition. Most patients with TB are infected with coccus bacteria, such as *Staphylococcus aureus*, that commonly attack the respiratory tract. However, the consumption of heme protein sources could improve the nutritional status of patients with TB. Fish comprise one of the most widely consumed sources of heme. The bloso fish (*Glossogobius giuris* sp.), considered a fish without economic value is a new alternative source of heme protein. This study aimed to develop supplements using bloso fish (*Glossogobius giuris* sp.). This study used an experimental pretest-post-test control group design. Seven male Wistar rats were used as the negative control group. Twenty- eight male Wistar rats were administered *S. aureus*, fed a protein-deficient diet, and divided into the positive control group, the K1 group, which received up to 675 mg/200 g of bloso fish flour, the K2 group, which received up to 67.5 mg/200 g of bloso fish oil, and the K3 group, which received up to 675 mg/200 g of bloso fish fluor from oil extraction dregs. Treatment was administered for 28 days. The hemoglobin (Hb), hematocrit (Ht), platelet, and albumin levels in blood serum from the retroorbital vein were measured. Data were processed using a paired t-test and one-way analysis of variance. The results showed differences in Hb, Ht, platelet, and albumin levels were observed before and after treatment. Additionally, differences in Hb, Ht, platelet, and albumin levels were observed in the groups that received bloso fish flour and bloso fish oil. Bloso fish flour and bloso fish oil increased the Hb, Ht, platelet, and albumin levels of rats with hypoalbuminemia.

Keywords: hemoglobin, hypo albumin, bloso fish, tuberculosis, fish oil

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* that affects the lungs. TB is the second leading cause of death worldwide, and it was declared a global public health emergency by the World Health Organization in 1993. As many as 8 billion new cases of TB are diagnosed each year, resulting in as many as 2 million deaths. In 2017, 10.4 million new cases of TB were diagnosed, resulting in 1.7 million deaths. *Mycobacterium tuberculosis* exposure causes various conditions, such as infection resistance, latent infection without active disease, active pulmonary disease and active extra-pulmonary disease. The TB incidence is higher in low and middle-income countries. Research has shown that as many as 91% of the sputum of patients with TB contains Gram-positive coccus bacteria from the *Staphylococcus sp*. and *Streptococcus sp. Genera* **[1]**.

In Indonesia, the rate of TB is high, and there is an increasing trend of severe TB. The 2017 World Health Organization report indicated that the number of new TB cases in Indonesia is approximately 1,020,000 per year

(399 cases/100,000 population), resulting in 100,000 deaths per year (41 cases/100,000 people). Furthermore, the estimated prevalence of human immunodeficiency virus among patients with TB is 6.2%. Additionally, the number of drug-resistant TB cases has been estimated to be 10,000; these cases comprise 1.9% of drug-resistant TB cases associated with new TB cases and 12% of drug-resistant TB cases associated with TB with repeated treatment **[2]**. Based on the 2018 Basic Health Research, the magnitude of the incidence of pulmonary TB has decreased; however, at 321 cases per 100,000 population, the incidence of pulmonary TB is still much higher than the national target. Furthermore, this number is too high according to the 2019 strategic plan, which indicates a target prevalence of pulmonary TB of 245 cases/ 100,000 population **[3]**.

TB is closely associated with malnutrition and anemia **[4]**. Moreover, anemia exacerbates TB and malnutrition. Hemoglobin, hematocrit, platelet, and albumin levels can reflect the nutritional status of patients with TB. Hemoglobin is a routine blood parameter and an illustration of the nutritional status related to the body's iron status. Hemoglobin can bind to iron, which is directly associated with anemia. Albumin is the main plasma protein component, which can reversibly bind with normal levels of 3.5 g/dL to 5.5 g/dL. The main function of albumin is maintaining osmotic pressure in the body; its half-life is 14 to 21 days **[5]**. Previous studies have shown that serum albumin levels decrease significantly in patients with TB and nutritional problems. Nutritional factors such as low food intake, anorexia, and increased catabolism, including acute phase protein reactions, also affect hypoalbuminemia in patients with TB **[6]**.

It is possible that increased albumin levels in patients with TB who experience hypoalbuminemia can increase the antimicrobial effect of anti-TB drugs and inhibit the production of inflammatory cytokines. Anti-TB drugs include rifampicin and isoniazid, which can bind strongly to albumin in pulmonary patients with TB, thereby improving their clinical condition **[7]**. Improvements in food consumption, especially protein, can help improve hypoalbuminemia in patients with TB. Fish is one source of heme protein; therefore, the bloso fish (*Glossogobius giuris sp*.) has the potential to be developed into a formula that could improve the nutritional status of patients. Individuals living in the coastal areas of the island of Java believe that consuming bloso fish benefits children and pregnant women. The bloso fish (*Glossogobius giuris sp*.) is an endemic species in Indonesia **[8]** that lives in brackish waters, is often considered a pest, and has low economic value.

Therefore, developing an appropriate form of processed bloso fish is necessary to help improve the hemoglobin, hematocrit, platelet, and albumin levels of rats with hypoalbuminemia and TB.

Scientific Hypothesis

Bloso fish flour and bloso fish oil can improve the hemoglobin, hematocrit, platelet, and albumin levels of rats with hypoalbuminemia.

MATERIAL AND METHODOLOGY

Samples

Blood samples obtained from 35 Wistar rats.

Animals, Plants and Biological Materials

This study used male Wistar rats. The inclusion criteria included: Wistar strain; male sex; age 8 to 12 weeks; the weight of 160 to 250 g; no anatomical abnormalities; and healthy and active during the adaptation period. Exclusion criteria included pain or inactivity during the adaptation period, extreme weight loss before treatment, and diarrhoea. The drop-out criterion was death during the treatment period. Thirty-five rats were randomly selected and divided into five groups of seven. A total of thirty-five experimental animals were required.

Instruments

Hematology Analyzer, Sysmex KX-21, Kobe, Japan.

Laboratory Methods

The Automated hematology analyzer (AHA) was used to measure hemoglobin, hematocrit, platelet, and albumin levels **[9]**.

Description of the Experiment

Sample preparation: Whole blood was obtained from each Wistar rat and stored with an anticoagulant to measure the hemoglobin, hematocrit, platelets, and albumin.

Number of samples analyzed: Blood samples from thirty-five Wistar rats were collected from the retroorbital vein (before and after intervention)

Number of repeated analyses: In duplicate.

Number of experiment replication: In duplicate.

Design of the experiment: The experimental animals were treated at the Center for Food and Nutrition Studies Laboratory of the Universitas Gajah Mada (Yogyakarta, Indonesia). Nutrient testing was performed in the Center for Food and Nutrition Studies Chemical Laboratory of the Universitas Gajah Mada (Yogyakarta,

Indonesia). The Nutrition Laboratory of Universitas Negeri Semarang (Semarang, Indonesia) and the Food Engineering Laboratory of Soegijapranata Catholic University (Semarang, Indonesia) were used to produce bloso fish flour. Hypoalbuminemia in this study used 1% *Staphylococcus aureus* to induce infection. The isolated culture from Center for Food and Nutrition Studies of the Universitas Gajah Mada, Yogyakarta, Indonesia.

A randomized, controlled, pretest-posttest design was used for this study. Wistar rats were randomly divided into a negative control group (K-group), a positive control group (K+ group), and the following three treatment groups: the K1 group, which received 675 mg/200 g of bloso fish fluor; the K2 group, which received 67.5 mg/200 g of bloso fish oil; and the K3 group, which received 675 mg/200 g of bloso fish flour from oil extraction dregs. During this study, three servings of animal protein per day, equivalent to 150 g of animal food per day, were administered **[10]**. The wet bloso fish were floured using a spray dryer. This drying process involved low-temperature evaporation at 40 °C for 8 to 12 hours. The dried bloso fish subjected to the spray dryer shrank by 25%. Bloso fish oil was obtained using a drying method involving a cabinet dryer. Pressing was performed until the bloso fish oil yield reached 10% of the dry weight. The residue from the pressing was referred to as oil extraction dregs. Treatment was administered using a gastric tube once daily during the morning for 28 days. The duration of administration was influenced by the half-life of albumin, which is 14 to 21 days. The negative control group received an AIN93M feed. The positive control and treatment groups received AIN93M modified feed and were injected with 1% *Staphylococcus aureus* to induce infection. Modifying the AIN93M feed was performed to induce protein deficiency, which involved removing the casein component. As a result, the feed contained only corn flour, sucrose, corn oil, alpha cell (non-nutritive bulk), and mineral mix. Rat feed was produced as pellets, similar to rat feed and consumed orally. The rats received 20 g of feed each day.

Statistical Analysis

The mean ±standard deviation (SD) was calculated for each group of seven rats. As all data were normally distributed, the significance of differences before and after treatments was determined using the paired t-test. The significance of differences between the groups was assessed by one-way analysis of variance (ANOVA), calculated by the SPSS version 20 program, with a significance level of $p < 0.05$ by the Tukey HSD Test.

RESULTS AND DISCUSSION

The conditions of protein deficiency and cell resistance to pathogenic bacteria allowed cells to form cellular defences by mediating T cells and macrophages **[11]**. Nutritional components, such as carbohydrates, proteins, fats, and vitamins, can activate T cells and macrophages. Fat can regulate the immune response against pathogenic bacteria **[12]**. Fatty acids can increase the ability of macrophages to kill bacteria through phagocytosis **[13]**. Eicosapentaenoic acid and polyunsaturated fatty acid (PUFA) can increase mycobacterial growth by reducing tumour necrosis factor-α secretion in macrophages. The fish oil used during this study was a source of n-3 PUFA. The anti-inflammatory content of n-3 PUFA can be used as therapy for chronic inflammatory diseases, such as TB. The n-3 PUFA can repair interferon-γ and stimulate the immune response. The activation of n-3 PUFA to kill bacteria occurs through chemotaxis, antigen presentation, adhesion molecule expression, and the major histocompatibility complex. Fats, especially n-3 PUFA, can influence the maturation process of phagolysosomes and endosomal membrane lipid composition during a critical phase of mycobacterial clearance **[14]**. The n-3 PUFA can also integrate with cell membranes, including effector cells in the immune system, thereby affecting functional changes that can be referred to as resistance to disease **[15]**.

The bloso fish is a source of heme iron. Bloso fish flour, bloso fish oil, and the dregs of bloso fish oil extraction increased hemoglobin levels during treatment under TB conditions (Table 1). Heme iron is easily absorbed during metabolism **[16]**. The conditions of our study were in agreement with those of studies of experimental animals with inflammatory anemia conditions that showed that iron mobilization and increased iron absorption could be mediated by erythroferrone suppression. The key regulator of iron homeostasis in humans is hepcidin which acts as ferroportin (FPN-1). Ferroportin is a membrane protein that has a role as the leading exporter of iron in mammalian cells. This ferroportin will have a different position in mammalian cells, such as macrophages which play a role in iron recycling, duodenum as an organ tasked with absorbing iron and hepatocytes which play a role in iron storage. Hepcidin will bind to ferroportin which causes internalization and is followed by cellular degradation which ultimately blocks the release of iron by enterocytes and reticuloendothelial cells to the bloodstream, which can ultimately maintain iron homeostasis. Increased extracellular and iron stores or inflammatory stimulation can trigger hepcidin expression. Hepcidin expression can be inhibited through hypoxia and erythropoiesis **[17]**. Hepcidin-modulated erythropoiesis to improve anemia. Patients with active TB who lack iron sources can experience anemia and hypoxia and require systemic signalling to enterocytes through the hepcidin-ferroportin axis induced by inflammation and regulation through intestinal hypoxia-inducible factor-2α **[18]**.

Anemia is common in patients with TB, with an incidence of up to 88%. Furthermore, anemia in patients with TB is caused by chronic inflammation **[19]**. Research conducted in Rio de Janeiro, Brazil, even showed that the proportion of patients with anemia due to chronic disease reached 75.9%. This proportioned figure is higher than iron deficiency anemia which only reaches 2.4%. This study also shows that the incidence of anaemia can be corrected without iron supplementation after TB therapy, either through food or medicine, **[20]**. Studies have shown a decrease in serum concentrations of C-reactive protein; and acute phase reactants; and an increase in the production of pro-inflammatory cytokines, such as interleukin-6, tumor necrosis factor-α, and interferon-γ, which contribute to the occurrence of anemia by reducing erythropoietin products. Erythropoiesis disorders begin with suppression of the bone marrow response to erythropoietin, thus affecting iron metabolism **[21]**. TB is a chronic inflammatory condition that begins with increased hepcidin levels.

Furthermore, TB can trigger hepcidin in patients with anemia. Hepcidin levels are affected by iron deficiency, hypoxia, and erythropoiesis. Anemia is related to low hemoglobin levels or red blood cell concentrations caused by major hematological findings with chronic diseases **[22]**. Table 1 shows that Bloso fish can increase hemoglobin levels. Bloso fish is one of the animal proteins which is rich in iron. Incidents of infection trigger competition between hosts and pathogens for iron reserves. Dysregulation of iron is associated with infectious diseases, including TB. Iron is a micronutrient required by *Mycobacterium tuberculosis* to survive in the host. The ability of *Mycobacterium tuberculosis* to use available iron can change the host's response to infection. During infection, *Mycobacterium tuberculosis* can convert excess Fe into Fe reserves which are then converted into siderophores and used to support their growth and multiplication **[16]**. Therefore, additional food sources of protein are needed to compensate for the use of iron by *Mycobacterium tuberculosis*.

Anemia affects the quality of life and increases the morbidity and mortality of patients with TB. The cell-mediated immune response and bactericidal capacity of leukocytes of patients with anemia are also observed with suppressed conditions **[23]**. Research using a cross-sectional and case-control design showed that the TB incidence of patients with anemia was 3.56 times higher than that of patients without anemia. This is possible because, among patients with anemia, immuno-compromised patients have an imbalance of nutrients. Phagocytic macrophage activity is an important immunological response that controls TB infection through granuloma formation **[24]**. Granuloma formation is characterized by a collection of immune cells and mycobacterium walls that limit the replication and spread of tubercle bacilli. Anemia conditions, especially iron deficiency anemia, can interfere with the immune response mediated by T cells, thereby causing disturbances in the function of polymorphonuclear neutrophils and the intracellular bactericidal activity of immunological cells. Iron deficiency can change the balance between Th1 and Th2 cytokines, ultimately triggering the Th2-dominant response associated with TB **[25]**.

Platelets are effector cells that have a role in the process of homeostasis and the initiation of wound healing. Furthermore, they are important in chronic infection and inflammation **[26]**. Additionally, they are one of the important markers of pulmonary cavitation. Platelets appear to support the host response to extracellular infection; however, with intracellular infections, they contribute to immune evasion **[27]**. Platelets can convert monocytes to an M2-like macrophage phenotype, thus creating a permissive environment for mycobacterial growth. Platelets can also induce foamy multinuclear epithelioids and macrophages with immunosuppressive capacities in vitro. Platelets contribute to TB pathology by increasing tissue damage by induction of matrix metalloproteinases in infected monocytes **[28]**. A study conducted by Fox- et al. showed that patients with TB had increased activated platelet markers and platelet-related factors that could be reduced through antibiotic therapy. Changes in platelet count, especially during TB infection, will be associated with death and severity of infection. Acute phase reactants and proinflammatory cytokines will affect megakaryocytes, reducing platelet size and platelet production from bone marrow in patients infected with *Mycobacterium tuberculosis* **[29]**. The provision of bloso fish in flour or oil can reduce the number of platelets (Table 1). Therefore, bloso fish have a role in reducing the inflammatory process and tissue damage. Platelets may mediate immunological mechanisms by forming immune cells and releasing chemokines and growth factors. Platelets can mediate the formation of granulomas by producing chemokines that involve innate cell responses such as neutrophils, monocytes, and macrophages in TB patients. Platelets can cause monocyte induction through collagenase activity, which will then differentiate from monocytes to become multinucleated giant cells. Research shows that the secretion of proinflammatory cytokines in TB patients will be activated directly by platelets after discovering *Mycobacterium tuberculosis* through receptors such as toll-like receptor-2 (TLR-2) and TLR-4. TLR-2 in the monocytes of TB patients will increase in the same direction as interleukin-1-beta (IL-1β), IL-6 and IP-10. Proinflammatory cytokines can trigger the production of platelets by triggering megakaryocytopoiesis. In vitro studies involving murine animal models indicate that vascular endothelial growth factor (VEGF-A) can trigger platelet production by accelerating megakaryocyte maturation when interacting with the VEGFR1 receptor via a paracrine or autocrine mechanism. Platelets and monocytes can produce this receptor **[30]**.

Figure 1 Picture bloso fish (*Glossogobius giuris sp*.).

Bloso fish is a local fish in Indonesia and is well known with buto cina. Bloso fish are included in demersal fish, having a cigar-shaped body, round or slightly flattened, head pointed and depressed, and the muzzle is wider than their length. The general colour is brown above and silver at the bottom. Bloso fish spread in Indonesia until the West Pacific region.

Table 1 Hemoglobin, hematocrit, platelet, and albumin levels of Wistar rats.

Parameters	**Groups**				
	K-[a]	**K+[b]**	**K1[c]**	**K2[d]**	**K3[e]**
Hb (g/dl)					
Pretest	15.8 ±0.4	11.7±0.1	11.6 ±0.3	11.4 ±0.1	11.5 ±0.2
Posttest	15.6 ±0.3	11.6±0.1	14.1 ±0.4	14.9 ±0.2	14.1 ±0.2
Δ	-0.2 ±0.1^{c,d,e}	-0.1 ±0.1^{c,d,e}	2.5 ±0.5^{a,b,d}	3.5 ±0.3^{a,b,c,e}	2.6 ±0.3^{a,b,d}
p	0.001*	0.001*	0.001*	0.001*	0.001*
Ht (%)					
Pretest	48.7 ±0.3	35.2 ±0.5	35.1 ±0.6	34.8 ±0.2	34.9 ±0.4
Posttest	48.6 ±0.3	35.0 ±0.5	46.2 ±0.4	47.8 ±0.5	42.3 ±0.9
Δ	-0.1 ±0.0^{c,d,e}	-0.1 ±0.1^{c,d,e}	11.1 ±0.6^{a,b,c,d,e}	12.9 ±0.6^{a,b,c,d,e}	7.5 ±0.9^{a,b,c,d,e}
p	0.006*	0.041*	0.001*	0.001*	0.001*
Platelets (10^3/µL)					
Pretest	166.0 ±4.2	787.6 ±3.1	784.4 ±5.3	787.0 ±5.2	784.4 ±3.3
Posttest	163.9 ±4.3	789.6 ±3.4	190.0 ±4.9	175.7 ±5.6	192.3 ±5.1
Δ	-2.1 ±0.7^{c,d,e}	2.0 ±0.6^{c,d,e}	-594.4 ±8.8^{a,b,d}	-611.3 ±7.5^{a,b,c,d,e}	-592.1 ±6.8^{a,b,d}
p	0.001*	0.001*	0.001*	0.001*	0.001*
Albumin (g/dl)					
Pretest	5.9 ±0.2	1.1 ±0.3	0.9 ±0.1	1.0 ±0.1	0.8 ±0.1
Posttest	5.9 ±0.2	1.0 ±0.2	5.0 ±0.1	5.7 ±0.1	4.1 ±0.1
Δ	-0.0 ±0.0^{c,d,e}	-0.1 ±0.1^{c,d,e}	4.1 ±0.1^{a,b,c,d,e}	4.7 ±0.1^{a,b,c,d,e}	3.3 ±0.1^{a,b,c,d,e}
p	0.001*	0.001*	0.001*	0.001*	0.001*

Note: *Sampling was performed 14 days after the induction of hypoalbuminemia and 28 days after the beginning of treatment. K-: negative control group (normal); K+: positive control group with hypoalbuminemia; K1: hypoalbuminemia group; treated with 675 mg/200 g of bloso fish flour; K2: hypoalbuminemia group, treated with 67.5 mg/200 g of fish oil; K3: hypoalbuminemia group, treated with 675 mg/200 g of the remaining oil extracted from bloso fish that was processed into fish flour. Values represent the mean ± standard deviation of the observation mode for seven rats in each group. Statistical analysis: *p; paired t- test; (significant difference at

(p <0.05). One-way analysis of variance; when significant, post hoc testing (least significant difference) was performed for intergroup comparisons. [a]Statistically significant difference (p <0.05) when compared with K- values; [b]Statistically significant difference (p <0.05) when compared with K+ values; [c]Statistically significant difference (p <0.05) when compared with K1 values; [d]Statistically significant difference (p <0.05) when compared with K2 values; [e]Statistically significant difference (p<0.05) when compared with K3 values.

Food and nutrition management can reduce TB's incidence and mortality rates **[31]**. Research conducted by Matos et al. indicated that low serum albumin levels at hospital admission are a strong risk factor for death. Furthermore, a study performed in Korea a relationship between malnutrition and death attributable to TB for hospitalized patients **[32]**. TB can cause malnutrition due to decreased food intake and increased use of energy and nutrients in the body. Nutritional support is needed for patient survival and the body's functional abilities. Nutritional support provided to patients should be a single component considering the amount of nutrients and composition. A study of HIV-infected TB patients who were given energy and protein supplementation in the form of biscuits showed an improvement in hand-grip strength but not in body weight or body composition **[33]**. Hand-grip strength is a measure of work capacity in TB patients. Measurement of hand-grip strength also shows the ability to improve food intake and TB care carried out.

The condition of malnutrition in TB patients is characterized by insufficient intake of protein and total calories. A study conducted in China showed that there was a condition of protein-calorie malnutrition in TB patients. This condition can reduce the effectiveness of components that play an important role in cell immunity. Malnutrition makes a negative contribution to TB care. Malnutrition can reduce protein level in the patient's body, slowing down the lesions' healing process. Adequate protein content not only provides benefits in repairing lesions during TB treatment but is able to increase the amount of TB drug carrier protein so as to increase the concentration of anti-tuberculosis drugs in the blood and assist in sputum conversion **[34].**

The condition of TB is capable of losing weight so that the patient is in a state of undernutrition. This undernutrition condition can be caused by decreased food intake or factors related to TB such as cachexia, due to metabolic dysfunction, poor absorption, fever, and anorexia. Metabolic changes due to TB can trigger a condition called anabolic block. This anabolic block allows the intake of protein sources to act as a source of energy but not as an ingredient in the anabolic process. Research shows that supplementation with macronutrients affects weight gain in TB patients so that they can improve their quality of life, including reducing the risk of hypercatabolism due to febrile illness **[35]**.

Serum albumin is an important marker of the nutritional status of a patient with TB **[13]**. The serum albumin level with TB can be used as a marker of liver function during the therapy process, is inexpensive, and used as a monitoring tool to determine the success of TB therapy **[36]**. The albumin serum concentration of the experimental animals injected with S. *aureus* was decreased compared to that of the control group. Albumin levels increased after the administration of bloso fish, as either flour or oil (Table 1). Hypoalbuminemia is a risk factor for death for elderly individuals with TB **[37]**. Hypoalbuminemia can change the total and relative numbers of T-lymphocytes and various immune system cells, ultimately helping the host fight against *Mycobacterium tuberculosis*. Low serum albumin levels are generally associated with an increased risk of inflammation because of the increased load of tubercle bacilli at pulmonary sites in patients with TB **[38]**. The administration of bloso fish preparations may be an alternative method of reducing the bacterial load and inhibiting bacterial replication **[39]**.

CONCLUSION

Hemoglobin, hematocrit, platelets, and albumin are markers used to detect TB. Patients with TB have a variety of haematological manifestations with features of anemia, hypoalbuminemia, and thrombocytosis. Therapy using bloso fish flour and bloso fish oil improved hemoglobin, hematocrit, platelets, and albumin levels. The weakness of this study is that it does not examine proinflammatory cytokines or the components responsible for inducing the inflammatory process. Examination using ferritin and serum transferrin should be advised to determine anemia activation of tuberculosis patients. Future research requires processed forms made from bloso fish flour or bloso fish oil to better understand its effects on TB patients who are experiencing malnutrition.

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Conflict of Interest:

The authors declare that they have no conflict of interest.

Ethical Statement:

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The use of buckwheat flour in the technology of semi-smoked sausage

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ABSTRACT

This article aims to substantiate the use of buckwheat flour in the technology of semi-smoked sausage based on the study of physicochemical, functional and technological, structural and mechanical and organoleptic parameters. It has been found that a small amount of buckwheat flour in semi-smoked sausage samples (up to 10.0% by weight of unsalted raw material) increases the moisture-binding capacity of the control sample by 1.1-1.8%. The study of the shear stress limit of the finished experimental samples showed that the maximum value of this parameter is 758 Pa. With increasing the dosage of hydrated buckwheat flour, the minced meat loosens, and the value of the shear stress limit in samples No.3 and No.4 is 420 and 390 Pa. The appearance, color, smell, aroma, consistency, taste and juiciness were studied in the produced samples of semi-smoked sausage. Histological examination of an experimental sample of semi-smoked sausage with a level of hydrated buckwheat flour of 6% was carried out. It has been found that introducing hydrated buckwheat flour into the minced meat up to 6% of the mass of raw meat material has a positive effect on the physical and chemical, functional and technological, structural and mechanical and organoleptic parameters of semi-smoked sausage.

Keywords: beef, semi-smoked sausage, meat product, buckwheat flour, pH, moisture-binding capacity

INTRODUCTION

The application of vegetable raw material in the technology of meat products is a topical direction. Vegetable raw materials are applied to manufacture functional products, including dietary, therapeutic and prophylactic, children's, and gerodietic products, etc.

It is proved by many scientists that the introduction of vegetative raw materials into recipes and technology of meat products improves physical and chemical, functional and technological, structurally mechanical and organoleptic parameters **[1]**, **[2]**, **[3]**.

The combination of raw materials of meat and vegetative origin provides high food and biological value of meat products, allowing to minimise losses during thermal processing.

Using inexpensive vegetative raw materials as partial replacements of meat raw materials decreases the final cost of a product.

Creating recipes and technologies for new meat products will expand the assortment of functional products for wide layers of the population **[4]**.

For the use of vegetative raw materials in the technology of combined meat products, it is necessary to define its dosage.

The finished product's quality depends on the components' composition and properties of raw materials. Analysis of literature data has shown that in the modern production of meat products, various herbal supplements are effectively used to regulate the properties of raw materials and finished products. In the development of new recipes and technologies of meat products, chickpea flour, sprouted rape seeds, pumpkin paste, carrot puree, apple powder, kelp, whey protein concentrate and so on are used **[2]**, **[5]**, **[6]**, **[7]**. Scientists have proved the possibility

of using buckwheat flour in the technology of functional meat products. It was found that the use of buckwheat flour affects the quality indicators of meat products **[8]**, **[9]**, **[10]**. Buckwheat groats are rich in vitamins, minerals and starch and contain much protein and fiber compared to wheat. Buckwheat flour has a protein structure with high biological value and does not contain gluten. Studies show it is rich in antioxidant compounds such as polyphenols **[8]**.

In this regard, it is assumed that the use of buckwheat flour will improve the physical, chemical, functional, technological, structural, mechanical, and organoleptic indicators of ground meat in the production of sausage products. To develop a new meat product (semi-smoked sausage), it was decided to use buckwheat flour as a vegetable filler.

Scientific Hypothesis

This article aims to substantiate the use of buckwheat flour in the technology of semi-smoked sausage based on the study of physicochemical, functional and technological, structural and mechanical and organoleptic parameters.

MATERIAL AND METHODOLOGY

Samples

For the manufacture of experimental samples of sausages, the following raw materials were used: 1st grade beef **[11]**, lean pork, pork brisket **[12]**, and buckwheat flour **[13]**. In the recipes of the experimental samples, additional raw materials were used: salt, sodium nitrite, granulated sugar, fresh garlic, ground black pepper, and ground pepper. All raw materials were purchased at the food market of Almaty.

Chemicals

The following chemical substances were used to obtain histological sections:

Technical formalin GOST 1625-89, grade FM, the highest grade (Chemical Industrial Reagent LLP, Shymkent, Kazakhstan).

Ethyl alcohol rectified from food raw materials (manufacturer: "DOSFARM LLP", Kazakhstan).

Paraffin GOST 23683-89, mark P 2 (food grade) (manufacturer: Turkey).

Hematoxylin regression and eosin alcohol staining kit "MEDIX" (manufacturer: Russia).

Fir balsam (manufacturer: Russia).

Instruments

For salting, the meat was spritzed with a salting solution. The salted meat was stored in a "Biryusa" refrigerated cabinet. Meat was chopped in a MP-300 meat grinder, and minced meat was mixed in a HO-25V mixer. Minced meat was dosed with an ASAN syringe. Active acidity in meat products was determined using a pH-410 device.

Heat treatment of semi-smoked sausage samples was carried out in SPAKO universal heat chamber for all sausage products. The temperature of the finished samples was controlled by an infrared thermometer with a laser pointer and a Testo 826-T4 penetrating food probe.

Samples were cut on an MSM-2850 semi-automatic microtome-cryostat. The obtained preparations were studied using a Biolam P1U4 microscope under 3.2-40 objective lenses with an eyepiece magnification of 13×

Laboratory Methods

Moisture content was determined by drying **[14]**. The concentration of hydrogen ions of meat and meat products (pH) was determined by a potentiometric method based on measuring the difference of electric potentials between the glass electrode and the reference electrode placed in the meat or meat products sample. Determination of moisture-binding capacity (MBC) – by pressing a sample under a 1 kg load and subsequent calculation by the difference of masses before and after pressing and the area of a wet spot determined by a planimeter according to the method of Grau and Hamm in modification of Volovinsky and Kelman and expressed in % to the total mass of moisture in the product. Shear stress limit (Pa) in sausage minced meat was determined by the dip cone method. Histological studies of raw sausage with enzyme and buckwheat flour were conducted using standard methods **[15]**, **[16]**. Histological studies of finished semi-smoked sausage products with enzyme and buckwheat flour were carried out according to the same standards as for raw sausage **[15]**, **[16]**.

Description of the Experiment

Sample preparation: In the experiments, the research object was semi-smoked sausage in a casing with a diameter of 45 mm. To study the influence of buckwheat flour on the quality and yield of semi-smoked sausage, buckwheat flour produced according to TU 9293-002-43175543-03 was used. Organoleptic parameters of the flour were as follows: color – light brown, homogeneous and without extraneous inclusions; smell – peculiar to the culture from which it was made; taste – fresh, peculiar to the culture. To determine the amount of water required for the hydration of buckwheat flour for its use in the recipe of semi-smoked sausage, this vegetable raw

material has determined the physicochemical, functional and structural-mechanical parameters: pH, moisture-binding capacity and the shear stress limit of minced meat.

Number of samples analyzed: A total of 18 samples were analyzed.

Number of repeated analyses: All measurements of instrument readings were carried out three times.

Number of experiment replication: Number of repetitions of each experiment to determine one value was three times.

Design of the experiment: Studies of pH, moisture-binding capacity, shear stress limit, organoleptic characteristics, and microstructure of meat products were conducted according to standard methods.

Statistical Analysis

In conducting research, a complex of standard and modified methods of definition of physical and chemical, functional and technological, and histological properties of raw materials and ready products was applied. The reliability of the results is confirmed by multiple repetitions and reproducibility of experimental data, mathematical processing and approbation of the technology of a new meat product in conditions of the joint-stock company "Almaty Technological University". Statistical processing was performed in Microsoft Excel 2016 and with Statistica 12.0 (USA). The accuracy of the obtained experimental data was determined by the Student's test with a confidence probability of ≤ 0.05 for the number of parallel determinations of 5 minimum. The linear programming problems were solved using the MS Excel spreadsheet "Solution search" (Excel Solver).

RESULTS AND DISCUSSION

The complete recipes for the model samples of semi-smoked sausages are shown in Table 1. The salting of raw meat in all variants of the experiment was carried out with a previously prepared brine under the recipe of experimental samples of semi-smoked sausage **[17]**. Following the research methodology, five samples of cased semi-smoked sausage were produced **[17]**.

Table 1 Recipes for semi-smoked sausage samples (100 kg).

Name of raw material	Control, (Ukrainian) sausage, 1 sort	Sample No.1 (4% buck-wheat flour)	Sample No.2 (6% buck-wheat flour)	Sample No.3 (8% buck-wheat flour)	Sample No.4 (10% buck-wheat flour)	Sample No.5 (12% buck-wheat flour)
Beef, 1st grade	50.0	50.0	50.0	50.0	50.0	50.0
Half fat pork	25.0	21.0	19.0	17.0	15.0	13.0
Pork brisket	25.0	25.0	25.0	25.0	25.0	25.0
Buckwheat flour	-	4.0	6.0	8.0	10.0	12.0
Salt	3.0	3.0	3.0	3.0	3.0	3.0
Sodium nitrite	0.05	0.05	0.05	0.05	0.05	0.05
Sugar	0.1	0.1	0.1	0.1	0.1	0.1
Fresh garlic	0.065	0.065	0.065	0.065	0.065	0.065
Black pepper ground	0.05	0.05	0.05	0.05	0.05	0.05
Allspice ground	0.06	0.06	0.06	0.06	0.06	0.06

Working out and defining organoleptic and physicochemical indicators of the ready products (pH, moisture-binding capacity) according to the standard methods also were spent in conditions of the educational-production laboratory of the Almaty technological university **[17]**. Physico-chemical, functional-technological, structural-mechanical and histological properties of experimental combined minced meat determine the quality of finished sausage products **[17]**. The quantitative content of key nutrients determines meat systems' functional and technological properties, primarily myofibrillar proteins and lipids, and their qualitative (amino- and fatty-acid) composition **[18]**. Functional and technological properties of meat raw materials change in time during the development of autolytic changes, during mechanical processing (massing, tendering, grinding of varying degrees), during curing in salting, heat treatment and other technological influences **[19]**. Moisture-binding capacity and the minced meat components' cohesiveness significantly influence the finished product's properties

[20]. The results of the pH of the control and experimental samples of semi-smoked sausages depending on the level of introduction of buckwheat flour are presented in Figure 1.

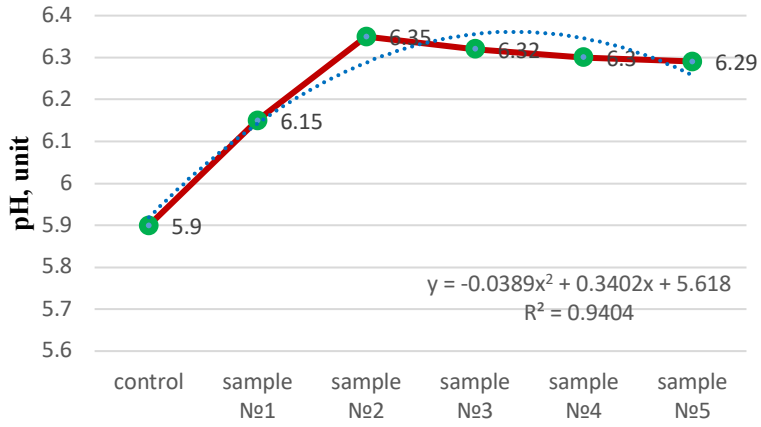


Figure 1 The change in pH in the control and experimental samples of semi-smoked sausage, depending on the level of introduction of buckwheat flour.

(Ukrainian) smoked sausage was used as a control. In the control sample, the pH value was 5.9 units. The maximum pH value was observed in sample No. 2 - 6.35 units. With increasing the dosage of buckwheat flour, the active acidity gradually decreased from 6.32 to 6.29 units. The moisture-binding capacity index determines the degree of moisture retention inside the product **[21**]. The moisture-holding capacity index in semi-smoked sausage samples was unequal in the experiments. The highest value was found in the experimental sample, 71.3% (Figure 2).

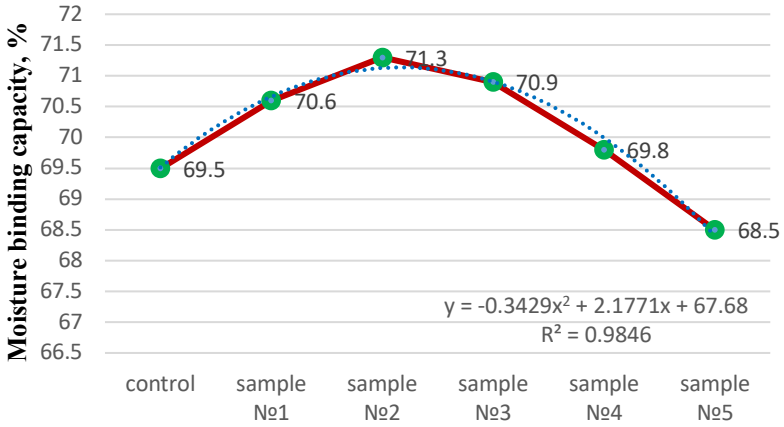


Figure 2 Changes in moisture-binding capacity in the control and experimental samples of semi-smoked sausage, depending on the level of introduction of buckwheat flour.

The results showed that the moisture-binding capacity of the minced meat in the experimental samples differed **[17]**. Thus, the highest value of the moisture-binding capacity was found in the experimental sample No. 2 – 71.3%. A relatively small amount of buckwheat flour in semi-smoked sausage meat samples (up to 10.0% by weight of unsalted raw material) increases the moisture-binding capacity of the control sample by 1.1-1.8 **[22]**. At the same time, the lowest values of the moisture-binding capacity have been noted in the samples with flour in the amount of 10 to 12.0% (69.8 and 68.5%, respectively). This fact is explained by the inability of buckwheat flour under heat treatment to transfer some of the released free moisture into the gel-like state **[22]**. The moisture-binding capacity gradually decreased by increasing the hydrated buckwheat flour content in the experimental samples' minced meat **[23]**. The lowest moisture-binding capacity values were obtained for samples with 10.0 and 12.0% buckwheat flour content – 69.8 and 68.5%, respectively. The decrease in the moisture-binding capacity in the experimental products is explained by the increase in the combined minced meat mix of the amount of moisture contained in the hydrated buckwheat flour **[22]**. Therefore, the combined minced meat mixture in the experimental samples becomes crumbly **[17]**.

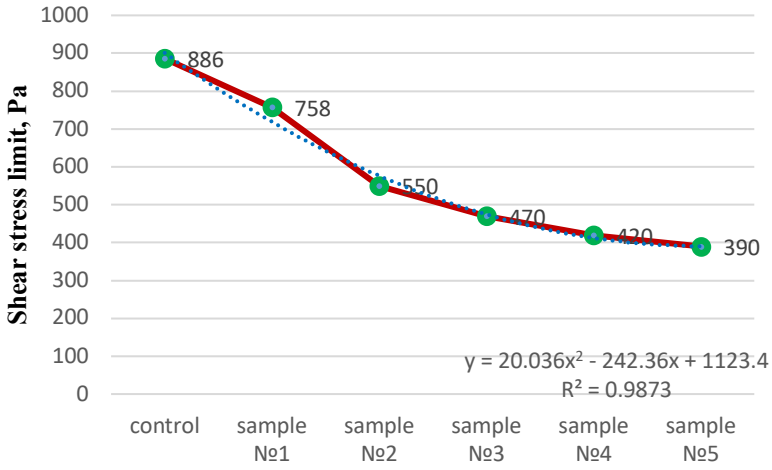


Figure 3 Change of shear stress limit in the control and experimental samples of semi-smoked sausage, depending on the level of introduction of buckwheat flour.

The prepared test samples' shear stress limit showed that this parameter's maximum value is 758 Pa. With increasing the dosage of hydrated buckwheat flour, the minced meat loosens, and the value of shear stress limit in samples No. 3 and No. 4 is 420 and 390 Pa (Figure 3). The studied physicochemical, functional-technological and structural-mechanical indicators of the minced meat system correlate with organoleptic indicators of the experimental product **[17]**.

The appearance, colour, smell, aroma, consistency, taste and juiciness were studied in the produced samples of semi-smoked sausage. The results of the organoleptic evaluation of the tested samples are shown in Table 2. The appearance of the examined samples differed significantly from each other by the variants of the experiment. The best was the control sample and variants with hydrated buckwheat flour in the quantity of 6 and 8% to the weight of meat raw material (5 points), and the worst were variants with 10 and 12% of buckwheat flour (4 points) **[17]**. The colour characteristics of semi-smoked sausage (on the cut) also differed between the variants. In this respect, preference was given to the control and experimental samples with 6.0% hydrated buckwheat flour (5 points) **[17]**.

Table 2 Organoleptic assessment of experimental variants of smoked sausages.

Sample and control variants	Appearance	Colour	Smell/aroma	Consistency	Taste	Juiciness	Total score
Control	great	attractive	inherent in the meat product	tender	delicious	juicy	great
I sample -4% buckwheat flour	excellent	attractive	inherent in the meat product	tender	delicious	juicy	great
II sample-6% buckwheat flour	excellent	attractive	inherent in the meat product	tender	tasty enough	juicy	great
III sample-8% buckwheat flour	good	good	insufficiently meaty	insufficiently	tasty enough	moderately juicy	good
IV sample-10% buckwheat flour	satisfactory good	satisfactory	no meat smell	tender	insuffici-ently tasty	not too juicy	average
V sample-12% buckwheat flour	unsatisfactory	unsatisfactory desirable	no meat odour	loose	unpalatable, tastes of buck-wheat flour	dry	average

Samples of semi-smoked sausage with 8 and 10.0% buckwheat flour scored 5 and 4 points, respectively. The lowest score was given to experiment No. 5 with 12% buckwheat flour to the weight of unsalted raw material.

When evaluating the samples of semi-smoked sausage in terms of smell and aroma the best indicators were noted in the control and the experimental sample with the content of buckwheat flour in the amount of 6% (5 points) (Table 2). Increasing the proportion of buckwheat flour in the raw meat material to 10% results in a slight aroma and aftertaste, reducing the meat product's taste (4 points) **[17]**. Significant differences characterized the flavour index of the semi-smoked sausage samples under study. When buckwheat flour was added to the raw meat up to 8%, the taste of the additive was barely perceptible in the product samples (5 points). A further increase in additive content in the samples resulted in a stronger taste of buckwheat flour and worsened the taste of the meat product **[17]**. The remaining samples each received a score of 3 for smell and flavour (Figure 4).

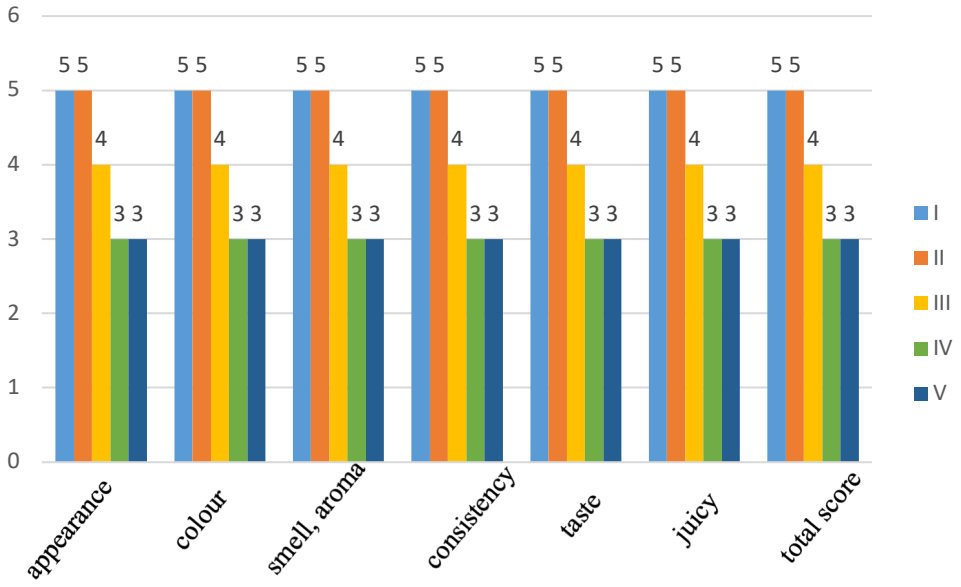
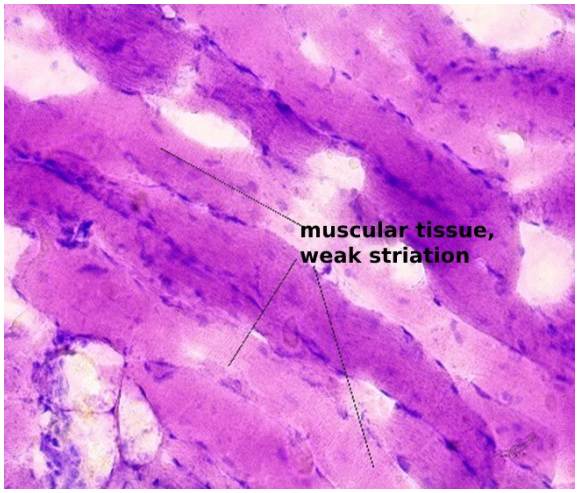
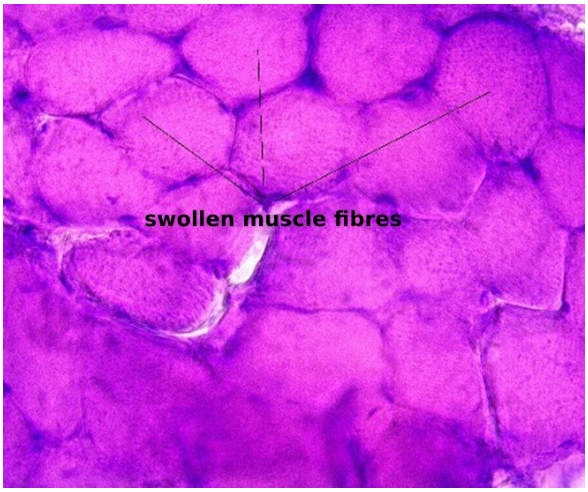


Figure 4 Histogram of organoleptic evaluation of experimental variants of semi-smoked sausage. Note: I sample; II sample; III sample; IV sample; V sample.

The consistency of semi-smoked sausage samples was not the same across the test variants. The samples absence or relatively small amount of additive ensured a fairly gentle consistency (5 points). With increasing the quantity of additive in the variants to 10,0 and 12,0% this indicator significantly decreased (4 and 3 points, respectively), and its maximum quantity (12.0%) worsened the product consistency to an unsatisfactory value (3 points) **[17]**. Regarding juiciness, all the samples tested were about the same (4-5 points). Thus, based on the total score of experimental samples, it was found that the introduction of buckwheat flour in an amount of up to 6-8% does not worsen the organoleptic quality of semi-smoked sausage **[8]**, **[9]**, **[10]**, **[17]**, **[23]**, **[24]**. At the next stage of experimental research, histological studies were carried out an experimental sample of semi-smoked sausage with a level of hydrated buckwheat flour 6% **[17]**. The sample studied includes muscle, connective, fatty tissue, fine-grained protein mass, buckwheat flour fragments, and rounded muscle fibres. Muscle tissue is the main functional component of raw meat and a source of protein and consists of muscle fibres – a kind of multinucleated cells with an elongated shape **[25]**, **[26]**, **[27]**, **[28]**, **[29]**, **[30]**. Figure 5 shows changes in the muscle tissue of thermally untreated and finished semi-smoked sausages. Weak striation disrupted tinctorial properties, and no nuclei of fibres were preserved. There are areas with indistinct boundaries between muscle fibres (sarcolemma and endomysium destroyed) (Figure 5) **[29]**, **[30]**, **[31]**, **[32]**, **[33]**, **[34]**, **[35]**, **[36]**, **[37]**.



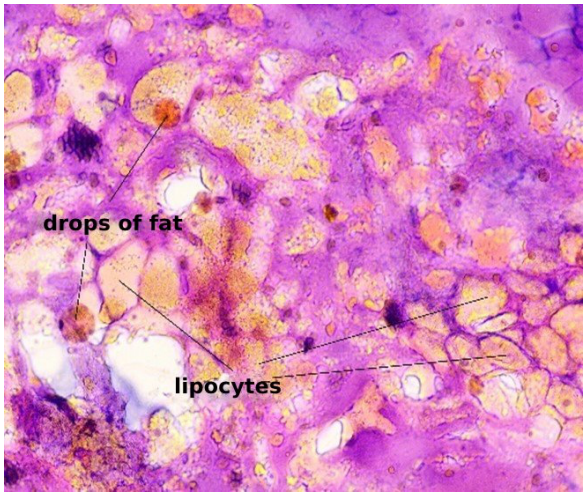
Raw sausage muscle tissue (sarcolemma destroyed, weakly distinguishable striations), ×200
a) in a raw sample



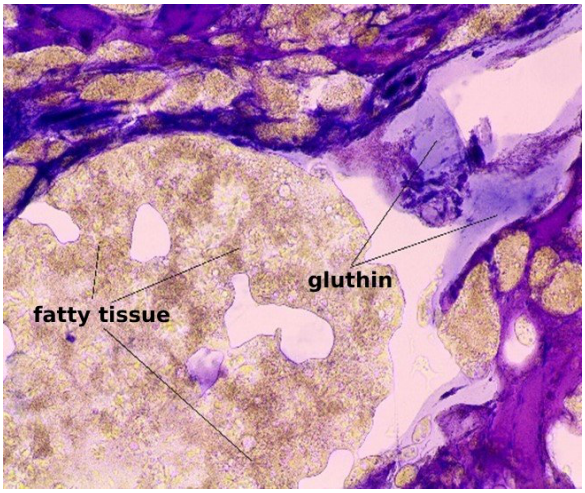
The muscle tissue of semi-smoked sausage (muscle tissue with swollen fibers) ×400
b) in the finished sample

Figure 5 Changes in the muscle tissue of raw and finished semi-smoked sausages.

Fatty tissue in the form of lipocytes and fat droplets (Figure 6). Loosening and destruction of connective tissue elements were detected, and fibrous structures were subject to lysis **[28]**. There are fragments with destroyed edges among buckwheat flour particles with pronounced boundaries (Figures 7, 8, and 9). The distribution of the flour throughout the sample is uniform **[29]**.

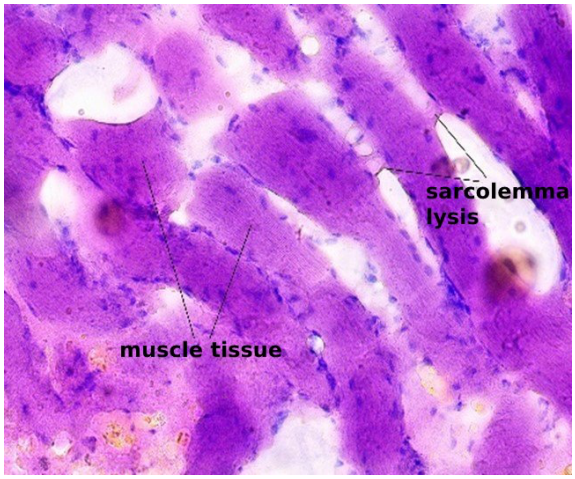


Fatty tissue, lipocytes, drops of fat in minced sausage, ×100
a) in a raw sample

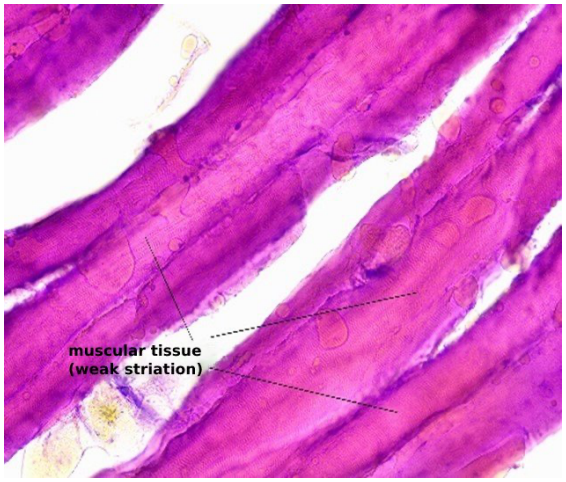


Fatty tissue and gluthin in semi-smoked sausage, ×100
b) in the finished sample

Figure 6 Change in the fatty tissue of thermally unprocessed and finished semi-smoked sausages.

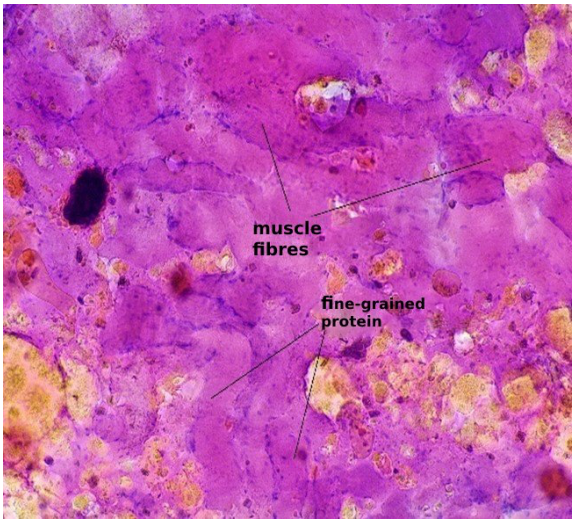


Muscle fibers with lysed sarcolemma and endomysium in minced sausage, ×200
a) in a raw sample

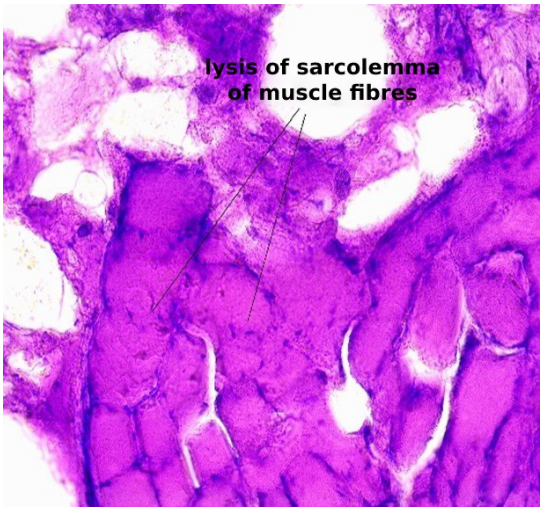


Muscle tissue of semi-smoked sausage, no nuclei, weak striation, ×200
b) in the finished sample

Figure 7 Change in muscle tissue of thermally untreated and finished semi-smoked sausages.

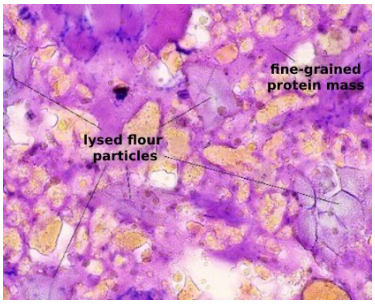


Fine-grained protein mass with lysed muscle fibers, ×100
a) in a raw sample

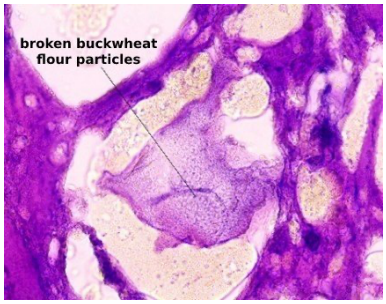


Lysis of the sarcolemma of muscle fibers of semi-smoked sausage, ×200
b) in the finished sample

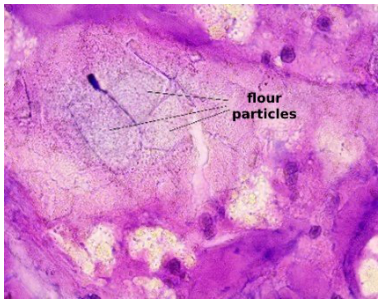
Figure 8 Lysis of sarcolemma muscle fibres of thermally untreated and finished semi-smoked sausages.



Fine-grained protein mass with lysed buckwheat flour particles, ×100
a) in a raw sample



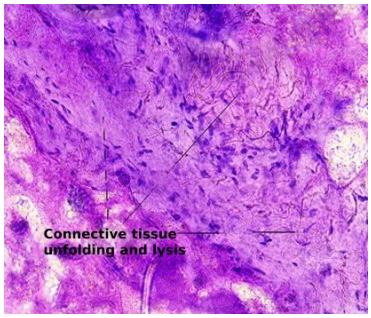
Broken buckwheat flour particles of semi-smoked sausage, ×200
b) in the finished sample



Flour particles with lysed edges in raw sausage, ×200
c) in a raw sample

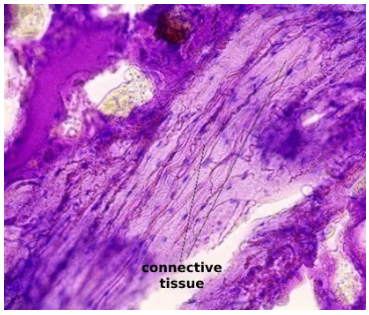
Figure 9 Changes in buckwheat flour particles of thermally untreated and finished semi-smoked sausages.

Figure 10 shows that the fibrous structures of the connective tissue are swollen, in some places, have lost their structure and acquired the appearance of a granular mass. In some cases, a mass with glutin was formed. Fragments with broken edges were visible among buckwheat flour particles with pronounced boundaries. Flour distribution throughout the sample is uniform **[29]**.



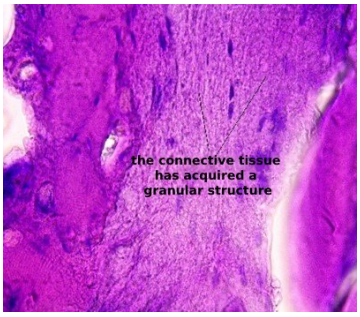
The connective tissue of raw sausage (unfolding and lysis), ×200

a) in a raw sample



The connective tissue of semi-smoked sausage, ×200

b) in the finished sample



Connective tissue in semi-smoked sausage (loss of fibrous structure and acquisition of granular mass), ×400

(c) in the finished sample

Figure 10 Changes in the connective tissue of thermally untreated and finished semi-smoked sausages.

Figure 11 Experimental sample of semi-smoked sausage with 6% buckwheat flour.

Thus, it has been established that adding hydrated buckwheat flour to the mass of meat raw materials positively affects the physico-chemical, functional and technological, structural and mechanical and organoleptic parameters of semi-smoked sausages **[17]**, **[29]**, **[38]**.

CONCLUSION

The study of active acidity of forcemeat samples of semi-smoked sausages showed that the maximum pH value was observed in experiment No.2 – 6.35 units. With an increasing dosage of buckwheat flour active acidity gradually decreased from 6.32 to 6.29 units. A relatively small amount of buckwheat flour in semi-smoked sausage samples (up to 10.0% by weight of unsalted raw material) increases the moisture-binding capacity of the control sample by 1.1-1.8%. At the same time the lowest values of the moisture-binding capacity have been noted in samples with flour in the amount of 10 to 12.0% (69.8 and 68.5%, respectively). The moisture-binding capacity gradually decreased with a further increase in the content of hydrated buckwheat flour in the minced meat of the experimental samples. The lowest moisture-binding capacity values were obtained for samples with 10.0 and 12.0% buckwheat flour content – 69.8 and 68.5%, respectively. A study of the shear stress limit of the finished test samples showed that the maximum value of this parameter was 758 Pa. With increasing the dosage of hydrated buckwheat flour, minced meat loosens, and the value of shear stress limit in samples number 3 and 4 is 420 and 390 Pa. The studied physico-chemical, functional-technological and structural-mechanical indicators of the minced meat system correlate with organoleptic indicators of the experimental product. The appearance, colour, smell, aroma, consistency, taste and juiciness were studied in the produced samples of semi-smoked sausage. Based on general organoleptic evaluation of experimental samples, it was established that the introduction of buckwheat flour in an amount of up to 6-8% does not worsen organoleptic quality indicators of semi-smoked sausage. Histological studies have been conducted on an experimental sample of semi-smoked sausage with 6% hydrated buckwheat flour. In general, it has been established that introducing hydrated buckwheat flour into the minced meat up to 6% of the raw meat material's mass positively affects the physicochemical, functional and technological, structural and mechanical and organoleptic parameters of semi-smoked sausages.

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

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Food safety and food security through predictive microbiology tools: a short review

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ABSTRACT

This article discusses the issues of food safety and food security as a matter of global health. Foodborne illness and deaths caused by pathogens in food continue to be a worldwide problem, with a reported 600 million cases per year, leading to around 420,000 deaths in 2010. Predictive microbiology can play a crucial role in ensuring safe food through mathematical modelling to estimate microbial growth and behaviour. Food security is described as the social and economical means of accessing safe and nutritious food that meets people's dietary preferences and requirements for an active and healthy life. The article also examines various factors that influence food security, including economic, environmental, technological, and geopolitical challenges globally. The concept of food safety is described as a science-based process or action that prevents food from containing substances that could harm human health. Food safety receives limited attention from policymakers and consumers in low- and middle-income countries, where food safety issues are most prevalent. The article also highlights the importance of detecting contaminants and pathogens in food to prevent foodborne illnesses and reduce food waste. Food and Agriculture Organization (FAO), an institution belonging to World Health Organization (WHO) presented calls to action to solve some of the emerging problems in food safety, as it should be a concern of all people to be involved in the pursue of safer food. The guarantee of safe food pertaining to microbiological contamination, as there are different types of active microorganisms in foods, could be obtained using predictive microbiology tools, which study and analyse different microorganisms' behaviour through mathematical models. Studies published by several authors show the application of primary, secondary, or tertiary models of predictive microbiology used for different food products.

Keywords: food safety, food security, predictive microbiology

INTRODUCTION

According to the World Health Organization (WHO), around 600 million cases of foodborne illness are reported around the globe every year. The number of deaths in the year 2010 was estimated to be around 420,000 people, and such a number tends to increase year after year due to the difficulty of estimating statistics on foodborne diseases **[1]**. Several agents are responsible for these infections, such as chemicals, parasites, and pathogenic and spoilage microorganisms **[2]**. Food safety refers to processes or actions that prevent foods from containing substances that could harm one's health and is designed to guarantee safe food for consumption. With the assurance of safe food, food security is improved by reducing hunger and malnutrition **[3]**, **[4]**. The quality and safety of foods can be determined through predictive microbiology, which uses mathematical modelling to estimate microbial growth and behaviour **[5]**. Many are applications of predictive microbiology in the food cycle production to guarantee safe food, from risk assessment to employee training. The result from its mathematical modelling helps to prevent foodborne illness outbreaks **[6]**, **[7]**. This review aims to introduce and discuss the

concepts of food safety and food security as a matter of world health issue, and the use of predictive microbiology through its models, which could be a tool to prevent food contamination and foodborne illness diseases.

FOOD SAFETY AND FOOD SECURITY

Food Security: According to the Food and Agriculture Organization (FAO), food security occurs when people have the social and economical means to physical access to safe and nutritious food that meet their dietary necessities and preferences for the maintenance of an active and healthy life, at all times **[8]**, **[9]**. Food security has a new reality where several factors influence the access of all people to safe food, being economic, environmental, technological, and geopolitical challenges globally **[10]**, **[11]**.

FAO/UNICEF described food security with the perception based on four main concepts: food availability; physical and economic access to food; food usage, based on cultural and nutritional requirements; and food stability, as the balance of supply (Figure 1) **[8]**.

Figure 1 The four pillars of food security (Adapted from **[8]**).

Between 1961 and 2000, an exponential increase in the global population demanded developments in food production. Scientific and technological progress, government actions, institutional interference, business investment and innovation were able to meet the requirements **[8]**. The United Nations predicts an increase in global population annually of 0.96% from 2015 – 2030 and 0.63% between the years 2030 – 2050, reaching approximately 9.7 billion people by the year 2050. This population rise is expected to occur mostly in lower-income and less-developed countries **[8]**, **[10]**, **[12]**.

Some global megatrends are expected to meet the safe food requirements for all within the next years. Food production "industrial-scale and centralized production systems, together with large-scale farming, intense animal production, and large-scale food process and distribution" **[12]** continuously increased in the last few years. Climate change can also affect food security in many aspects, so the need for actions to be taken will most likely address some of the current problems in that area. Mega-cities and mega-regions could facilitate people's access to modern food chains and supermarkets, supplying safe food. The growing ageing population demands "extra-safe food", the impacts food can have on their welfare is a matter of public health. One of the trends is also technologically web-based food, the printing of food in 3D printers could revolutionize the food industry. At last, as consumers desire individualized food, nutrition, service, and experiences, new products and innovations will emerge to satisfy those needs **[12]**, **[13]**.

Food safety: Food safety refers to how food is handled, prepared, and stored to minimize the risk of contamination. FAO (2022) describes food safety as a "science-based discipline, process or action that prevents food from containing substances that could harm a person's health, aiming to have safe food". Furthermore, improving food safety decreases hunger and malnutrition, associating safe food with food security **[14]**, **[15]**.

Food is the most basic human need, coming in third place after air and water, and is a necessity for everyone, meaning everyone should be involved in the pursuit of safer food. Those involved in growing, harvesting, transporting, processing, and packing food, also consumers and people responsible for providing laws, regulations, institutions, and inspections, should guarantee the production of safe and nutritious food for all **[16]**, **[17]**, **[18]**.

Most safety issues can be observed among low- and middle-income countries (LMIC) and could be related to poor infrastructure and lack of information on food handling, causing pathogenic contamination. Food safety receives limited attention from policymakers and consumers in LMICs. Governmental institutions often in charge of these functions in such countries deal with a problem with funds and other constraints, reducing and restraining any action **[19]**, **[20]**.

Also, disadvantaged and underprivileged populations are less willing to pay for safer food, probably due to low knowledge, than those with a higher income. According to studies, low-income and marginalized populations in Europe and the USA presented higher incidences of foodborne illnesses **[19]**, **[21]**, **[22]**.

Contaminants that present as a hazard to food safety if not detected prior to consumption include heavy metals, veterinary residues, pesticides, environmental organic residues, mycotoxins, microorganisms, and many others. Food spoilage can also be considered a food safety concern, contributing to food waste and food poisoning. Approximately one-third of all food produced (1.3 billion tons per year) is wasted globally **[23]**, **[24]**.

Antibiotic resistance is one of the most pressing issues in food safety. Antibiotic-resistant microorganisms that could cause diseases are becoming more prevalent. Enteric viruses provide a significant hazard to the spread of foodborne illnesses. Unintentional chemical pollution is dangerous for both people and wildlife. Food adulteration, motivated by economic factors, has a terrible impact on health. Around 3.5–4% of the world's population suffers from allergies and intolerances; nanotechnology is still under investigation but may pose risks to food production; genome editing may have an impact on global food production; and there may be a need for food safety regulations in this area **[12]**, **[25]**.

According to the SPS Agreement, which was established by the World Trade Organization (WTO) in 1994 to determine sanitary and phytosanitary procedures to protect public health in a way that causes the least amount of trade disruption, preventive actions in food safety systems should follow key criteria, such as systematic; risk-based, following a set of priorities and risk management methods; being open and participatory; being cost-effective; and causing the least amount of trade disruption. How institutions work together also impacts outbreak management success, as food safety is equally an institutional challenge as it is academic and scientific, corporate, or legislative **[26]**, **[27]**.

WHO, with FAO in 2021, organized the campaign “World Food Safety Day 2021” with the theme “Safe food now for a healthier tomorrow”, uniting the community globally to aid in the prevention, detection, and management of foodborne illness with actions presented in Figure 2 **[3]**.

Figure 2 Calls to action on food safety (Adapted from **[3]**).

MICROBIOLOGICAL ASPECTS

In fresh products, a series of microbiological, enzymatic, chemical, and physical reactions occur, simultaneously or consecutively, during storage time **[28]**, **[29]**, **[30]**. Product features determine the predominance of one spoilage effect over the other production activities, packaging, and storage conditions, among other factors **[31]**, **[32]**, **[33]**, **[34]**, **[35]**, **[36]**.

The analysis is carried out through comprehensive monitoring of variations in the critical indicator when the product is stored under specific circumstances, and considering the deterioration caused by microorganisms, simultaneously evaluating changes in sensory and physicochemical characteristics **[37]**, **[38]**, **[39]**.

Microorganisms: Microorganisms present in food are divided into three categories:

- Technologically beneficial: added to foods to enhance technological and sensory qualities. Selected microorganisms are added to preserve and extend the product's shelf life **[40]**.
- Foodborne pathogen: produces toxins and infects living cells, causing foodborne disease. These microbes can be found in the product's flora or can be transferred to food by contamination during processing, storage, or transportation **[41]**, **[42]**, **[43]**.
- Deteriorating microorganisms: create sensory changes in the product's colour, odour, flavour, and texture due to microbial proliferation and metabolism **[44]**, **[45]**.

Due to the severity of diseases caused by pathogenic microorganisms, a minimum limit or absence in food products is estimated to avoid foodborne illness epidemics **[46]**, **[47]**. The behaviour of spoilage microorganisms represents a challenge for shelf-life determination as they cause physical, chemical, and biological changes, making the food unpleasant to the human senses and unsuitable for consumption **[44]**, **[48]**, **[49]**, **[50]**, **[51]**.

Microbiological indicators: Microorganisms in food can cause sensory changes in a variety of ways: when microbial growth reaches a certain threshold, a large number of specific spoilage organisms (SSO) can cause opacity in liquids or viscous, mucous surfaces, as well as colour changes, which are typical of meat and fish products. Furthermore, sensory changes caused by enzymatic reactions will result in the degradation of proteins, carbohydrates, and fats, increasing metabolites **[44]**, **[52]**, **[53]**.

A sigmoid curve characterizes microbial growth, divided into four sections: lag phase, exponential or log phase, stationary phase and decline or death phase **[44]**, as shown in Figure 3.

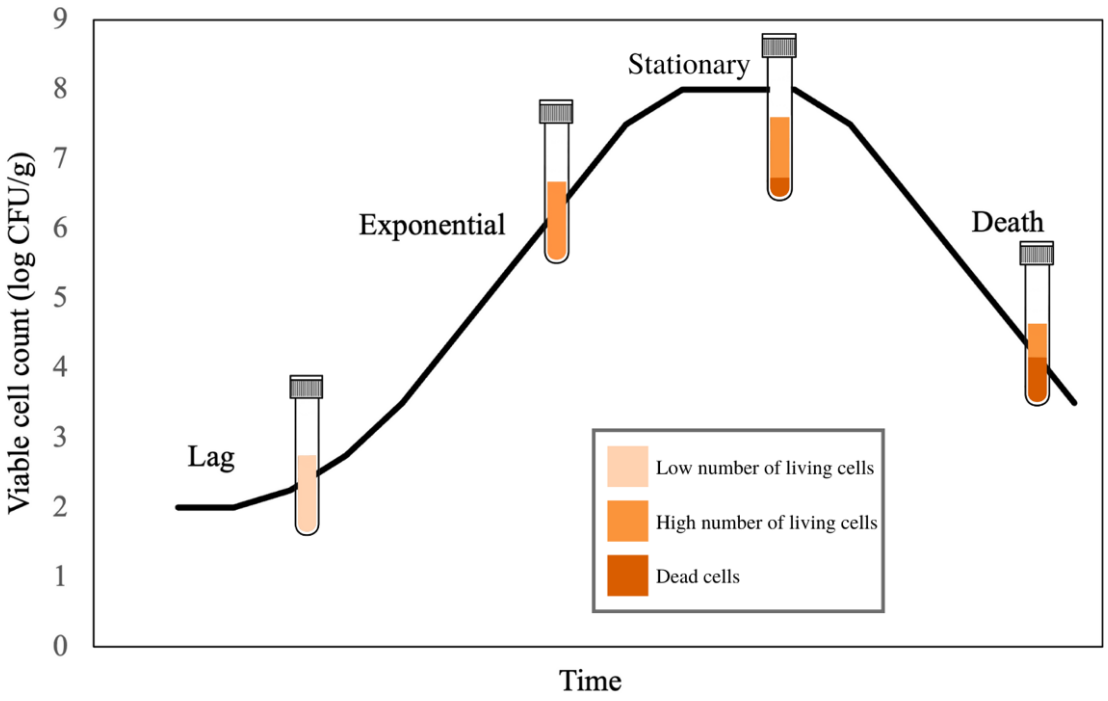

Figure 3 Microbiological growth curve.

The lag phase is the period of cell adaptation to the environment, and there is no multiplication. Increasing the duration of this phase is an important aspect of product shelf life. There are no changes in the sensory characteristics of food during the lag phase. In the exponential phase, the division of cells takes place, and the number of microorganisms grows at an exponential rate. During this phase, when the slope of the curve is at its steepest, the growth rate reaches its maximum value. Sensory characteristics change as a result of metabolic activity and microorganism growth. The stationary phase shows a decrease in cell division, resulting in some cells dying, some dividing slowly, and some ceasing to grow. The exhaustion of essential nutrients and space and the accumulation of inhibitory products cause this effect. It is also at this point that the cell count reaches its maximum amount. The death phase is characterized by the reduction in the population of microorganisms because during this stage, the number of cells dying outnumbers the number of cells born **[44]**, **[54]**, **[55]**.

Some studies indicate that odours caused by spoilage microorganisms become apparent when they reach values of 7 to 7.5 log CFU/g. The maximum number reached by spoilage organisms in fresh foods is estimated at and 10 log CFU/g **[44]**.

PREDICTIVE MICROBIOLOGY

Food safety standards in the food industry could develop risk analysis in public and private sectors to establish regulations and predictions. Applying quantitative and qualitative model systems allows prognosis and information about operations and quality, addressing the microbiological issues in food causing spoilage and health risks **[56-59]**.

Predictive microbiology is a tool used to study the behaviour of microorganisms under specific conditions for scientific analysis, as well as to determine the lifespan of products and the safety of foods **[60]**, **[61]**, **[62]**. Using mathematical models, it is possible to calculate the growth of microorganisms and draw conclusions about product quality and shelf life. This concept was first introduced in 1937 but has gained more use in recent years **[44]**, **[63]**, **[64]**.

The models used in predictive microbiology approaches have different evaluations. The most usual was the proposal by authors Whiting and Buchanan who characterized modelling as primary, secondary, and tertiary **[65]**.

The primary model elucidates the behaviour of microorganisms at a specific time under certain conditions **[56]**, **[66]**. They enable the calculation of relevant growth parameters such as maximum growth rate, lag phase duration, initial count, and maximum count of microorganisms. Most common models are modified Gompertz, Baranyi and Roberts, logistic, and three-phase linear models **[44]**, **[67]**.

Secondary models describe primary model parameters that are affected by environmental factors such as temperature, pH, and water activity. They also propose the time required to reduce the lag phase by ten times in response to changes in these factors **[44]**, **[54]**. The models used are the second-order response surface equation, the square root model, and the Arrhenius equation **[54]**. Table 1 shows an overview of various primary and secondary models used to predict the growth of microorganisms in foods, extracted from the studies of Kreyenschmidt and Ibald **[68]**.

Tertiary models generate representations from primary and secondary models and provide algorithms capable of calculating the microbial response to different applied conditions and comparing the effects of these different conditions **[73]**, **[74]**, **[75]**. There is several existing software to be used in the development of tertiary models **[44]**, **[54]**, **[56]**, **[76]**, **[77]**,

Besides the classifications above, the models can also be divided into four groups as presented in Table 2. Kinetic models predict the concentration levels of a microbial strain related to the rates of growth and death response; probability models predict the production of toxins of microorganisms and are related only to growth rate and not speed; empirical models relate two variables through polynomial equation offering a mathematical relationship between inputs and outputs; and mechanistic models allow to determine different parameters and present the effectiveness of predicted experimental conditions **[56]**.

Table 1 A correlation of various primary and secondary models (Adapted from **[44]**).

Primary Models	Equations	Source
Modified logistic model	$N(t) = A + \frac{C}{1 + e^{-B.(t-M)}}$	**[69]**
Modified Gompertz model	$N(t) = A + C.e^{-e^{-B.(t-M)}}$	**[69]**
Baranyi and Roberts model	$N(t) = A + \mu_{max}.a(t) + \ln\left[1 + \frac{\exp(\mu_{max}.a(t)) - 1}{\exp(N_{max} - A)}\right]$	**[70]**
Parameter	*N(t)* = microorganism count at a time t; *A* = lower line of the asymptotic growth curve; N_{max} = maximum population count; $C = N_{max} - A$, distinction between upper and lower lines; *B* = maximum rate of growth time *M*; *M* = time when maximum specific growth rate is achieved and *t* is time, m_{max} is maximum specific growth rate; *a(t)* = adjustment function, which takes into account the lag phase (adaptation to the new environment); *q(t)* = physiological conditions of the cells at time t.	
Secondary Models	**Equations**	**Source**
Arrhenius equation	$ln(B) = \ln F - \left(\frac{E_a}{R.T}\right)$	**[71]**
Square root equation	$\sqrt{B} = b * (T - T_0)$	**[72]**
Parameter	*B* = relative rate of growth at time *M*; *F* = preexponential factor; E_a = activation energy for the bacterial growth; *R* = gas constant; *T* = absolute temperature; *B* = slope (steepness) of the regression; T_0 = theoretical minimum cell growth temperature.	

Table 2 Classification of mathematical models in the food industry (Adapted from **[56]**).

Model category	Prediction	Publication
Kinetic	Growth or death response rate (concentration level of microbial strain)	**[78]**
Probabilistic	Toxin contaminant production by microorganisms or sporulation	**[79]**, **[80]**, **[81]**
Empirical	Interactions between inputs and outputs. A polynomial equation is used to describe a two-variable relationship.	**[82]**, **[83]**, **[84]**
Mechanistic	Prediction index under modified conditions, determination of various parameters	**[85]**, **[86]**

Predictive microbiology application in food: A selection of articles from the last ten years was conducted to verify the many applications of *predictive microbiology* analysis for microbiological contamination in different food products, as presented in Table 3. Pin and Baranyi from 1998 were also included because it was one of the initial publications presented for predictive microbiology, displaying the behaviour of several microbiological contaminants found most in meat products.

Table 3 Predictive microbiology analysis in different food products.

Food	Microorganism	References
Cantaloupe	*Listeria monocytogenes*	**[87]**
Cheese	*Escherichia coli* and *Staphylococcus aureus*	**[88]**
	Listeria monocytogenes and *Pseudomonas fluorescens*	**[89]**
Cheese - *Gorgonzola*	*Listeria monocytogenes*	**[90]**
Cheese – *Kochkäse*	Yeasts and molds, Aerobic mesophilic bacteria, and Lactic acid bacteria	**[91]**
Cheese - *Minas*	Lactic acid bacteria and *Listeria monocytogenes*	**[92]**
Cheese - *Paneer*	*Listeria monocytogenes*	**[93]**
Cheese - *Ricotta fresca*	*Enterobacteriaceae, Listeria monocytogenes,* Mesophilic lactic acid bacteria, molds, *Pseudomonas* spp., *t*otal bacterial count, and yeasts	**[94]**
Chicken meat	Total viable count	**[95]**
	Lactic acid bacteria, *Pseudomonas,* and total viable count	**[96]**
	Salmonella	**[97]**
	Salmonella spp.	**[98]**
Ham	Lactic acid bacteria	**[99]**
	Weissella viridescens	**[100]**
Hot Pepper Sauce	*Pichia manshurica, Lactobacillus, Escherichia coli, Salmonella enterica* and *Listeria monocytogenes*	**[101]**
Infant Formula	*Salmonella spp.*	**[102]**
Lettuce	*Salmonella* spp., *Escherichia coli*	**[103]**
	Salmonella spp.	**[63]**
Meat	*Acinetobacter*, *Brochothrix*, *Carnobacterium*, *Kurthia* spp., *Lactobacillus*, *Leuconostoc*, *Pseudomonas*, *Psychrobacter*, and *Shewanella*	**[104]**
	Lactobacillus plantarum	**[105]**
	Listeria monocytogenes and *Pseudomonas putida*	**[106]**
Meat - *Morcilla*	*Leuconostoc mesenteroides and Weissella viridescens*	**[107]**
Milk	*Listeria innocua*	**[108]**

Table 3 Continue.

Food	Microorganism	References
	Listeria monocytogenes and *Pseudomonas putida*	**[109]**
Mushroom – *Agaricus bisporus*	*Pseudomonas* spp.	**[110]**
Octopus	*Enterobacteriaceae*, *Pseudomonas* spp., total viable count	**[38]**
Pork meat	*Salmonella*	**[111]**
Potato Salad	*Salmonella* Enteritidis	**[112]**
Poultry	*Listeria monocytogenes* and *Pseudomonas fluorescens*	**[89]**
Rice	Molds, Aerobic plate count	**[113]**
Salmon	*Listeria monocytogenes*	**[89]**
Scrambled egg mix	*Salmonella*	**[114]**
Tomato	*Bacillus coagulans*	**[115]**

Predictive microbiology software: Tertiary models are applied in software that generates the model response in equations and graphs. There is a range of software with different databases and applications; a few of them are described in various articles, as presented in Table 4.

Table 4 Use of predictive microbiology softwares.

Software	Study	Reference
ComBase	Use of ComBase data to develop an artificial neural network model for nonthermal inactivation of *Campylobacter jejuni* in milk and beef and evaluation of model performance and data completeness using the acceptable prediction zones method	**[116]**
DMFit	The suitability of the ISO 11290-1 method for the detection ofListeriamonocytogenes	**[117]**
GinaFit	GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves	**[118]**
IPMP Global Fit	IPMP Global Fit – A one-step direct data analysis tool for predictive microbiology	**[77]**
MicroFit	MicroFit: free software for the development and adjustment of mathematical models of bacterial growth	**[76]**
MicroHibro	'MicroHibro': A software tool for predictive microbiology and microbial risk assessment in foods	**[73]**
VaIT	Validation software tool (ValT) for predictive microbiology based on the acceptable prediction zones method	**[119]**

Software is presented online or as an extension for Microsoft Office Excel. Each program contains a unique database for equations and models presented and validated by various authors **[120]**. The most common one used online, with a complete database, is ComBase, as shown in Figure 4. ComBase allows its users to insert research data, demonstrates changes in microbiological curves with extrinsic parameters change and compares with existing data in software (Figure 5).

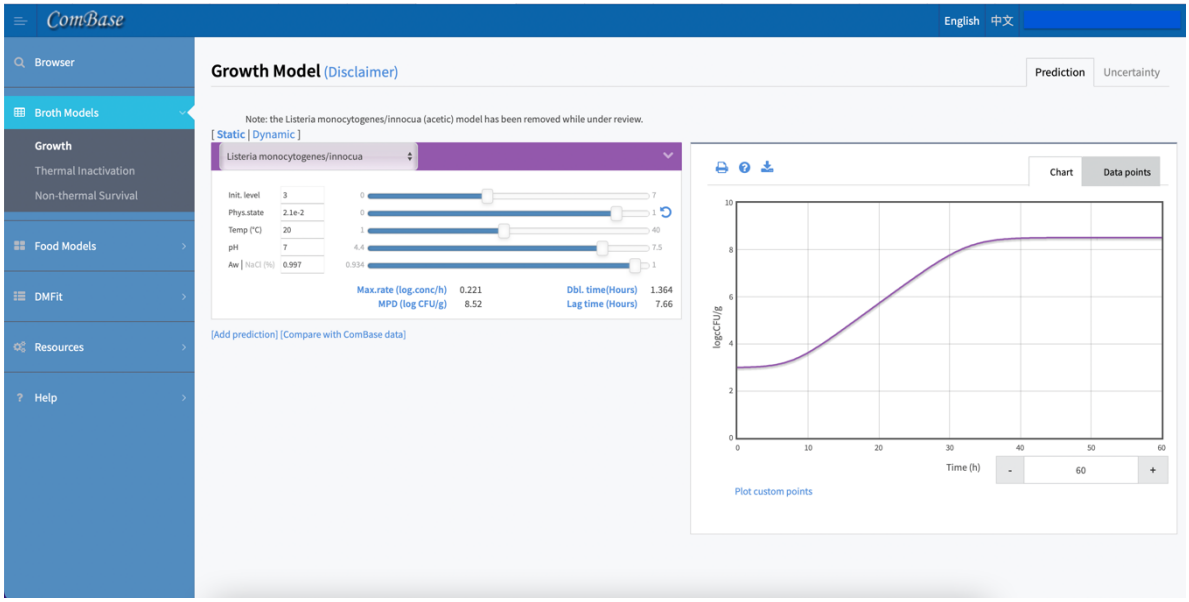

Figure 4 Interface of ComBase software.

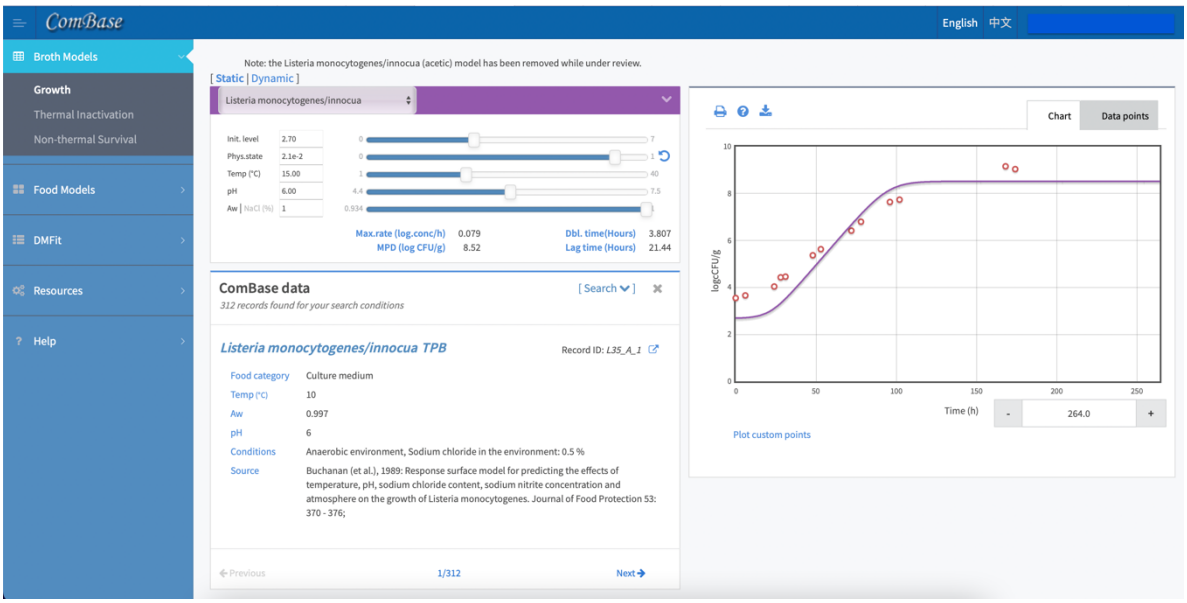

Figure 5 Interface of ComBase software with growth prediction and comparison with ComBase data.

Alternatively, software for Microsoft Excel has limited usage, but it is still one of the significant tools for calculating the parameters of the equation. GinaFit (Figure 6) presents several fixed models in which the user just needs to create a sheet with the data obtained in research, and, according to the model, results are presented. The reference for the model used is also presented **[120]**.

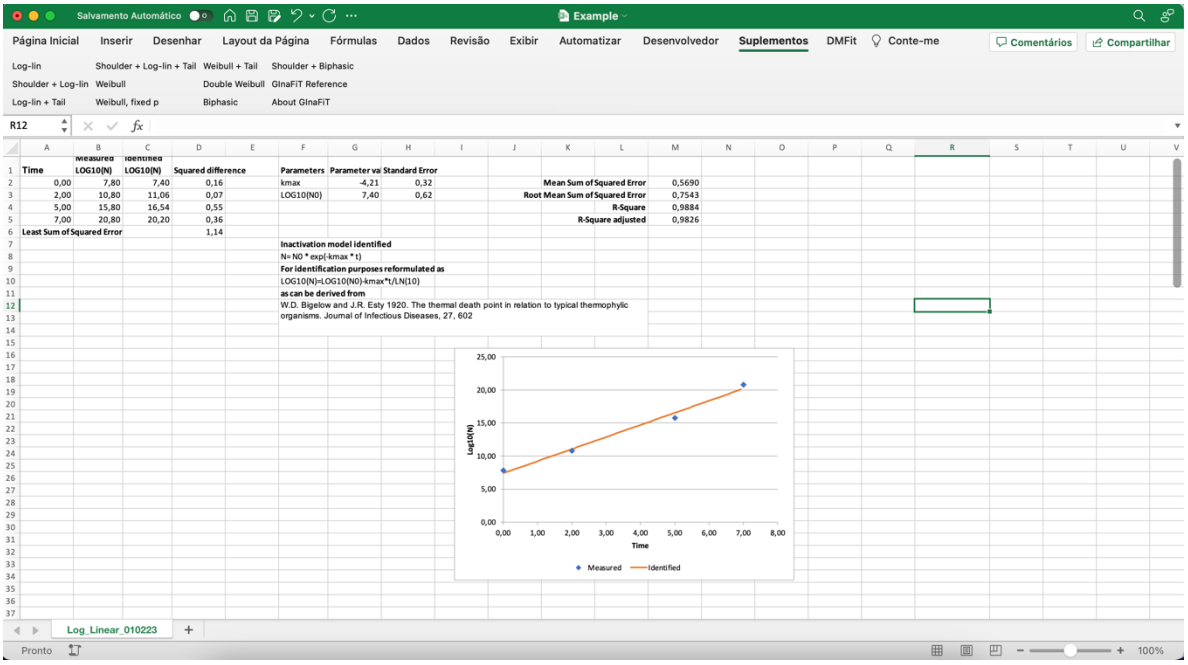

Figure 6 Interface of GinaFit within Microsoft Excel.

CONCLUSIONS

Food safety and food security are very important for public health. Predictive microbiology tools, using mathematical models to estimate microbial growth and behavior, are being used to prevent food contamination and foodborne illness diseases. The risks of unsafe food distributed to consumers could bring several problems to worldwide governments and organizations, as food safety and security are treated as public health issues and responsibilities. Predictive microbiology, a tool that was first addressed in 1937, has gained strength over the last twenty years between industry and academia, and can be utilized to establish a product shelf-life to reduce contamination from pathogenic and spoilage microorganisms. Acknowledging possible microbial contamination could reduce the problems regarding food safety and the waste of food that is generated annually. With safer food and waste reduction, food security becomes a closer reality for all people, as it should be according to the guidelines from FAO. Many studies have been conducted on different microorganisms in many food products. The approach between university and industry should be a reality to help prevent and solve the food safety and security problem, especially in economically developing nations, where food insecurity is often related to a lack of knowledge.

Food security is the state achieved when all people have the social and economic means to access safe, nutritious, and culturally acceptable food that meets their dietary requirements and preferences for an active and healthy life at all times. It is a complex issue influenced by various factors, including economic, environmental, technological, and geopolitical challenges. To address food security concerns, the concept is often based on four pillars: food availability, physical and economic access to food, food usage based on cultural and nutritional needs, and food stability. Achieving food security requires the involvement of all stakeholders, including policymakers, producers, distributors, and consumers.

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Income optimization of rice paddy farmers in the narrow fields during the covid-19 pandemic in South Sumatra province

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ABSTRACT

This study aims to analyze the amount of income through business diversification as well as scenarios for increasing income in business diversification during the Covid-19 pandemic. The research method used is the survey method, the sampling method used is a snowball and the number of respondents is determined by purposive sampling with 100 respondents. The study results show that the amount of optimization of the income of lowland rice farmers during the Covid-19 pandemic at a business diversification of 1 Rp. 29,130,500.00, business diversification 2 Rp. 19,007,006.29, business diversification 3 Rp. 8,301,257.48, business diversification 4 Rp. 14,877,500.00. The amount of farmer's income after the scenarios for business diversification 1 is carried out with additional capital of Rp. 1,870,000 so that the optimal allocation result will be an increase in income of Rp. 2,871,644.88 or 9.86%. Business diversification 2 is carried out with additional capital of Rp. 750,000 and a reduction of the workforce by 5 JOK so that the optimal allocation result will be an increase in income of Rp. 1,472,001.57 or 7.74%. Business diversification 3, it is carried out with additional capital of Rp. 370,000 and the addition of 4 JOK workers so that the optimal allocation result will be an increase in income of Rp. 978,173.65 or 11.78%. Business diversification 4 is carried out by increasing the land area by 0.25 so that it becomes 1 hectare and increasing capital by Rp. 500,000 so that the optimal allocation of income increases by Rp. 733,061.37 or 4.93.

Keywords: diversification, optimization, paddy, income, Covid-19

INTRODUCTION

Indonesia is also located in a tropical area with a climate suitable for extensive agricultural business so that Indonesia is an agricultural country **[1]**. The production of agricultural products for the provision of food, feed, industrial raw materials, and exports, as well as its role in the formation of GDP, employment, and sources of public income, make up the agricultural sector in Indonesia one of those that significantly contribute to the country's development **[2]**. Agriculture is one of the agribusiness industries that is regarded as an economic activity **[3]**. Agriculture is a process of producing food, livestock and agro-industrial products. Subsistence farming, who farms a small area with limited resource inputs, and produces only enough food to meet the needs of his/her family. Indonesia is a country with a fairly high vulnerability. One of the reasons is that the agricultural sector is a sector that is quite large in influencing this vulnerability **[4]**.

East OKU Regency is the main central rice area in South Sumatra Province and is a national food barn supported by technical irrigation. One of the agricultural enterprises that demands advanced growing methods is the rice field system. The area of rice paddy fields in 2019 was 638,198.79 ha with a production of 575,340.17 GKG and productivity of 62.24 %, the highest in South Sumatra Province. Meanwhile, East Buaymadang District is an area of East OKU Regency with the highest contributor to rice production, considering that this area has a very good area of irrigated rice fields. Rice farm is a source of livelihood and food security for a large proportion

of rural families. The domestic policies of the Indonesian governments have sought to achieve self-sufficiency in rice because they recognize the significance of rice in ensuring both household and global food security as well as its relevance as a key source of income for small-scale subsistence farmers **[5]**.

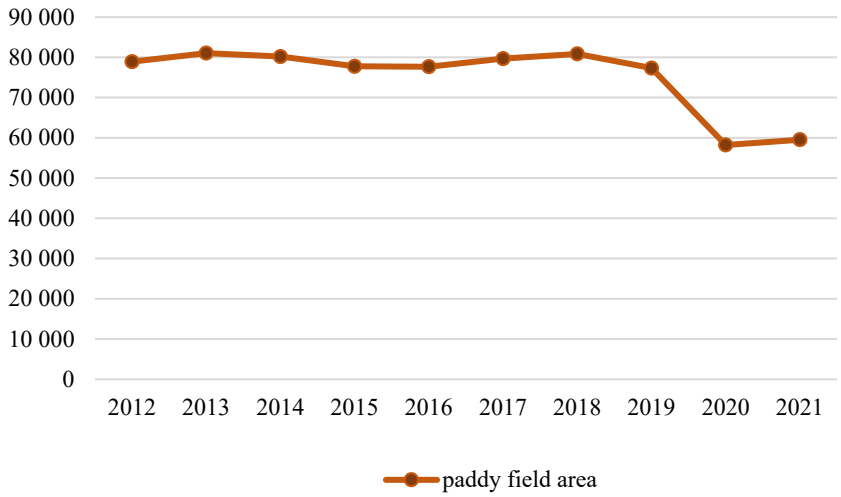

Figure 1 Development of changes in the area of paddy fields in East OKU Regency.

But on the other hand, the research results **[6]** show that tropical agriculture is an area that experiences a lot of fragmentation of paddy fields so that the existing land is narrow lands with an average of fewer than 0.5 hectares. Land is the main asset of farming, which tends to be narrower, affecting the production system and decreasing farm income. Consequently, we require a farming system that uses polyculture farming to maximize land use. Residential construction has supplanted agriculture **[7]**.

Urban regions' growing populations impact the requirement for housing. So many people exploit agricultural land and forests for houses and shelter to meet these needs. Construction of homes has been done on agricultural land **[8]**. Due to changes in regional spatial planning, population growth, and other factors, land factor, which has historically been the primary asset of farming, tends to drop over time**.** Indonesia's predominant farming system features are small farmers with a limited land ownership level **[9]**. Farmers in rural areas with small plots of land simultaneously put various plants there **[10]**.

The data in the field shows that small land farmers continue to make changes to the sengon farming business because the land around them has been planted with sengon so when farmers persist in sugarcane farming. This change continues to be made because their sugar cane will die and not get results, so they follow the change to sengon. When small land farmers switch to sengon farming, it is hoped that they will get better results with their narrow land. Small land farmers will continue to follow changes in sengon farming carried out by large land farmers because it is considered that the shift in sengon farming will be more profitable than staying in sugar cane farming which experiences price fluctuations every year and experiences the risk of loss. The informant's statement supports this statement: "My land is only narrow, if there is no one track sugar cane, the result is at least 10 bunches with a yield of less than 1.5 million, the maintenance costs have not been deducted, fortunately sengon is 4 years and it can be 15 million **[11]**.

Even though East OKU Regency is the capital city of South Sumatra, land fragmentation has occurred, with only 0.38 ha of available land per farmer. Rice farmers carry out their farming twice a year (IP 200), but the results obtained are still insufficient to meet the needs of the farmers. The implication of this shrinking land has an impact on decreasing the income of rice farmers so that farmers diversify their businesses to increase farmers income. **[12]** stated that based on research and discussion, the social capital of small land farmers is fulfilling household livelihoods. Small land farmers take advantage of their social capital. With this social capital, smallholder rice farmers are can other income alternatives outside of farming activities, thereby reducing the difficulty of living to fulfill household livelihoods. Rice farmers in Kolomayan Village carry out various income alternatives to fulfill household income by utilizing their social capital, such as raising livestock, taking debt, and working together on agricultural land. The capacity of farmers is influenced by the ability of agribusiness planning **[13]**.

In addition, the global condition is the COVID-19 pandemic, resulting in an economic crisis **[14]**, **[15]**. This pandemic does affect not only the health and education sectors but also the socioeconomic conditions of society. The COVID-19 pandemic also disrupted economic activity in all lines of business, including the agricultural

sector. One of the impacts that must be anticipated regarding the impact is food availability for all people **[16]**. The COVID-19 pandemic has triggered problems, especially in agriculture, such as low community productivity and also external problems, namely in the form of market and climate aspects that are difficult for farmers to overcome **[17]**, **[18]**, **[19]**. Impacts of the COVID-19 epidemic on farming households are significant **[20]**. It is destroying the agricultural production sector, which is the root of food system **[21]**.This reduces the welfare of farmers. For this reason, a mature strategic plan is needed to overcome problems and increase farmers' production and selling power **[22]**, **[23]**.

During the Covid-19 pandemic, small-land rice farmers in Buaymadang District, Ogan Komering Ulu Timur Regency, also experienced a significant impact in decreasing income due to restrictions on economic activities. This is in line with the findings **[24]**, who concluded that the Covid-19 pandemic significantly impacted all aspects of human life, including agriculture, due to government policies aimed at economic and non-economic development. As a result, one of the most important strategies is business diversification. Utilizing sustainable farming methods can increase productivity and farmer income **[25]**.

According to **[26]** strategic efforts that must be made are using machinery and reducing labour wages. This can reduce production costs which are quite large, increase the productivity of land to achieve more perfect land, and reduce losses due to loss of yields at harvest and make cooperation in the sale of production. Considering South Sumatra has an area of 87,421.24 km^2 and an agricultural area of 1,354,847 ha it is divided into 4 cities and 13 regencies. The agricultural sector is one sector that has a very important role in the economy in South Sumatra, this is because the agricultural sector is a job and a source of income for the community. Agriculture is the heart of the economy and rice remains its lifeblood **[27]**. According to the Central Statistics Agency **[28]**, one of the business fields that play a role in South Sumatra's GRDP is business from the agricultural sector 16.06 % **[29]**.

Recently, the findings of the **[30]** study in Nigeria, a developing country such as Indonesia, showed that the Covid-19 pandemic also experienced a surge in petitions and food support, leading to a decrease in the number of people living in poverty. The results of the **[31]** also showed that the COVID-19 crisis caused a surge in economic activity that had not previously occurred for governments around the world, with certain sectors becoming more vulnerable to pandemics. The plight of small migrant farmers in India has shown fault lines not only in the economic sphere but also in the community. Pandemics have changed the status quo. Based on the description, this study analyzed the optimization of narrow land rice farmers' income through business diversification at the time of the Covid-19 pandemic and scenarios of increasing income in business diversification during the Covid-19 pandemic.

Scientific Hypothesis

The study had two hypothesis:

1. The optimal utilization of agricultural resources such as land, capital, and labour increases income.
2. Farming diversification business is proven to increase farmers' income, and highest income is in the paddy-cucumber business diversification.

MATERIAL AND METHODOLOGY

Study Area

This research was conducted from January to February 2021. The research was conducted in two villages, namely Genuksuran Village and Nambuhan Village. Determination of the research location is done by the purposive method based on certain criteria, namely where farmers are fragmented, and currently, the land they own is narrow.

Data Collection

The research method used is a survey method, in which the data collection instrument is a questionnaire. Survey research involves gathering data from a sample and using it to characterize various facets of a population through questionnaires or interviews **[32]**.

Samples

The sampling method used was the snowball sampling method, and the number of respondents was determined by purposive sampling with a total of 100 respondents.

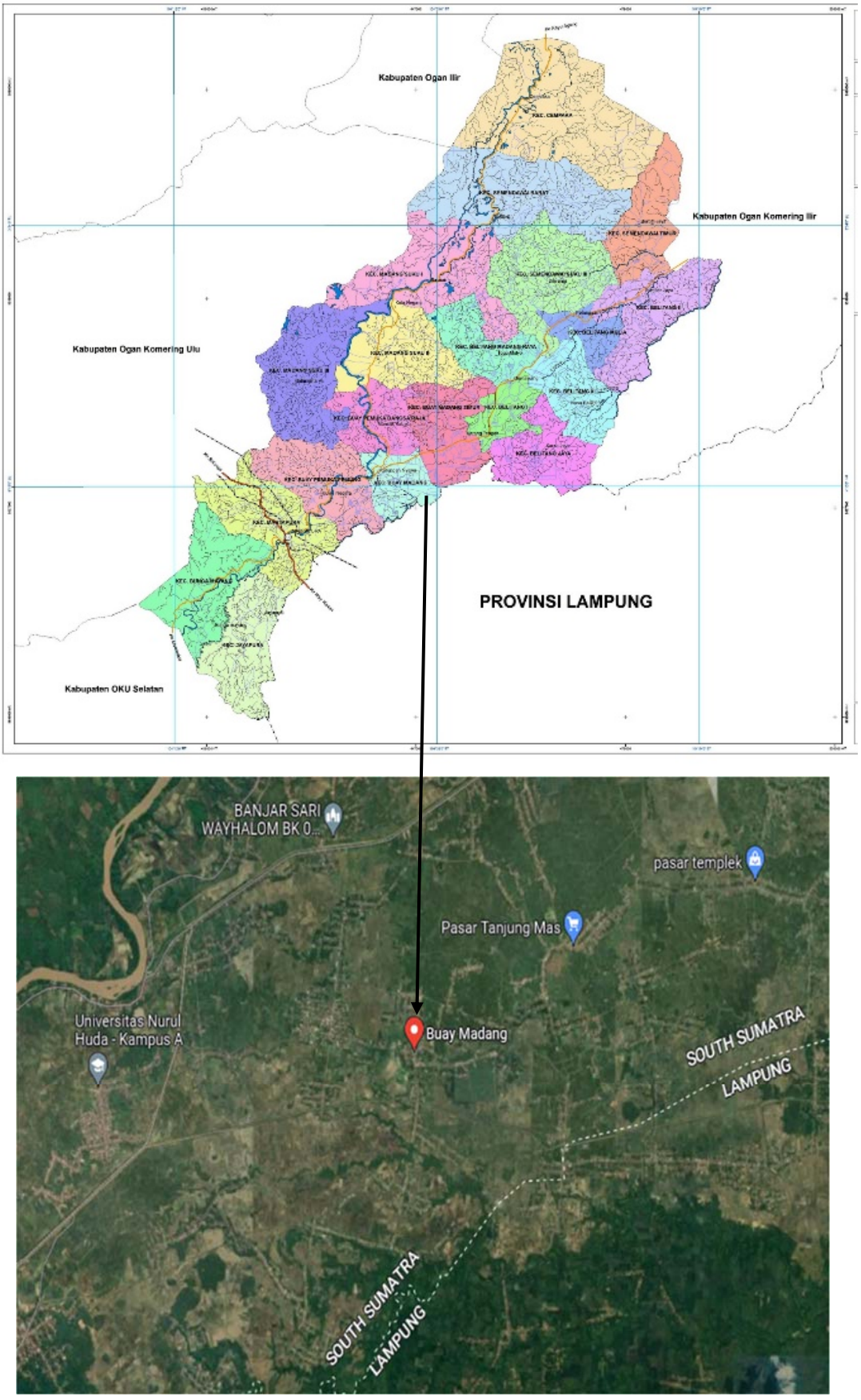

Figure 2 Map of research locations in Buay Madang East OKU District.

Statistical Analysis

The data was collected in the form of primary data and secondary data. The software used for data analysis in the study was the LINDO (Linear Interaktive Discrete Optimizer) software version 6.1. LINDO software was developed by a software development company called LINDO system Inc. in Chicago, Illinois. LINDO is software that can be used to find solutions to linear programming problems. Data processing is done using the following formula:

$$Z = \sum Cj \times X$$

Where:
Z = Purpose function; Cj = Objective function parameters to-j; Xj = Activity level to j n.

$$\text{Maximum } Z = C1X1 + C2X2 \ldots + CjXj - \ldots. + CnXn \text{ or } Z = \sum_{j=1} Cj\ Xj$$

With a constraint:

$$a11x1 + a12x2 + \ldots a1jxj + \ldots a1nxn \leq b1$$
$$a21x1 + a22x2 + \ldots a2jxj + \ldots a2nxn \leq b2$$
$$a31x1 + a32x2 + \ldots a3jxj + \ldots a3nxn \leq b3$$
$$.. .. \quad .. .. \quad .. .. \quad .. ..$$
$$.. .. \quad .. .. \quad .. .. \quad .. .. \quad n$$
$$am1x1 + am2x2 + \ldots amjxj + \ldots amnxn \leq bm \text{ or } \sum_{j=1} aij\ Xj \leq bi$$

Where:
i = 1, 2, 3 … m is the number of limiting factors; j = 1, 2, 3 … n is the number of production activities; activity is not negative: xj ≥ 0 for the whole j.

Where:
Z = objective function, which is farm income which is maximized; C = prices of production (C) and prices of inputs (-C); xj = production and consumption activities carried out by households farmer; aij =input coefficient of each production and consumption activity; bij =constraint value or available resource limit.

RESULTS AND DISCUSSION

Optimizing the available resources is very important. Land area, labour and capital, if able to be optimized, can generate optimal income.

Optimal Income: Based on the results of the analysis with Linear Programming through computerization of optimal income, business diversification for each activity is obtained as in Table 1.

Table 1 Optimal income, business diversification for each activity.

Activity	Optimal Income (Rp/th)
Business diversification 1	Z = 29,130,500.00
Business diversification 2	Z = 19,007,006.29
Business diversification 3	Z = 8,301,257.48
Business diversification 4	Z = 14,877,500.00

Note: Sources: Analysis results.

Based on Table 1 It shows that the highest income is in the rice-cucumber business diversification and the lowest is Padi-Kale. If we look further, the income from business diversification that farmers have carried out is a form of farmers' strategy of narrow land in increasing income. Low-land tropical and subtropical agriculture frequently employs many crops as a method of managing land use **[33]**, **[34]**. The primary benefit of employing a multiple cropping system is that it entails combining crops while making better use of both space and labor **[35]**, **[36]**. Which is still far from the level of welfare, therefore farmers also still take advantage of their free time outside of farming to look for additional jobs, namely as farm labourers in other places. These results are in line with research **[37]**, showing that small land farmers, due to their fragmentation, still have a lot of free time. Farmers use this free time as farm labourers **[38]**. This is in accordance with research **[39]** that the land use will be maximized if you pay attention to the type of plant and performance of farmers. With access to cash and optimal land usage, the land will be more productive and promote food security **[40]**.

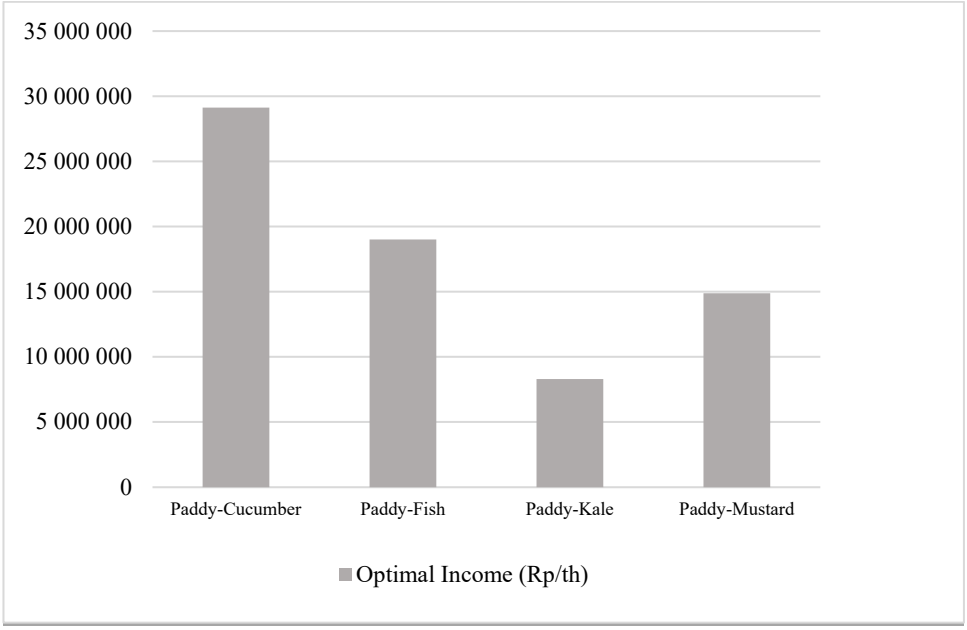

Figure 3 Optimal income based on diversification business variations.

Identification of Linear Programming Model: Business diversification carried out by land farmers during the COVID-19 pandemic in East Buay Madang District consisted of several business diversification activities. A small portion of the rice fields, rice field bunds, the yards of rice fields, or other land that is not planted with rice is taken in order to plant some of these plants **[41]**. Diversified farms have proven to be more crisis-resistant and able to handle the pandemic than other types of specialized farms **[42]**. In line with research **[43]**, **[44]** Agricultural diversification is done by intentionally adding functional biodiversity to cultivation agriculture and multiple cropping can enhance agricultural systems' efficiency and lessen the occasionally negative environmental effects of crop production. Increasing agricultural diversity is a key method being studied to improve agricultural systems' resilience to shocks and variability **[45]**, **[46]**.

Table 2 Business diversification carried out by land farmers during the COVID-19 pandemic.

Nr.	Business Diversification	Description
1.	Paddy-Cucumber	
	$Z = 9{,}831{,}500X1 + 19{,}299{,}000X2$	
	C1 $0.37X1 + 0.12X2 \leq 0.50$	Land
	C2 $3{,}200{,}000\ X1 + 11{,}500{,}000 \leq 14{,}700{,}000$	Capital
	C3 $92X1 + 15X2 \leq 107$	Labour
2.	Paddy-Fish	
	$Z = 8{,}746{,}000X1 + 10{,}297{,}000X2$	
	C1 $0.25X1 + 0.12X2 \leq 0.37$	Land
	C2 $2{,}500{,}000\ X1 + 4{,}250{,}000 \leq 6{,}750{,}000$	Capital
	C3 $90X1 + 26X2 \leq 115$	Labour
3.	Pady-Kale	
	$Z = 5{,}629{,}500X1 + 4{,}415{,}000X2$	
	C1 $0.12X1 + 0.10X2 \leq 0.22$	Land
	C2 $1{,}470{,}000\ X1 + 1{,}670{,}000 \leq 3{,}140{,}000$	Capital
	C3 $31X1 + 16X2 \leq 47$	Labour
4.	Paddy-Mustard	
	$Z = 10{,}702{,}000X1 + 4{,}175{,}500X2$	
	C1 $0.25X1 + 0.12X2 \leq 0.37$	Land
	C2 $5{,}000{,}000\ X1 + 2{,}600{,}000 \leq 7{,}600{,}000$	Capital
	C3 $102X1 + 11X2 \leq 113$	Labour

Note: Sources: The results of the analysis of the linear equation programming.

To determine the optimum combination of these activities, it is necessary to do calculations using linear programming techniques and computer aids. The purpose of the linear programming arrangement is to maximize the income obtained by farmers by finding the optimum combination of business diversification carried out by farmers in East Buay Madang District. The results of the analysis of the linear equation programming model are present in Table 2.

Optimal Business Pattern: Optimization analysis using linear programming consists of primal-dual analysis and sensitivity analysis. Primal analysis shows a combination of types of businesses that can provide maximum income, and dual analysis assesses resource use by looking at the level of sensitivity to changes made **[47]**.

Primal-Dual Analysis: Based on the results of data processing analysis with LINDO analysis, it shows that of the six types of existing business activities, only four business activities are selected types of business that can maximize profits with limited resources.

Table 3 Selected business activities in optimizing business patterns in Buaymadang.

Activity	Types of crops	Variable	Value	Reduce Cost
Business diversification 1	Paddy	X1a	1.00	0.00
	Cucumber	X2a	1.00	0.00
Business diversification 2	Paddy	X1b	0.98	0.00
	Fish	X2b	1.00	0.00
Business diversification 3	Paddy	X1c	0.00	3,323,307.48
	Kale	X2c	1.88	0.00
Business diversification 4	Paddy	X1d	1.00	0.00
	mustard	X2d	1.00	0.00

Note: Sources: Data analysis with LINDO Programme.

Based on Table 3 the suggested businesses to be cultivated by farmers in East Buaymadang District are in diversification 1, namely rice (X1a) and cucumber (X2a), in business diversification 2, namely rice (X1b) and fish (X2b), in business diversification 3, namely rice (X1c) and Kangkung (X2c), while in business diversification 4, namely Rice (X1d) and Sawi (X2d). Based on Table 2, shows that in business diversification 3 for the type of rice plant, it is a business that is not recommended or selected, this can be proven from the Reduce Cost value of 3,323,307.48, it can be interpreted that the cultivation of rice plants in diversification 3 will reduce the optimal profit obtained by 3,323,307.48.

Table 4 Use of resources for the optimal solution for smallholder farmers in East Buay Madang District.

Activity	Obstacles	Available	Resource used/ fulfilled	Unused/ not fulfilled
Business diversification 1	Land	0.50	0.00	0.34
	Capital	14,700,000	No limit	0.00
	Labour	107	No limit	0.00
Business diversification 2	Land	0.37	No limit	0.00
	Capital	6,750,000	No limit	3,555,555.55
	Labour	115	1.00	73,706
Business diversification 3	Land	0.22	No limit	0.03
	Capital	3,140,000	1.765.625	3,140,000
	Labour	47	No limit	16,916
Business diversification 4	Land	0.75	No limit	0,00
	Capital	7,600,000	No limit	2,060,784.31
	Labour	113	0.00	80,846

Note: Sources: Analysis result.

Meanwhile, for the use of resources (Table 4), some resources are not used up and resources that are used up. Resources that are not used up in business diversification 1 are 0.340 hectares of land or 68 % of the available land area. In business diversification, 2 resources that are not used up are 0.002 hectares of land or 0.89 % of the

available land area, then capital resources of Rp. 3,555,555.55 or 52.67 % of the total available capital and 64.09 JOK labour resources or 0.74 % of the total available working people.

In business diversification 3 some resources are not used up, namely a land area of 0.037 hectares, then capital resources as much as Rp. 3,140,000, and a workforce of 16,916 JOK. In business diversification 4, some resources are not used up, namely capital as much as Rp. 2,060,784.31 and 80,846 JOK manpower resources.

Based on Table 3. for resources that are used up, it shows that if the resource is added by one unit, it will increase the income by the shadow price. In business diversification, 1 resource that is used up is an area of 0.50 hectares, which means that each additional unit of land will increase farmers' income by Rp. 13,113,214.05. Besides that, the resource that is used up is a workforce of 107 JOK, which means that each additional unit of labour will increase farmers' income by Rp. 1,536.

In business diversification, the 2 resources that are used up are 115 JOK, which means that each additional unit of labour will increase farmers' income by Rp. 2,203, in addition to the capital of Rp. 6,750,000 which means that each additional unit of capital will increase the income of Rp. 35,993.70. For business diversification, 3 resources that are used up are capital of 3,140,000, which means that each additional capital of one unit will increase the income by Rp.2,644. Meanwhile, for business diversification, 4 resources that are used up are 113 workers, which means that each additional unit of labour will increase income by Rp. 1,466 besides that, the capital is 7,600,000, which means that each additional capital of one unit will increase the income of Rp. 33,052.80. According to **[48]** that to optimize income, it is necessary to increase the area and reduce labour costs so that this research is in line with previous research studies. In line with research according to **[49], [50]** increasing crop productivity per hectare and per labor unit, as well as the efficiency of the agri-food sector at all organizational levels, has been the primary agricultural challenge.

The excess resources, except for land and capital resources in diversification 1, capital and labour resources in diversification 2, capital resources in diversification 3, and capital and labour resources in diversification 4, can be allocated to other uses to contribute to farmers' income. An increase in the stability of farm revenue is correlated with expanding agricultural activity diversity, lowering input intensity, and earning larger rewards from agri-environment programmes **[51]**. By choosing a variety of crops with low or negative productivity correlations and nutritional importance for the household diet, diversification in agricultural activities lowers the overall production risk **[52]**.

Table 5 Shadow Price Resource use on the optimal allocation of smallholder farmers in East Buaymadang.

Activity	Obstacles	Resource	Slack/ Surplus	Shadow Price
Business diversification 1	C1a	Land (0,5 ha)	0.00	13,113,214.05
	C2a	Capital (Rp/0,5)	0.00	1,536
	C3a	Labour (JOK)	0.00	0.00
Business diversification 2	C1b	Land (0,375 ha)	0.00	0.00
	C2b	Capital (Rp/0,375)	0.00	2,203
	C3b	Labour (JOK)	0.00	35,993.70
Business diversification 3	C1c	Land (0,225 ha)	0.00	0.00
	C2c	Capital (Rp/0,225)	0.00	2,644
	C3c	Labour (JOK)	0.00	0.00
Business diversification 4	C1d	Land (0,5 ha)	0.00	0.00
	C2d	Capital (Rp/0,5)	0.00	1,466
	C3d	Labour (JOK)	0.00	33,052.80

Note: Sources: Analysis result.

Sensitivity Analysis: Sensitivity analysis will provide information about how many changes (increase or decrease) in prices or activity costs are allowed so as not to change optimal results and how many changes (increase or decrease) the number of resources that are still allowed so that optimal results do not change.

Table 6 Sensitivity analysis of the resource objective function on the optimal allocation of land farmer narrow.

Activity	Commodity	Decrease	Present value	Increase
Business diversification 1	Paddy	5,370,156.52	9,831,500	No limit
	Cucumber	No limit	19,299,900	35,331,953.12
Business diversification 2	Paddy	6,057,058.82	8,746,000	No limit
	Ikan	2,526,622.22	102,977,000	14,868,200
Business diversification 3	Paddy	No limit	5,629,500	3,886,257.48
	Kale	639,541.83	4,415,000	No limit
Business diversification 4	Paddy	8,029,807.69	10,702,000	No limit
	mustard	1,154,137.25	4,175,500	5,565,040

Note: Sources: Analysis result.

Based on the results in Table 6, for business diversification 1 to 4 all business diversification can be increased from Rp. 3,886,257.48 until the limit is not determined as well as a decrease in income starting from Rp. 1154,137,255 to an indefinite limit.

Table 7 Sensitivity analysis of the right-hand side of the optimal allocation of smallholder farmers.

Activities	Commodity	Impairment	Present Value	Increase
Business diversification 1	Land	0.16	0.50	0.50
	Capital	14,700,000	14,700,000	No limit
	Labour	107	107	No limit
Business diversification 2	Land	0.37	0.37	No limit
	Capital	3,194,444.44	6,750,000	No limit
	Labour	41.29	115	116
Business diversification 3	Land	0.18	0.22	No limit
	Capital	0.000	3,140,000	4,905,625
	Labour	30.98	47	No limit
Business diversification 4	Land	0.75	0.75	No limit
	Capital	5,539,215.68	7,600,000	No limit
	Labour	32.15	113	113

Note: Sources: Analysis result.

Based on the results in Table 7, the overused resource can be increased to an unspecified extent. In diversification 1 is capital and labour, diversification 2 is land and capital, diversification 3 is land and labour, while diversification 4 is land and capital.

Optimal Business Diversification Scenario: After the data were analyzed primal-dual and sensitivity analysis so that the scenario that had to be done by rice farmers on narrow land with a pattern of farming diversification to obtain an optimal increase in income, in business diversification 1, it was carried out with additional capital of Rp. 1,870,000 so that the optimal allocation of income increases by Rp. 2,871,644.88 or 9.86%. In business diversification 2, the additional capital is Rp. 750,000, and a reduction of the workforce by 5 JOK so that the optimal allocation result will be an increase in income of Rp. 1,472,001.57 or 7.74%. Business diversification 3, it is carried out with additional capital of Rp. 370,000 and the addition of 4 JOK workers so that the optimal allocation result will be an increase in income of Rp. 978,173.65 or 11.78%. Business diversification 4 is done by increasing the land area by 0.25 so that it becomes 1 hectare and increasing capital by Rp. 500,000 so that the optimal allocation result will increase the income by Rp. 733,061.37 or 4.93. In order to increase farmers' revenue and provide for their families, a farming system that can use land as efficiently as possible is required. This relates to the claim made by **[53]** that, for small and marginal farmers, their farming revenue is essentially insufficient to support their farming family. This is in line with research **[54]**, **[55]**, **[56]** which says that land optimization can be used as optimally as possible by combining capital and labour input factors so that the income obtained is maximized. Efficiency in the use of inputs is crucial and has a significant impact on the generation of outcomes and profit **[57]**.

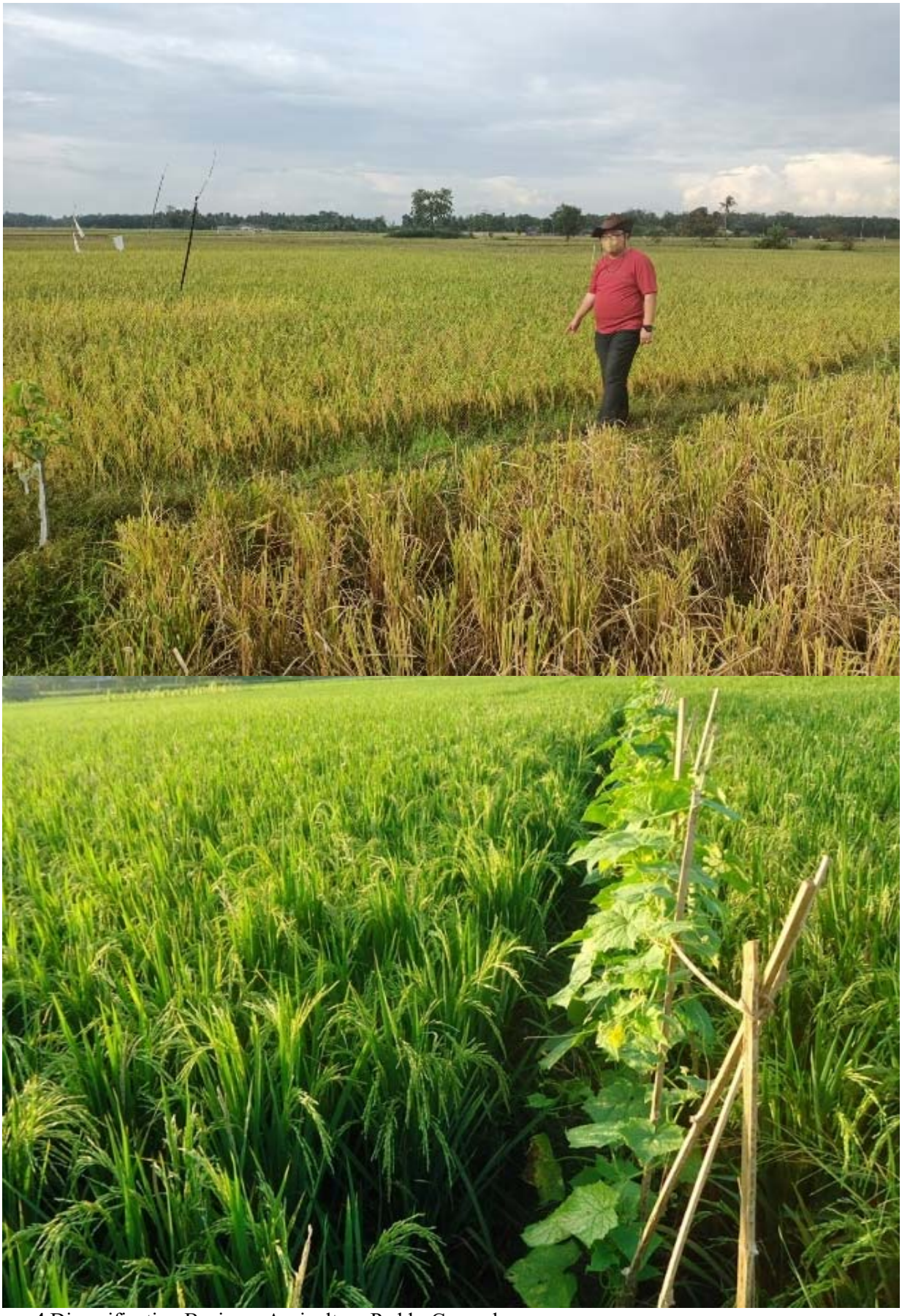

Figure 4 Diversification Business Agriculture Paddy-Cucumber.

CONCLUSION

Based on the results of the study, the following conclusions can be drawn:

1. The amount of optimization of the income of lowland rice farmers during the Covid-19 pandemic is:
   a. For business diversification 1 (Paddy-Cucumber) Z = 29,130,500
   b. For business diversification 2 (Paddy-Fish) Z = 19,007,006.29
   c. For business diversification 3 (Paddy-Kale) Z = 8,301,257.48
   d. For business diversification 4 (Paddy-Mustard) Z = 14,877,500

2. The amount of farmers' income after carrying out the scenarios on business diversification, namely:
   a. In business diversification 1, it is carried out with additional capital of Rp. 1,870,000 so that the optimal allocation result will be an increase in income of Rp. 2,871,644.88 or 9.86%.
   b. Business diversification 2 is carried out with additional capital of Rp. 750,000 and a reduction of the workforce by 5 JOK so that the optimal allocation result will be an increase in income of Rp. 1,472,001.57 or 7.74%.
   c. Business diversification 3, it is carried out with additional capital of Rp. 370,000 and the addition of 4 JOK workers so that the optimal allocation result will be an increase in income of Rp. 978,173.65 or 11.78%.
   d. In business diversification 4, it is carried out by increasing the land area by 0.25 so that it becomes 1 hectare and increasing capital by Rp. 500,000 so that the optimal allocation of income increases by Rp. 733,061.37 or 4.93 In business diversification 2, it is carried out with additional capital of Rp. 750,000 and a reduction of the workforce by 5 JOK so that the optimal allocation result will be an increase in income of Rp. 1,472,001.57 or 7.74%.

The suggestions given based on the results of this study are as follows:

1. Farmers should be more selective in choosing the type of business diversification that will be sought to increase optimal income.
2. The allocation of costs should be improved by reducing excessive costs and shifting to increase the availability of costs that are the main constraint.

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

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The expressiveness of meat forms of cattle depending on the content of adipose tissue under the skin and between the muscles

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ABSTRACT

The paper covers the peculiarities of the degree of meat shapes in the bulls of the Ukrainian meat breed, depending on the adipose tissue content under the skin and between the muscles. They were evaluated according to their productivity from 8 to 18, 21, and 23 months. Bulls with better development of meat shapes are characterized by fat deposition in the carcass and between the muscles earlier and more intensively. They have from 15.1 to 44.7% more fatty tissue in the carcass, including under the skin – from 3.8 to 44.1%. With a different degree of meat shapes, subcutaneous fat is deposited more than between muscles. The content of adipose tissue under the skin relative to its total amount in the body of animals tends to decrease by 6.5 points with age for a better degree of meat shapes, and on the contrary, to increase by 2.6 points for a worse degree. If the fat under the bull skin at 18 months in the best shapes is 72.1% of the fat in the carcass, and in the worst – 72.3%, then at 23 months, its amount decreases by 13.6 and 4.4 points, respectively. The fat between the muscles, on the contrary, increases from 27.9 and 27.7% by the same amounts, respectively. With a greater degree of meat shapes and subcutaneous fat thickness on the carcasses of 18-month-old bulls, intramuscular fat (marbling) content is lower by 75.0%. 18-month-old bulls with better-developed meat shapes have fat cuts off from the carcass by 15.2% more than animals with less developed shapes, 23 – by 11.3%. A large amount of produced waste in the body of animals in the best meat shape leads to excessive (from 0.9 to 14.5%) feed consumption (feed unit) for the increase in live weight. The subcutaneous fat content and the number of cuts off from 16 to 24 months positively correlate with the degree of meat shapes in bulls at 15 months and have correlation coefficients of 0.26 and 0.17, respectively.

Keywords: degree of meat shapes development, subcutaneous fat, adipose tissue, muscle, bulls, Ukrainian meat breed

INTRODUCTION

The degree of meat shape development is affected by the development of adipose tissue under the skin and between the muscles and inbreeding **[1]**. The covering of adipose tissue on the carcass is related to the beef quality by protecting the muscles from drying out during the cooling of the carcass in the refrigerating chamber, which can lead to their stiffness. The carcass must have a sufficient fat thickness to guarantee its preservation and the desired quality for consumption **[2]**. Deposition of a large amount of fat under the skin contributes to an increase in the sexual precocity of animals **[3]**, excessive feed consumption **[4]** and its costs for live weight gain **[5]**. A low tendency to deposit fat is a problem for animals breeds with less adipose tissue under the skin and in the middle of the muscles **[6]**. The amount of adipose tissue in cattle varies depending on the breed and stock **[7]** and

homozygosity **[8]**. Beef fat has a low nutritional value in the processing industry. The healthy nutrition of people is now aimed at the partial replacement of animal fats with triglycerides with polyunsaturated fatty acids, and the introduction of raw materials of plant origin into recipes **[9]**. The biological value of beef proteins is improved **[10]** by the enzymatic method, and its use by people and health-promoting properties are improved by the addition of rosemary extract **[11]** and iodine compounds **[12]**. The issue has not been sufficiently resolved regarding the formation of the degree of meat shapes in animals with different fat content under the skin and between the muscles. This information would help explain its differences. Since the distribution of fat by fat depots is also the subject of accounting for the generation of waste, the disclosure of the features of the formation of the degree of meat shapes in cattle is necessary for the effective and purposeful production of beef for the optimal yield of its valuable quantitative and qualitative components.

This paper aims to establish the relationship between the degree of meat shapes in bulls of the Ukrainian meat breed and the content of various types of adipose tissue in their carcasses and cuts off from them.

Scientific Hypothesis

Previous studies have shown that the better degree of meat shapes in bulls harms their growth rate and breeding value, and the factors of its formation have not been confirmed. It is assumed that animals with less developed meat shapes acquire the shallow-bodied type, closely related to the body's increased metabolic processes. They should have a lower growth rate, a mass of fat in the middle of the muscles and their cuts off because the worse degree of meat shapes affects the development of individual organs that participate in them. Deposition of fat for different degrees of meat shapes may differ from the general trend of increasing the growth of animals.

MATERIAL AND METHODOLOGY

Samples

For research before slaughter, at 18, 21 and 23 months old, two groups of experimental animals were formed using the method of balanced groups by age. The first group included the animals with a degree of meat shape development above the average value for the herd. At 18 months, this value was 56.0 points, at 21 – 56.5, and 23 – 54.2 points. The second group included young animals with a degree of meat shape development less than the average value for the herd. 18 months – 49.2 points, 21 – 50.5, and 23 – 48.0 points. The research was conducted using Ukrainian meat animals (Figures 1, 2) at the Volia stud farm of the Zolotoniskyi district of the Cherkasy region. They were raised from birth to slaughter at 18, 21 and 23 months. After slaughter, fat (subcutaneous, intermuscular, its cuts off) was selected and a piece (300 g) of meat for weighing from *M. Longissimus dorsi*.

Chemicals

Solution of hydrochloric acid, 1.5%, (Khimlaborreaktyv LLC, Ukraine).
Solution of sulfuric acid, 5%, (Khimlaborreaktyv LLC, Ukraine).
Chloroform, (Khimlaborreaktyv LLC, Ukraine).

Figure 1 Bull Pavlyn 7604 CHUM – 62 has slightly better worse expressed meat forms (53.5 points). Live weight is 677 kg at the age of 18 months.

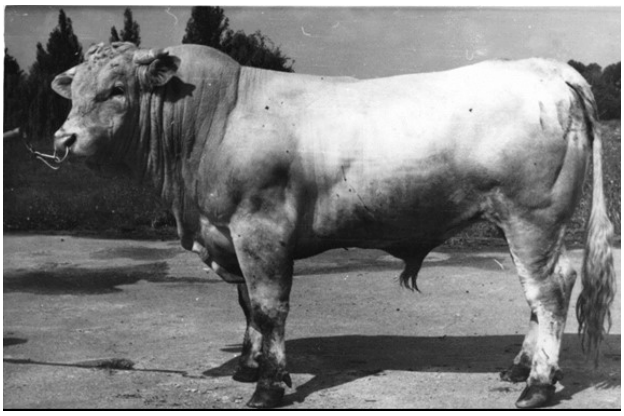

Figure 2 Bull Navodghik 6887 CHRUM – 61 with slightly better expressed meat forms (59.5 points). Live weight is 610 kg at the age of 18 months.

Animals, Plants and Biological Materials

The research was conducted with the use of Ukrainian beef bulls: Bull Pavlyn 7604 CHUM – 62 and bull Navodghik 6887 CHRUM – 61.

Instruments

Static scales 4BDU-1500X-P (Axis, Ukraine). Weight unit ≥0.5 kg, weighing range from 10 to 1500 kg.

Scales Prok (Axis, Ukraine). Weighing ranges up to 150 kg. Weighing of subcutaneous and intermuscular fat and cuts off.

Gaschromatograph (Kupol_55,Shimadzu Corporation, Japan).

Dryingcabinet (SNOL, Khimlaborreaktyv LLC, (Ukraine)

Distiller for steam distillation (Velp Scientifica UDK 129, Khimlaborreaktyv LLC, Italy)

Laboratory Methods

The formation of balanced peer groups was carried out following the requirements of Fundamental concepts of experimentation in breading. Study guide.

To determine the amount of fat in animal carcasses, bulls were slaughtered at the Cherkasy meat processing plant following the requirements of DSTU 4673:2006 **[13]** and DSTU 3938-99 **[14]**. Before that, the pre-slaughter live weight of bulls was determined by weighing them before and after a 24-hour starvation period with free access to water. After the animals were slaughtered and cleaned, their carcasses were weighed in even condition (slaughter weight) and the absolute weight of their cuts off. The left half-carcasses went through skinning and eviscerating. After that, the adipose tissue was weighed and divided into subcutaneous and intermuscular fat according to DSTU 3938-99. The absolute value and percentage of subcutaneous and intermuscular adipose tissue from the slaughter mass (carcass) were determined.

Estimation of total fat content in *M. Longissimus dorsi* was performed following DSTU ISO 1443:2005 **[15]**, protein – GOST 25011-81 **[16]**, mass total ash – DSTU ISO 936:2008 **[17]**, moisture content –DSTU ISO 1442:2005 **[18]**.

Description of the Experiment

Sample preparation: The research was conducted during the second calendar day after skinning and eviscerating slaughtered cattle at 18, 21 and 23 months. For this, 7 subcutaneous and intermuscular fat samples were selected, including 4 samples, each from animals with a better degree of meat shapes and 3 samples each with a worse degree. At the age of 21 and 23 months, samples were taken from 3 animals in groups.

Number of samples analysed: 19 samples from three conducted experiments were used for analysis. For chemical analysis of minced meat from *M. Longissimus dorsi* was taken in 18-month-old animals from both groups by three samples

Number of repeated analyses: Using the carcasses of slaughtered bulls, the weight of subcutaneous fat was determined 3 times, fat between the muscles 3 times and cuts off 3 times, which amounted to 3 repetitions, respectively, at the age of 18, 21 and 23 months.

Number of experiment replication: The study was repeated 3 times, and the experimental data were processed using mathematical statistics methods.

Design of the experiment: Research plan: the research was conducted at the "Volia" stud farm of the Zolotoniskyi district of Cherkasy region. Well-developed bulls of the Ukrainian meat breed from birth to 6-7 months were kept with their mothers as sucker bulls. After weaning, they got typical food and maintenance until 8 months. After weaning, the animals were tested for their performance from 8 to 18 months, 8 to 21, and 8 to 23 months. They were kept on a leash under individual control of the amount of given and consumed feed. Using the herd for slaughter at the age of 18, 21 and 23 months, two groups were formed, including well-developed bulls with different degrees of meat shapes. The first group included animals with a value of this indicator above the herd average, and the second group included animals below the herd average. Bulls were grouped by age using the method of balanced peer groups **[19]**. The degree of meat shape development was evaluated at 15, 18, 21, and 23 months of age on a 60-point scale following methodological instructions **[20]**.

The general level of the feeding of bulls was calculated on receiving average daily gains from 1000 to 1200 g. During this period, the animals were fed with a feed of their products according to the rations prepared according to the norms. The mass of fodder eaten by each bull was calculated every decade (two days in a row) by weighing the given fodder and its residues. Their energy value (in oat fodder units) and costs per 1 kg of live weight gain were calculated based on the consumed fodder. There was no discernible difference in the amount of feed consumed by the bulls of the groups from 8 to 18 months, 8 to 21 months, or 8 to 23 months (Table 1).

Table 1 Fodder consumption by bulls by periods.

Fodder	From 8 to 18 months		From 8 to 21 months		From 8 to 23 months	
	better (56.0 points); n = 4	worse (49.2 points); n = 3	better (56.5 points); n = 3	worse (50.5 points); n = 3	better (54.2 points); n = 3	worse (48.0 points); n = 3
Concentrated, fodder unit	1342 ±48.0	1310 ±34.5	2031 ±109.1	2049 ±72.5	2703 ±1.0	2678 ±26.0
Concentrated, %	46.3 ±26.3	48.0 ±1.95	47.5 ±0.50	47.1 ±0.50	48.7 ±0.20	48.6 ±0.45
Roughage, fodder unit	548 ±109.8	378 ±140.5	900 ±124.3	907 ±91.3	853 ±26.0	942 ±34.0
Roughage, %	18.9 ±2.79	13.8 ±4.78	21.0 ±1.77	20.9 ±1.71	15.4 ±0.40	17.1 ±0.30
Juicy, fodder unit	431 ±54.4	344 ±23.2	655 ±41.8	707 ±12.4	816 ±84.5	831 ±31.5
Juicy, %	14.9 ±1.24	12.6 ±0.82	15.3 ±0.95	16.3 ±0.65	14.7 ±1.40	15.1 ±0.25
Green, fodder unit	578 ±52.9	699 ±81.6	692 ±63.8	685 ±25.6	1174 ±80.0	1059 ±16.0
Green, %	19.9 ±1.54	25.6 ±3.31	16.2 ±1.66	15.7 ±10.10	21.2 ±1.55	19.2 ±0.10
Total, fodder unit	2899 ±208.0	2731 ±60.9	4278 ±263.3	4347 ±130.2	5546 ±29.5	5510 ±107.5
For 1 kg of grain, the fodder unit	9.5 ±1.17	8.3 ±1.44	11.1 ±0.02	11.0 ±0.04	13.2 ±0.70	13.1 ±0.85

Statistical Analysis

Variational statistics processed the obtained data according to the methods adopted in breeding and biology. Statistical processing was performed by Microsoft Excel 2016 in combination with XLSTAT. The average value and standard deviation evaluated indicators. The arithmetic mean (unweighted) value (M) and arithmetic mean were calculated. The accuracy of the obtained experimental data was determined using the Student's test for a confidence probability of ≤0.05 based on the number of parallel determinations at least 5. The results were analyzed using the ANOVA.

RESULTS AND DISCUSSION

Bulls with higher meat shapes are characterized by forming fat earlier and more intensively in the carcass (Table 2). This causes higher (from 0.9 to 14.5%) consumption of fodder (fodder unit) for live weight gain (Table 1). With the different degrees of meat shape development, subcutaneous fat is deposited more than between muscles. Bulls have better-defined meat shapes at 18 months; 44.7% more fatty tissue in the carcass, including 44.1% under the skin. A similar feature is observed in the 21st and 23rd months. For animals with a worse degree of meat shapes at the age of 21 months, compared to peers with a better degree of meat shapes, there is a tendency (by 17.6%) to increase the adipose tissue between the muscles, which in this period has the highest natural growth.

Table 2 The content of adipose tissue under the skin and between the muscles of bulls after slaughtering, depending on the degree of meat shapes.

Degree of meat shapes (points) in age, months		n	Adipose tissue, kg				
			total in the carcasses	under the skin		between muscles	
			M ±m	M ±m	to total, %	M ±m	to total, %
18	**Better (56.0)**	4	6.8 ±0.50	4.9 ±0.70	24.6	1.9 ±0.50	9.5
	Worse (49.2)	3	4.7 ±1.30	3.4 ±0.90	19.3	1.3 ±0.70	7.4
21	**Better (56.5)**	3	6.1 ±0.60	4.4 ±0.40	21.3	1.7 ±0.20	8.2
	Worse (50.5)	3	5.3 ±1.30	3.3 ±0.40	17.0	2.0 ±1.00	10.3
23	**Better (54.2)**	3	9.4 ±0.67	5.5 ±0.39	18.1	3.9 ±0.28	12.9
	Worse (48.0)	3	7.8 ±0.59	5.3 ±0.38	21.3	2.5 ±0.78	10.0

From 18 to 23 months, the content of adipose tissue under the skin relative to its total amount in the body of animals tends to decrease by 6.5 points in the case of a better degree of meat shapes, and vice versa to increase by 2.6 points in the case of the worse degree of meat shape. In animals, with different degrees of meat shape development, adipose tissue between muscles increases curvilinear, unevenly. If the fat under the bull skin at 18 months is 72.1% for the best meat shapes, and 72.3% for the worst, then at 23 months its share decreases by 13.6 and 4.4 points, respectively. The fat content between the muscles, on the contrary, increases by 27.9 and 27.7% by the same values, respectively.

The obtained data on adipose tissue content in animals under the skin and between the muscles indicate that they achieve a better degree of meat shape due to the excess deposition of subcutaneous fat, which to some extent

smooths out the defects of the exterior. With its greater accumulation, cattle is characterized by less angularity and better development of their meat shapes. A higher quantity of fatty tissue in the carcass between the muscles, which causes their displacement on the surface of the carcass, is thought to be why animals with better-defined meat forms have an advantage over peers with worse shapes **[21]**. The yield of edible portions in the carcass diminishes when a lot of fat is under the skin. It is not utilized to enhance meat's tenderness and other aspects of its quality **[22]**, **[23]**. Subcutaneous fat is viewed as **[24]** waste since it has little commercial worth.

Intramuscular or marble fat is necessary to improve beef's juiciness, tenderness and taste **[25]**. During cooking, it melts, soaking the meat. As a result, it is juicy and tender. The content of intramuscular fat in beef is the most important factor that determines its quality in Japan, Korea, Australia and the USA, while in the countries of the European Union, including France and Germany, leaner meat (with less marbling) is preferred **[26]**, **[27]**. With more meat shapes in 18-month-old bulls, the total fat content (marbling) in *M. longissimus dorsi* is lower by 75.0% compared to peers due to their poorer development (Table 3).

Table 3 Chemical composition of beef in 18-month-old animals with different degrees of meat shapes development.

Chemical composition of beef	Better (58.7 points), n = 3		Worse (49.2 points), n = 3	
	M ±m	Cv, %	M ±m	Cv, %
Total fat content	0.37 ±0.17	8.0	0.6 5±0.19	51.6
Protein	20.83 ±0.53	4.4	20.26 ±0.70	6.0
Mass total ash	1.12 ±0.04	5.4	1.04 ±0.02	3.4
Moisture	77.68 ±0.42	0.94	78.05 ±0.13	0.29

Ukrainian meat breed animals are characterized by rapid growth at the expense of muscle tissue and the formation of fat at an older age **[28]**, **[29]**, **[30]**. There was no significant difference in other components of beef's chemical composition between the groups' animals. The obtained data concerning 18-month-old animals of the Ukrainian meat breed do not confirm the connection between subcutaneous fat thickness and the marbling of *M. longissimus dorsi*, discovered **[31]**, **[32]** in bulls from Ukrainian black-spotted dairy cattle at 22 months. Its data shows it is positive and high (r = 0.68). A greater amount of fatty tissue under the skin and between the muscles in animals with better-developed meat shapes is also subject to their excessive waste formation (Table 4).

Table 4 The quantity (kg) of cuts off from the carcasses of bulls depends on the degree of the meat shapes.

Age of slaughter,	Better meat shapes			Worse meat shapes		
months	n	M ±m	Cv, %	n	M ±m	Cv, %
18	4	5.3 ±0.30	12.6	3	4.6 ±0.20	6.5
21	3	4.4 ±0.60	24.2	3	5.5 ±0.60	18.3
23	3	5.9 ±4.20	14.3	3	5.3 ±3.71	12.2

18-month-old bulls with better-developed meat shapes have 15.2% more fat cuts off from the carcass than animals with less developed forms at 23 – by 11.3%. A large amount of waste (internal fat, under the skin and between the muscles) from the body of animals with the best meat shapes, for which the processor does not pay the producer, leads to an increase (from 0.9 to 14.5%) of feed costs (feed units) on the increase in live weight (see Table 1). Thus, these cattle are less efficient for beef production. **[33]**, **[34]**.

Recently, the population of Ukraine has had a demand for marbled beef. An analysis of the latest research and publications on this issue was carried out to understand how to solve the related problems. It is believed **[35]**, **[36]**, that any food, including meat, performs the main functions of nutrition, taste and disease prevention. Concerning beef consumption in Ukraine in 2022, 6.4 kg of the need (36 kg) per average citizen, now the issue is being solved by increasing its quantity, not quality, unlike many foreign countries, where it is consumed as a source of disease prevention. An important aspect of beef quality, identified **[37]** by parts of the production chain (producer, processor, consumer), is fat deposition in the middle of the muscles. The content of intramuscular fat in beef and its nutritional quality is affected by factors that are divided **[38]** into those that occur on the farm and in the period before slaughter (breed, sex, age at the time of slaughter, housing system, feed and handling before slaughter) and after death (handling after slaughter, packaging and cooling temperature), individual genetic predisposition of animals. Therefore, we will consider only a few factors that affect the deposition of intramuscular adipose tissue and cause the most problems during its production.

Genetic factors (breed, gender and heredity).

The black breed of Wagyu cattle in Japan has the highest intramuscular fat content (marbling) in the world (more than 30%) **[39]**, and the Korean Hanwoo has the second highest content **[40]**. In animals of European breeds, the content of intramuscular fat is only 0.6-4.7% **[41]**. Wagyu is known **[42]** for a significant content of monounsaturated fatty acids and a higher ratio of them to saturated than other breeds. This does not lead to health benefits for people who consume beef with high marbling rates. Increasing the content of useful polyunsaturated fatty acids and conjugated linoleic acid in the middle of beef muscles improves its taste properties and shelf life **[43]**. Aberdeen-Angus cattle meat has the highest intramuscular fat concentration and sensory properties among European breeds. In Ukraine, no beef is not produced from Wagyu animals, and Aberdeen-Angus is not a purebred of Scottish origin but a hybrid (1/2 Aberdeen-Angus x 1/2 black and spotted Holstein), from embryos, imported once from the USA.

Heifers deposit more fatty tissue in the body than steers and bulls. The insignificant ratio of omega n-6 to omega n-3 fatty acids indicates that beef from heifers contributes to human health. Castration of bulls increases adipose tissue deposition in the middle of the muscles **[44]**. The beef of uncastrated bulls is of the worst quality and is valued lower by the consumer **[45]**. During the delivery of young animals at the meat processing enterprises of Ukraine, bulls are rated the best, and heifers the worst. Prepared bulls are not practically sold because, on farms, bulls are not castrated at a young age. The markets sell mainly not beef but veal, which practically has no intramuscular fat.

The marbling of beef increases as the animals mature and their live weight increases **[46]**, but the ratio of omega n-6 to omega n-3 fatty acids is higher in heavyweights (p >0.05) compared to lightweight **[47]**. The deposition of adipose tissue in the muscles of cattle is facilitated by feeding them with concentrated feed with a high energy content **[48]**. However, concerning the beef of animals raised on concentrated feed, there is a lower level of mono- and polyunsaturated fatty acids than in bulls fed with grass on pastures **[49]**. Increasing unsaturated fatty acids in muscles makes them more susceptible to lipid oxidation. As a result, various aldehydes, ketones, alcohols, esters and carboxylic acids are formed, affecting beef's taste. Thanks to the fattening (grazing) of animals on the grass, extraordinary juiciness of the meat were achieved. Feed energy costs for feeding young animals increase during live weight gains due to the increase of internal, subcutaneous, and intermuscular adipose tissue, which has a low commercial value and is considered **[50]** waste from beef production. For its formation, animals spend 2.25 times more feed nutrients than for forming carcass muscles. Excess energy supplied to animals in the late period of fattening significantly reduces feed efficiency due to a decrease in its digestibility, increases the amount of fat waste from them and worsens the efficiency of livestock management.

In contrast to the complexities arising in enterprises during the production of marbled beef, the processor considers the excess fat deposited in the body a problem because it must be removed from the animal or an important ingredient in the processed product. The processor does not pay the producer for a significant amount of removed internal fatty tissue, which cuts off from the carcass. Payment is made only for slaughter weight (carcasses). And with an increase in the grade of marbling of beef, which appears simultaneously with an increase in the body's fat, the percentage of carcass yield decreases **[51]**.

Consumers have different opinions about the content of intramuscular fatty tissue in beef. Some prefer lean meat; others consume beef with more fat. It is important to understand these additional requirements of various industry segments to consider how fat is deposited during the animal's life. In specific depots, inedible internal fat is deposited first, which is located in the abdominal cavity around the internal organs, then under the skin, between the muscles and in their middle (marble) **[52]**. Intramuscular fat, which is desirable for improving the palatability of meat, has a general decrease in lipogenesis and an increase in the activity of the enzyme cholesterol-25 hydroxylase in cattle **[53]**, which is synthesized from the carbon of glucose, not acetate, during intensive fattening already after the accumulation of "excess" fat between the muscles.

Marbling (intramuscular fat) in cattle is considered **[54]** to be a sign of late maturation, which becomes noticeable only after other fat depots, even though the relative rates of its increase are similar to theirs. Marbled beef generally contains more saturated fatty acids than subcutaneous fat **[55]**. Probable differences in increasing the sensory parameters of beef steaks are manifested by lower indicators of the content of intramuscular fat in it **[56]**. Therefore, a minimum level of its marbling is necessary to detect differences in the juiciness of the beef.

Intramuscular fat is deposited between primary and secondary muscle bundles in the perimysium of cattle and muscle bundles **[57]**. However, the quality of beef depends not only on the content of fat inside the muscles but also on the coordinated relationship of three of their components – intramuscular connective tissue (general and insoluble collagen that surrounds each muscle fibre and their bundles and muscle as a whole), types of muscle fibres and intramuscular fat **[58]**.

The quality of marbled beef is affected by the duration of its ripening, which also imposes costs on the cost price of the manufactured products. Beef for steaks goes under dry or wet ageing. During wet ageing, it is vacuum packed and placed in a chamber for 3-5 days in a special microclimate, during which the fibres soften, and the

meat is saturated with juices. During dry maturation, beef cuts are placed in salt chambers at certain temperatures and humidity for up to 120 days.

Consumers are increasingly concerned about the negative effects of extremely marbling beef on the human body (cardiovascular diseases and atherosclerosis). Excess omega-3 polyunsaturated fatty acids (linolenic acid, docosahexaenoic acid, eicosapentaenoic acid) increase the risk of developing prostate cancer. The main polyunsaturated omega-6 fatty acids include linoleic and arachidonic acids. For human nutrition and good health, it is possible to use food containing fat (depending on the composition and number of fatty acids) compared to saturated with simple carbohydrates **[59]**.

Thus, there are many challenges to beef production of bulls with a better degree of meat shapes, including high feed costs, disposal of untreated excreta, and food safety risks that can result from diseases caused by fatty beef. Despite the recent achievements in the world regarding the regulation of fat deposition in the muscles of cattle in the Ukrainian meat breed, this problem remains unsolved and deserves further research. We have started to develop a strategy to optimize the amount of fat deposited in the body of these animals. To predict the content of adipose tissue under the skin and between the muscles after slaughtering bulls aged 16 to 24 months based on the severity of their meat forms at 15 months of age, correlation coefficients were calculated between these indicators. The degree of meat shapes development correlates with the adipose tissue content between muscles slightly and negatively (r = -0.08) (Table 5). The highest (r = 0.26 and 0.17) positive relationship exists between the degree of meat shape development, the content of fatty tissue under the skin, and the number of cuts off the carcass.

Table 5 Correlation coefficients between the degree of the meat shapes of bulls at the age of 15 months and the fat content in their carcasses, between muscles and cuts off from 16 to 24 months.

Indicator	n	r
Subcutaneous fat	20	0.26
Intramuscular fat	20	-0.08
Subcutaneous and intermuscular fat	20	0.11
Cuts off	34	0.17

Thus, according to the degree of meat shapes, it is only possible to a certain extent to predict the amount of deposited non-commercial fat (subcutaneous and between the muscles) and waste (cuts off) obtained during the handling of carcasses. But it is difficult to judge it in terms of the amount of deposited intramuscular adipose tissue (marbling) of beef. A change in the composition of adipose tissue by any factor depends mainly on a person's ability to control its relative amount depending on the development degree of shapes of meat animals. Modern methods of regulating the deposition of adipose tissue in the middle of the muscles should limit the increase in its total content in the body, including inedible. Additional research is needed to develop methods for the production of beef with appropriate levels of marbling to satisfy its quality and taste, following consumer preferences, preserve human health and support the economy of cattle breeding under the condition of justification of the optimal degree of the meat shapes of the bulls without increasing the deposition of total inedible fat in the body, including internal and subcutaneous fat.

A deeper understanding of these complex mechanisms of body composition regulation can improve beef quality and consistency prediction, improve the selection of animals that achieve the desired final meat quality, and identify those better suited to specific diets and consumer treatments. Because of this, meat producers will be able to use a regulatory mechanism to justify the optimal degree of meat shapes of animals, determined by the demand for beef of different qualities, which helps to optimize the accumulation of fat in muscles by improving its ratio to the protein. In addition, understanding the mechanisms of increasing fat deposition and the efficiency of beef production while simultaneously reducing the degree of meat shapes and general conditions of animals will lead to better processing of animals and the overall health of the consumer.

CONCLUSION

Bulls with different degrees of meat shapes, from 18 to 23 months, have more fat deposited under the skin than between the muscles. In animals with the best meat shapes, compared to the worst ones, there is more fat tissue in the carcass from 15.1 to 44.7%, including under the skin from 3.8 to 44.1%. The content of adipose tissue under the skin relative to its total amount in the body in animals with a better degree of meat shapes tends to decrease by 6.5 points, with a worse one – on the contrary, to increase by 2.0 points. 18-month-old bulls with better-developed meat shapes have 15.2% more fat cuts off from the carcass than animals with worse development, 23 – by 11.3%. The formation of a larger amount of internal adipose tissue under the skin and between the muscles in animals causes excessive (from 0.9 to 14.5%) feed consumption (feed unit) for live weight gain. With the content of fatty tissue under the skin and the number of cuts off from the carcass from 16 to 24 months, the degree

of meat shapes at 15 months correlates slightly positively (r = 0.26 and 0.17, respectively). The degree of the meat shape development may be used to predict, to a certain extent, only the deposition of non-commercial fat (subcutaneous, between the muscles) and production waste (cuts off). Further research is needed to develop methods for the production of marbled beef, using bulls with less pronounced meat shapes without increasing the deposition of total inedible fat in the body, including internal and external (subcutaneous) fat and the appropriate level of intramuscular, to satisfy its quality and taste and save people's health.

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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.

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Development of new technologies (recipes) to produce pasta with the addition of millet and the determination of organoleptic and physicochemical quality indicators

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ABSTRACT

The article presents the organoleptic and physicochemical (humidity and strength) quality indicators of pasta with the addition of millet at 7.7, and 15.5%, as a new recipe for pasta production. Millets can be used to supplement pasta because of their superior nutritional value and health advantages. On the territory of the Republic of Kazakhstan and the Eurasian Economic Union, the quality indicators were calculated while taking into account the practices outlined in the standardized documents. Express drying, accelerated drying, drying to a constant mass, and employing the MA-30 "SARTORIUS" apparatus following interstate standards were all employed. The study aimed to achieve appropriate organoleptic quality indicators and physicochemical indicators of humidity up to 28% (after processing pasta with the addition of millet 7.7, and 15.5%. Approximately 100 trials were carried out at the Federal State Autonomous Scientific Institution "Scientific Research Institute of the Bakery Industry" Russian Federation, Moscow. According to the study's findings, all quality indicators are within acceptable ranges, except for pasta with the addition of millet 23.3%, recipes for pasta with the addition of millet have been developed, a utility model patent has been obtained in the territory of the Republic of Kazakhstan No. 7071, issued by the Republican State Enterprise on the right of economic management "National Institute of Intellectual Property". In conclusion, pasta recipes with the addition of millet have been developed. According to the study's findings, all quality indicators are within acceptable limits except pasta with the addition of millet, which accounts for 23.3% of the total.

Keywords: pasta, indicators, millet, durum wheat, methods for determining quality

INTRODUCTION

On the global market today, there are more than 350 different types of pasta. Additionally, its astounding variety ranges from classic tubes to tennis rackets **[1]**. Italy is the uncontested world leader in pasta manufacturing, with an annual per-capita consumption of 24 kilos **[2]**. According to the International Pasta Organization, Greece is ranked number four in the world for pasta consumption, after Italy, Tunisia, and Venezuela **[3]**. Turkey's demand for pasta increased by 20% during the coronavirus pandemic, according to a survey conducted by the Turkish Pasta Manufacturers Association, and the average yearly pasta consumption among Turks is 8 kg. Based on this indication, Turkey was ninth on the list of nations that consumed pasta in 2021 **[4]**. In Britain, spaghetti is consumed by more than two-thirds (68%). The most often consumed variety of pasta in the UK is fusilli, which is shaped like a spiral. According to a recent YouGov **[5]** research, 19% of respondents favored it. Regarding pasta consumption, Russia rounds out the top 10 nations (Italian pasta). The analytical and statistical division of Barilla published these findings **[6]**.

About 95% of the country's adult population consumes macaroni products regularly **[7]**. According to the Statistics Portal for Market Data for 2022, the number of countries in the world that eat pasta is as follows **[8]**. Figure 1 shows that pasta consumers worldwide are increasingly appreciating pasta as the basis of delicious and nutritious food. Italy, and France, the United States are the largest pasta market. According to the IPO, Italians are a major consumer of pasta: their per capita consumption is 23.5 kg, a total of 1.4 million tons. Next in line: Tunisia (17 kg), Venezuela (12 kg), Greece (11.1 kg), Chile (9.4 kg), Argentina, Turkey, and Iran - only about 8.5-8.7 kg per capita, followed by Portugal and the Czech Republic. Markets are growing dynamically in Asia (growth of 8.6%) and Africa (growth of 2.6%).

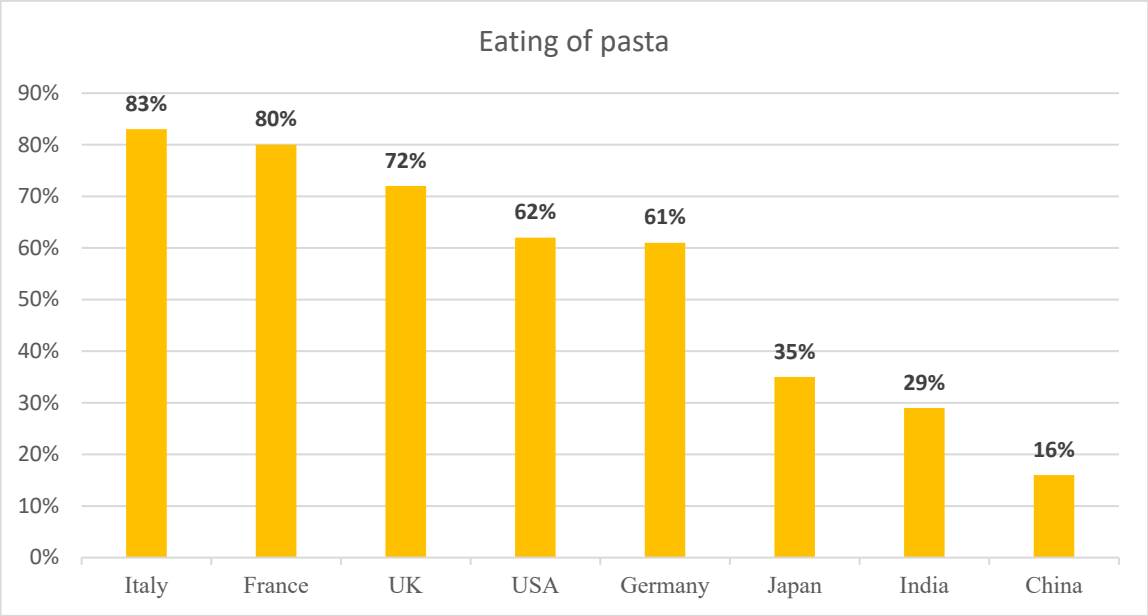


Figure 1 Eating percentages of pasta in the world in 2022.

According to 9 months of 2022, 122.8 million tons of products were produced in the Republic of Kazakhstan. There is an increase in the indicator compared to the same period last year by 5.8% (Figure 2). According to the ministry, 159.8 thousand tons of pasta were produced in 2021. It is noted that Kazakhstanis consume an average of about 130 thousand tons of pasta annually **[9]**.

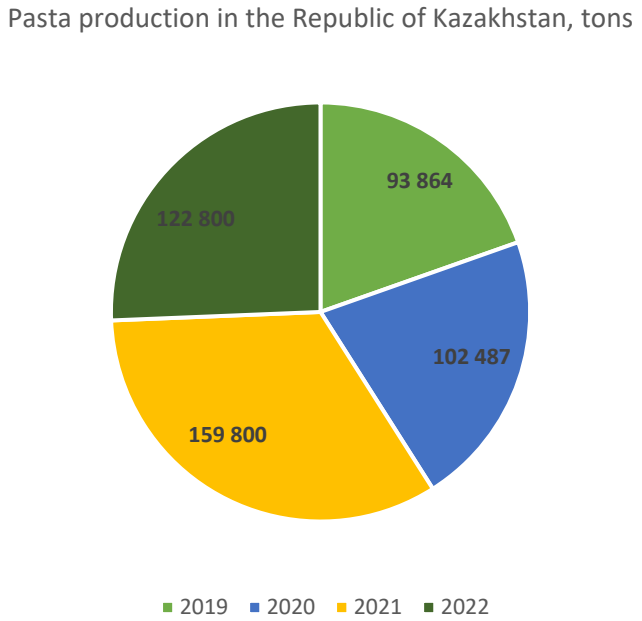


Figure 2 The production of pasta in the Republic of Kazakhstan in the period from 2019 to 9 months 2022, tons.

The Food and Agriculture Organization of the United Nations has published data on the forecast of grain production and trade in the world as of December 02, 2022 (Figure 3).

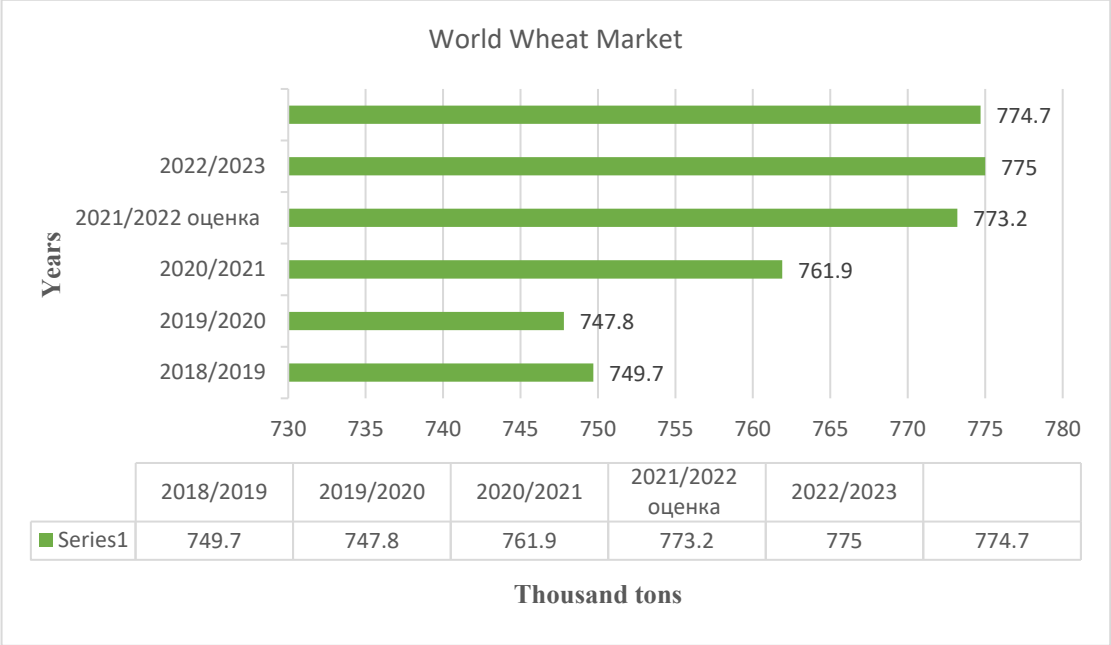


Figure 3 World Wheat Market, thousand tons.

The wheat consumption in the world in the 2022-2023 season remains unchanged at 775 million tons compared to last month, which is slightly higher (by 0.2%) than the level of the 2021-2022 season; at the same time, it is expected that an increase in food consumption of wheat can compensate for the expected decrease in its consumption for feed and, although to a lesser extent, consumption for other needs **[10]**. People of all ages, from young children to the elderly regardless of financial class consume pasta since it is a basic cuisine everywhere in the world, especially in emerging nations. High-quality pasta should have the following qualities: firmness, elasticity, minimized cooking loss, stickiness, ease of preparation, and good firmness after cooking **[11]**. Due to the rising number of Chinese customers who have lived abroad and who follow Western dietary customs, pasta is becoming a more popular Western food item in China. Pasta imports to China totaled $342 million in 2020 **[12]**. There are now several recognized standardized papers (Interstate Standard; further GOST) in the Republic of Kazakhstan that regulate the specifications for pasta, establish the guidelines for their acceptance, and lay out the criteria for determining their quality. GOST 31743-2017's paragraph 3.1 states that “Pasta Products”. General technical specifications " the phrase "Pasta: A food product made from the products of processing of cereals and non-grain crops using additional raw materials and without it, mixing with water, with further molding and drying in various ways" **[13]**.

Pasta is divided into group A (pasta made from durum wheat flour, primarily used for producing high-quality pasta products because of its superior characteristics) and into grades: the highest, first, and second; groups B and C - the highest and first. Depending on the type of source wheat and flour grade, pasta is divided into groups:

- Group A pasta: Pasta prepared from durum wheat flour.
- Group B pasta: Pasta produced from soft wheat flour.
- Group B pasta: Pasta prepared using bread wheat flour or all-purpose wheat flour.

Pasta can be cut, pressed, or stamped according to moulding technique. Pasta is divided into types: tubular, filamentous, ribbon and curly. Pasta of all varieties is separated into long and short pasta. Long pasta may be single- or double-bent, formed into skeins, bows, nests, and more. Long pasta may be cut into skeins, bows, and nests of any weight and size. Pasta must meet the requirements of GOST 31743-2017 "Pasta products. General technical conditions" as listed in Table 1 under organoleptic parameters.

Table 1 General technical conditions of pasta products according to organoleptic parameters (GOST 31743-2017).

Indicator Name	Characteristic

Color	Corresponding to the flour grade. The color of products using additional raw materials varies depending on the type of this raw material.
Form	Corresponding to the type of products
Taste	Characteristic of this product, without extraneous taste
Smell	Characteristic of this product, odorless

According to physicochemical parameters, pasta must comply with the standards of GOST 31743-2017 "Pasta products. General technical conditions" specified in Table 2.

Table 2. General technical conditions of pasta products according to physicochemical parameters GOST 31743-2017.

Indicator Name	Norm						
	Group A			Group B		Group C	
	Top Grade	First Grade	Second Grade	Top Grade	First Grade	Top Grade	First Grade
Humidity of products, %, no more*	13	13	13	13	13	13	13
The acidity of products, deg, no more:							
- tomato - the others	10 4	- 4	- 5	10 4	- 4	10 4	- 4
Mass fraction of protein in terms of dry matter, %, not less	10.5	10.5	10.5	-	-	-	-
Ash insoluble in 10% HCl solution, %, no more	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Mass fraction of ash in terms of dry matter, %, no more than vegetable, egg	0.90 1.40	1.20 1.70	1.90 2.40	0.60 1.10	0.75 1.25	0.56 1.10	0.75 1.25
The content of soft wheat flour, %, no more	15	15	15	-	-	-	-
Dry matter transferred to the cooking water, %, no more for small format and filamentous with a diameter of up to 1 mm	6.0 9.0						
Shape preservation of welded products, %, not less	100						
Metallogenetic impurity, mg per 1 kg of product, no more	3 when the size of individual particles is not more than 0.3 mm in the largest linear dimension						
The presence of contamination and contamination by pests of grain stocks	Not allowed						
* For the rest, sent to the Far North and hard-to-reach areas, as well as by sea - no more than 11%							

According to microbiological indicators, pasta must adhere to the requirements established in TR CU 021/2011 Technical Regulations of the Customs Union "On Food Safety" or regulatory legal acts in effect on the state's territory. The content of toxic elements, mycotoxins, pesticides, and radionuclides in pasta must comply with the requirements established in TR CU 021/2011 Technical Regulations of the Customs Union "On Food Safety" or regulatory legal acts in force on the territory of the state **[14]**. Because of increased demand from health-conscious customers, researchers and food producers are increasingly interested in developing pasta products high in minerals, vitamins, fiber, and low glycemic index. The Food and Drug Administration (FDA) and the World Health Organization (WHO) both see pasta as a good vehicle for adding nutritional supplements. Due to its low price, lengthy shelf life, and widespread consumption, pasta, among other functional foods, is suitable for health benefits. Additionally, millets stand out among cereals because of their high calcium, dietary fiber, polyphenol, and protein contents. Millets are a wonderful option for celiac disease patients affected by wheat and other gluten-containing cereal grains since they are gluten-free **[15]**. Being the first crop to be harvested in the year, it provides the indigenous people in many regions of the world with essential food grain. It should be regarded as a necessary

food for ensuring nutritional security since it is a high source of protein (7.7 g/100 g), very rich in carbohydrates (67.0 g/100 g), and low in fat (4.79 g/100 g).

In comparison to other millets and grains, little millet has phosphorus (220 mg), iron (9.3 mg), and fat (4.7 g) per 100 g, iron (9.3 mg), crude fiber (7.7 g), and phosphorus (220 mg) per 100 g which is similar to cereals and other millets. Little millet's high dietary fiber content contributes to its low glycaemic index, and a recent study on little millet found that this increased dietary fiber level causes little millet to have a hypoglycemic impact. It contributes significantly to the diet's supply of considerable levels of phytochemicals and antioxidants. There is a need to address the varied needs for millet-based food items since consumers are becoming more aware of the health advantages of millet **[16]**. Semolina, which is typically used to make pasta, is high in calories but low in dietary fiber, vital amino acids, minerals, and other nutrients **[17]**. According to research, the pasta matrix sustains nutritional stability and can be an excellent transporter to improve dietary components **[18]**. The popularity of multigrain meals has grown due to their health benefits, such as delayed digestion, cholesterol-lowering effects, antioxidant, anti-carcinogenic, and anti-inflammatory qualities **[19]**. Over the last decade, some fascinating research has been conducted to increase the nutritional potential of pasta by combining the flour of various kinds of cereals, such as quinoa and faba bean flour **[20]**, fermented quinoa flour **[21]**, plant proteins made from mushroom powder, Bengal gram flour, and defatted soy flour (DSF) **[22]**, **[23]**.

The current study aimed to develop new technologies (Recipes) for making pasta (as a new technology for pasta production) with millet and determination of organoleptic and physicochemical quality indicators.

Scientific Hypothesis

The purpose of the current study was to develop millet and determination of organoleptic and physicochemical quality indicators. The main scientific hypothesis is to increase traditional pasta's nutritional value and consumer properties by using appropriate organoleptic quality indicators and physicochemical indicators of humidity up to 28% (after processing pasta with millet 7.7 15.5 %). We are expecting to develop pasta recipes with good characteristics to solve some problems such as the fragility to solve the problem of transportation and others.

MATERIAL AND METHODOLOGY

Samples

Millets, wheat flour, starch, pea, soy, amaranth flour, gluten-free flour, durum wheat (genotype), and flax seeds have been purchased from local markets in Almaty, Kazakhstan. As a rule, traditionally, the composition of pasta is wheat flour and water.

Chemicals

The chemical composition (starch and amaranth flour, gluten-free flour, soy, pea) was determined.

Animals, Plants and Biological Materials

Animal and biological materials weren’t used in this research.

Instruments

Eleks - 7M, Russian Federation, Moscow (Manufacturer Limited Liability Company "Tagler"). Drying cabinet SESH-3M, Ukraine, Vinnytsia region, Mogilev-Podilskyi, instrument-making plant. Additionally, MA – 150 "SARTORIUS" infrared humidity analyzer MA-150 is designed to measure the humidity level of liquid, bulk, solid substances, and emulsions during input/output control of products and during scientific research, Germany, Göttingen, Weender Landstrasse 94-108, manufacturer "Sartorius Weighing Technology GmbH". Structurometer ST-1M", designed to determine the rheological characteristics of raw materials, semi-finished products, and finished products, Russian Federation, Moscow, manufacturer "Ochakov Combine of Food Ingredients".

To produce pasta for consumers with gluten intolerance, gluten-free flour was added, such as rice, buckwheat, and corn. Various vegetable and fruit powders such as starch, pea, soy, and amaranth were added to gluten-free flour. Increasing the biological value of pasta products and giving them therapeutic and prophylactic properties was a partial replacement of wheat flour of the highest grade with flour from flaxseeds. Flaxseed flour proteins significantly exceed wheat proteins in the amino acid composition. The fiber in flaxseed flour contains up to 30% of the total weight. Flaxseed flour also contains minerals and vitamins in an easily digestible form.

Many domestic and foreign scientists in search of new sources of raw materials and functional additives for pasta production, which would contribute to reducing calories, increasing nutritional value, and enriching with functional ingredients, talk about the relevance of this direction **[24]**, **[25]**. As part of our research, millet is used as a food additive.

Millet contains about 12-15% protein, 70% starch, and essential amino acids. There is 0.5-8% fiber in cereals, 2.6-3.7% fat, a little sugar – up to about 2%, vitamins PP, B1 and B2, as well as a large amount of potassium, magnesium, and phosphorus. Millet holds the record for the content of molybdenum and magnesium. It is millet

that is considered the least allergenic grain crop. The body easily absorbs this cereal – even people with sensitive digestion can include it in their diet.

Laboratory Methods

Following the requirements of GOST 31743-2017 "Pasta products, general technical conditions", GOST 31964-2012 "Pasta products, acceptance rules and methods of quality determination", studies were conducted on organoleptic parameters (color, shape, taste, smell) and physicochemical parameters, such as humidity.

There are the following methods for determining humidity according to GOST 31964-2012 (Figures 4-6):

- by drying to a constant mass,
- accelerated drying method,
- by the express method,
- on the MA-30 "SARTORIUS".

Following GOST 572-2016 "Millet grain ground. Technical conditions" the organoleptic indicators of millet, which consist of color, smell, and taste, are considered.

The color should be "yellow of different shades", the smell "characteristic of millet groats, without foreign odors, not musty, not moldy" and the taste "characteristic of millet groats, without foreign tastes, not sour, not bitter".

Figure 4 Device used for determining the humidity of food raw materials and products Eleks – 7M, Russian Federation, Moscow (Manufacturer Limited Liability Company "Tagler").

Figure 5 Drying cabinet SESH-3M for determining the moisture content present in pasta by drying to a constant weight (point 2.2), Ukraine, Vinnytsia region, Mogilev-Podilskyi, instrument-making plant.

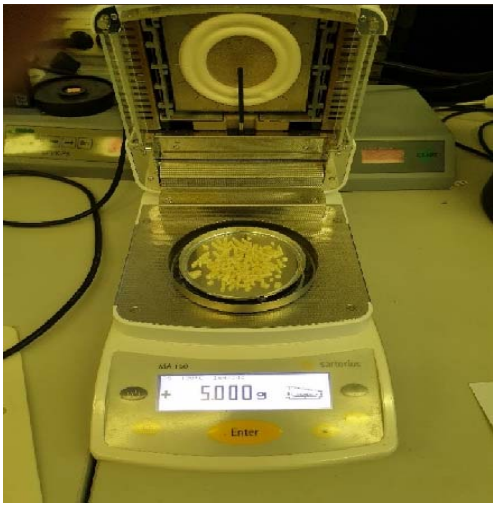


Figure 6 The device MA - 150 "SARTORIUS" infrared humidity analyzer MA-150 is designed to measure the humidity level of liquid, bulk, solid substances, and emulsions during input/output control of products and scientific research (Point 2.2), Germany, Göttingen, Weender Landstrasse 94-108, manufacturer "Sartorius Weighing Technology GmbH".

Description of the Experiment

Sample preparation: Mixing and testing: The research was carried out in one of the leading research institutes of the Russian Federation, Moscow, the Federal State Autonomous Scientific Institution "Research Institute of the Baking Industry". We are considering options for replacing wheat flour with 7.7 and 15.5% of the total mass. The calculation of the added raw materials is determined by the formula below (1):

$$M_D = M_c - \frac{M_c \times \%}{100} \quad (1)$$

Where:
M_D – the weight of the additive; M_c – the mass of raw materials; % - the percentage of input raw materials.

The grits weighed on the KERN 440-45N (Germany, Balingen, manufacturer "KERN & Sohn GmbH") device. Kneading the dough with the addition of millet was carried out for about 30 minutes on the model Sandorina, serial 1861 device (Made in Italy, 2002, Watt 400, Volts 220, Hz 50, Ph 1). The volume of the millet fraction was determined, which amounted to 670 microns. As a control copy, high-grade grits and water were used.

Number of samples analyzed: 36 samples.

Number of repeated analyses: Repeated analyses 9.

Number of experiment replication: Triple.

Statistical Analysis

All data are presented as the mean standard deviation (SD) of three independent experiments, and significance is defined as p <0.05. Utilizing Excel and STATISTICA 13 applications, the research's collected data were statistically analyzed (Dell, StatSoft).

RESULTS AND DISCUSSION

Developing new recipes to produce pasta with the addition of millet can be a great way to increase the nutritional value of this popular food item. Millet is a type of gluten-free cereal grain, high in fiber, and rich in various vitamins and minerals. Gluten protein in durum wheat semolina provides significant features such as low cooking loss, good texture, low surface stickiness, and resistance to surface disintegration **[26]**. Making millet pasta typically involves grinding the millet into a fine flour, which is then combined with water to form a dough. This dough can be shaped into various pasta shapes, such as spaghetti or penne, and then dried. Several organoleptic and physicochemical quality indicators can be measured to determine the quality of the resulting millet pasta. Organoleptic quality indicators include factors such as the pasta's appearance, flavor, texture, and aroma. Pasta is now available in countries all over the world, and it is one of the most popular dishes due to its nutritional content, organoleptic properties, and ease of preparation **[27]**. For example, when cooked, the pasta should have a uniform color and texture, a pleasant aroma, and a satisfying flavor and texture. Physicochemical

quality indicators include moisture content, protein content, and cooking quality measurements. Cooking qualities are an important factor in pasta evaluation **[28]**, **[29]**, **[30]**. Cooking loss is one of the most important criteria that influence consumer approval of this type of product, hence it is very useful in predicting the overall cooking performance of pasta. All fiber-enriched pasta samples had cooking losses that did not surpass the expected values for durum wheat pasta **[31]**, **[32]**, **[33]**.The following cooking parameters are used to evaluate pasta quality: optimum cooking time, cooking loss, water absorption index, and swelling index. The optimal cooking time (OCT), the time required to see the disappearance of the center core when pasta is gently squeezed between two glass slides, is usually one of the first technological criteria checked following pasta manufacturing. Most of the time, OCT is lowered following pasta fortification **[34]**, **[35]**. For example, the pasta should have a low moisture content to ensure a long shelf life, a high protein content to provide adequate nutritional value, and good cooking quality, meaning it should cook evenly and retain its texture and shape **[36]**. The optimal cooking time, according to the American Association of Cereal Chemists (AACC), is when the center core of the pasta simply disappears when squeezed between two glass plates. Pasta fortification increased optimal cooking time and water uptake while lowering the swelling index **[37]**, **[38]**, **[39]**. Water absorption capacity is critical in developing ready-to-eat foods, and a high absorption capacity may guarantee product cohesion **[40]**, **[41]**.

Structure: The millet colour was studied on the equipment CHROMA METERCR-410 (Japan, manufacturer "Konica Minolta Sensing Europe"). As a result of the measurement, the following data were obtained (Table 3):

Table 3 Color characteristics measurement of millet.

Number of measurements carried out	Received data
1	C2-22305 [0090] L* = 73.82 a * = 2.77 b * = 30.19 Δ L *= - 9.33 Δ a * = + 4.24 Δ b * = + 10.67 Δ E * = 14.80
2	C2-22305 [0091] L* = 73.76 a * = 2.73 b * = 30.58 Δ L *= - 9.39 Δ a * = + 4.20 Δ b * = + 11.06 Δ E * = 15.11
3	C2-22305 [0092] L* = 73.64 a * = 2.92 b * = 30.16 Δ L * = - 9.51 Δ a * = + 4.39 Δ b * = + 10.65 Δ E * = 14.94

Note: L – color brightness, measured from 0 to 100%; a – color range in the color circle from green (-120 °) to red (+120 °); b is the color range in the color circle from blue (-120 °) to yellow (+120 °); Δ E: Graph display and evaluation; Δ b: Graph display and evaluation; Δ a: Graph display and evaluation; Δ L: Graph display and evaluation; C2-22305 [0090]: Illuminator C; C2-22305 [0091]: Illuminator C; C2-22305 [0092]: Illuminator C only.

The millet with fractions obtained were 315 microns, 670 microns and 1.25 mm. The distinctive yellow color generated by the high carotenoid content of durum wheat semolina is one of the most essential parameters that define pasta quality. Color is incredibly essential and has a major influence on consumer decision. Some components may significantly alter the color of the new pasta compositions **[42]**, **[43]**, **[44]**, **[45]**. The product's

colour is an important quality element that is highly tied to consumer impression. An increase in pearl millet flour in the blend resulted in a change in the color of uncooked pasta, which is regarded as an undesirable attribute. Pasta with a brilliant yellow color is preferred by most pasta customers **[46]**, **[47]**, **[48] [49]**, **[50]**.

Installation Time: Water, 7.7% millet and premium grain. The dough is kneaded for 30 minutes (according to the timer).The amount of water used to obtain a moisture index of 28% at the "press" stage is given below (Table 4):

Table 4 The amount of water used in kneading the dough to achieve a humidity of 28% after the press.

The amount of added water for kneading the dough						
Volume (mL)	117	119	120	125	128	133
Time (Min)	30	30	30	30	30	30

After the pasta is released, they are laid out on a sieve for drying. An increase in moisture content can accelerate biochemical and microbiological activities, decreasing product quality. The results show that none of the samples exceeds the allowable humidity levels (Table 4) **[51]**. The high moisture content has been related with short shelf life of composite millet flour as they encourage microbial proliferation that cause spoilage **[52]**, **[53]**.

Research method No. 1.: We determine the moisture content of pasta on the ELEKS – 7M device (Russian Federation, Moscow, manufacturer Limited Liability Company "Tagler").
Calculation method:

$$B = \frac{H - C}{H - B} * 100\%$$

Where:
B – raw material moisture, %; H – the weight of the raw material with a paper bag before drying, g; C is the weight of the raw material in a paper bag after drying, g; B is the weight of the dried paper bag, g.

Sample No. 1:

$$B = \frac{5.97 - 4.55}{5.97 - 0.90} * 100\% = \frac{1.42}{5.07} * 100\% = 0.28 * 100\% = 28\%$$

Sample No. 2:

$$B = \frac{5.99 - 4.57}{5.99 - 0.92} * 100\% = \frac{1.42}{5.07} * 100\% = 0.28 * 100\% = 28\%$$

Sample No. 3:

$$B = \frac{5.98 - 4.55}{5.99 - 0.92} * 100\% = \frac{1.43}{5.06} * 100\% = 0.28 * 100\% = 28\%$$

Research method No. 2.: Humidity determination was performed on the device MA-30 "Sartorius". Humidity is 27%.

Research method No. 3.: Determination of humidity by the express method – 28%. The formula calculates the mass fraction of moisture W, %:

$$W = \frac{(m_1 - m_2)}{m} \cdot 100$$

Where:
m_1 – the mass of the bux with a sample for analysis before drying, grams; m_2 – the mass of the bux with a sample for analysis after drying, grams; m – the mass of the sample for analysis, grams.

$$W=\frac{(m_1-m_2)}{m}\cdot 100 = \frac{6.20-4.82}{5}\cdot 100 = \frac{1.38}{5}\cdot 100 = 0.276\cdot 100 = 27\%$$

Research method No. 4.: Determination of humidity by drying to a constant mass – was not carried out. The results are as follows (Figures 5-6):

We continue experimenting with 15.5% millet. The weight was determined on the device KERN 440-45N (Germany, Balingian, manufacturer "KERN & Sohn GmbH"), max 1000 g, d = 0.1 g. Kneading is carried out within 30 minutes on the device Sandorina1861 (according to the timer) (Table 5). Sandorina 1861 made in Italy, 2002, Watt 400, Volts 220.

Table 5 The amount of water used in kneading the dough to achieve a humidity of 28% after the press.

	The amount of added water for kneading the dough	
Volume (mL)	113	117
Time (min)	30	30

Research method No. 1.: We determine the moisture content of pasta on the ELEKS – 7M device (Russian Federation, Moscow, manufacturer Limited Liability Company "Tagler").
Calculation method:

$$B = \frac{H - C}{H - B} * 100\%$$

Where:
B – raw material moisture, %; H – weight of the raw material with a paper bag before drying, g; C is the weight of the raw material with a paper bag after drying, g; B is the weight of the dried paper bag, g.

Sample No. 1

$$B = \frac{5.99 - 4.53}{5.99 - 0.90} * 100\% = \frac{1.46}{5.09} * 100\% = 0.28 * 100\% = 28\%$$

Sample No. 2

$$B = \frac{6.0 - 4.55}{6.0 - 0.94} * 100\% = \frac{1.45}{5.06} * 100\% = 0.28 * 100\% = 28\%$$

Research method No. 2.: Humidity determination was performed on the device MA-30 "Sartorius". The humidity is 28.41%.
Research method No. 3.: Determination of humidity by express method – was not carried out.
Research method No. 4.: The method of drying to a constant mass was not carried out.
The results are as follows (Figures 7-10): Figure 7 shows the findings obtained after adding 7.7% millet to pasta and the influence of humidity using three different methods: MA-30 (Sartorius), express technique, and ELEKS 7M devise. It is obvious that the Sartorius method yielded a humidity of 27% when compared to the other two procedures, which yielded the same findings (28%). Figure 8 represents the form of the pasta after 7.7% millet was added.

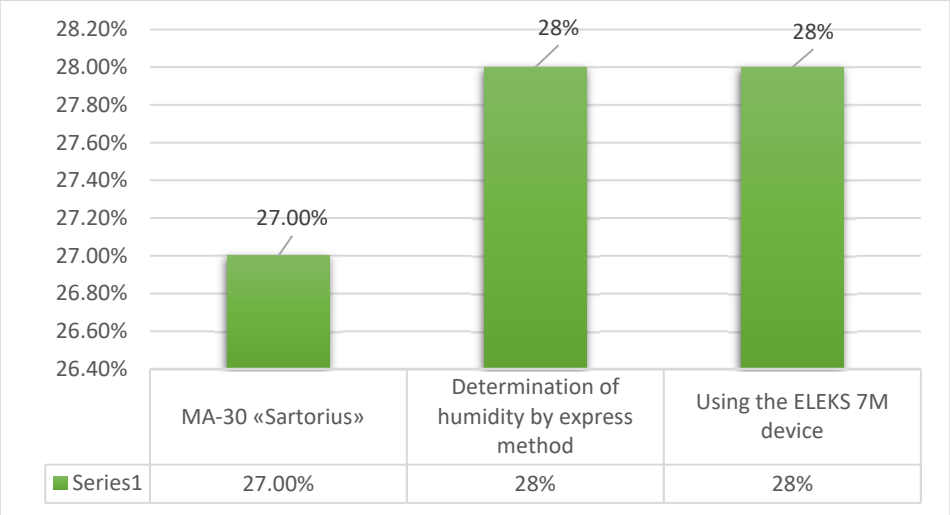


Figure 7 Moisture indicators of pasta with the addition of millet 7.7%.

Figure 8 Pasta with the addition of millet 7.7 %.

Figure 9 displays the outcomes of adding 15.5% millet to pasta and examining the impact of humidity using the MA-30 (Sartorius) and ELEKS 7M devices. It is obvious that the Sartorius method, which yielded a result of 28.41%, was superior to the ELEKS 7M device, which produced a nearly identical result (28%). Figure 10 shows the pasta's shape after being mixed with 15.5% millet.

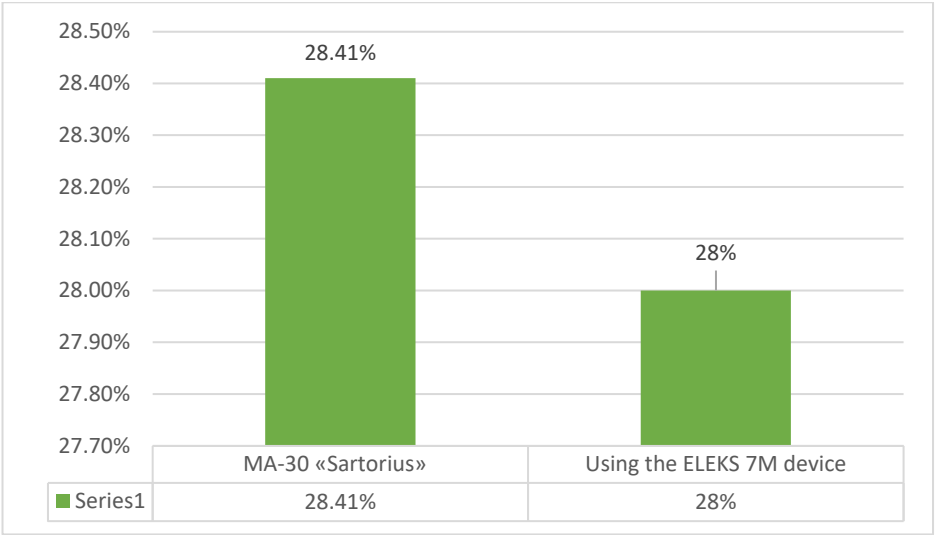
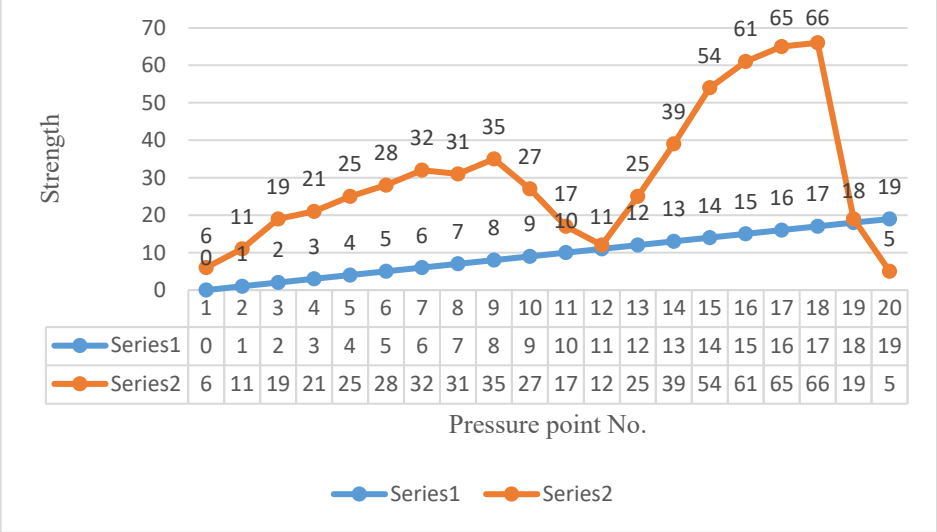


Figure 9 The moisture content of pasta with the addition of millet 15.5%.

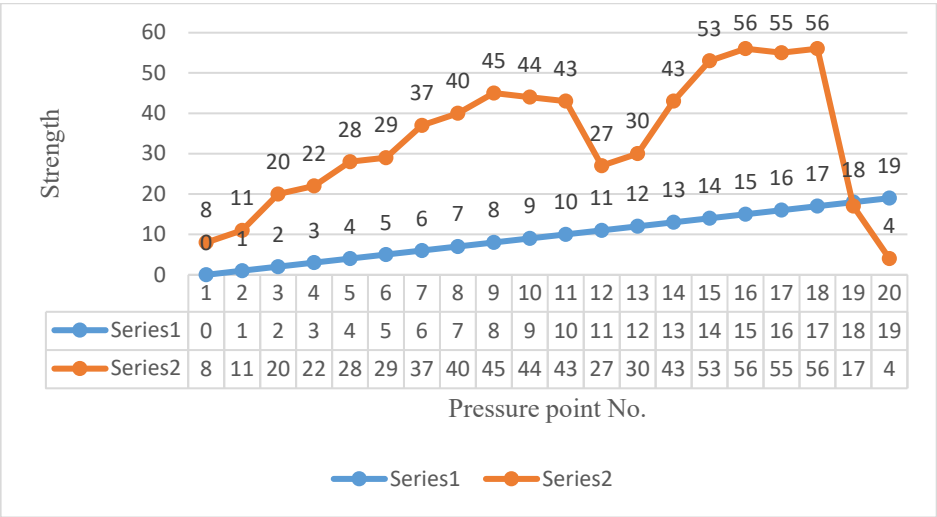
Figure 10 Pasta with the addition of millet 15.5%.

We determine the pasta's strength with adding millet 7.7%, and 15.5%. We preliminarily determine the strength of pasta of the control sample prepared with the highest-grade grits (Samples No. 1, 2, 3) as indicated in Figures 11-13. The studies were carried out on three samples with the addition of 7.7% (Samples No. 4, 5, 6) (Figures 14-16), 15.5% (Samples No. 7, 8, 9) (Figures 17-19).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Series1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Series2	6	11	19	21	25	28	32	31	35	27	17	12	25	39	54	61	65	66	19	5

Figure 11 Strength indicators of pasta made from the highest-grade twist (Sample control No. 1).



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Series1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Series2	8	11	20	22	28	29	37	40	45	44	43	27	30	43	53	56	55	56	17	4

Figure 12 Strength indicators of pasta made from high-grade twist (Sample control No. 2).

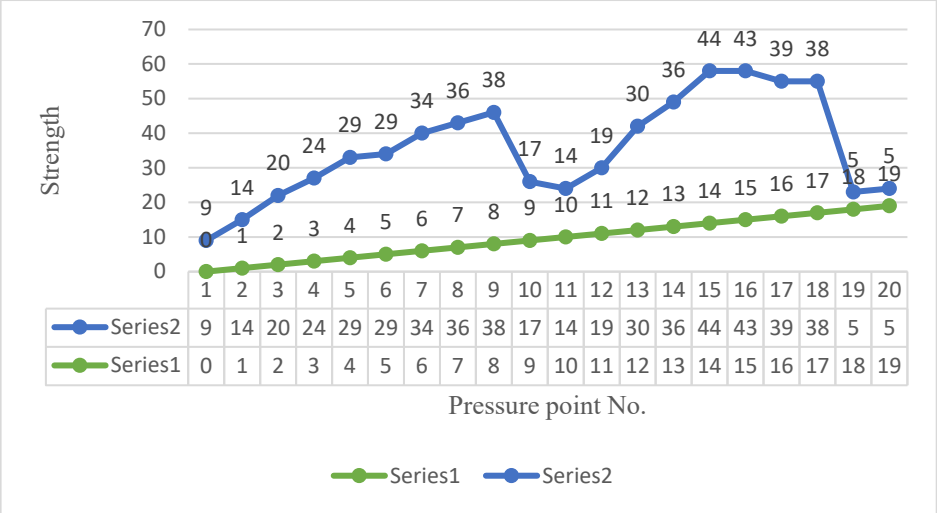


Figure 13 Strength indicators of pasta made from high-grade twist (Sample control No. 3).

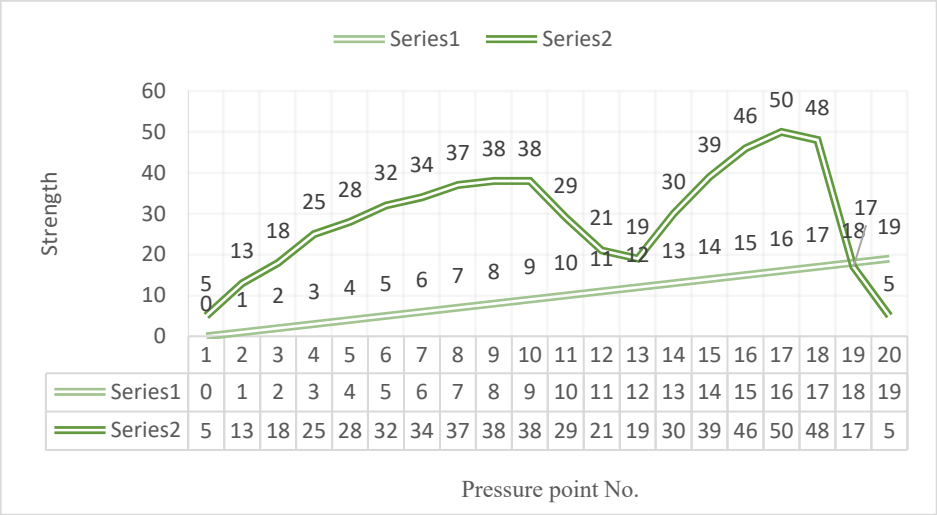


Figure 14 Strength indicators of pasta with the addition of millet 7.7% (sample No. 4).

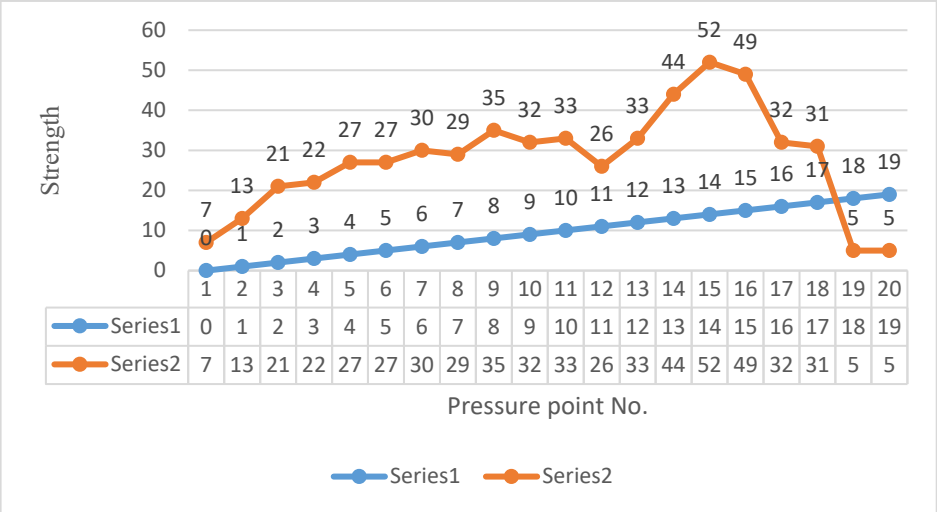


Figure 15 Strength indicators of pasta with the addition of millet 7.7% (sample No. 5).

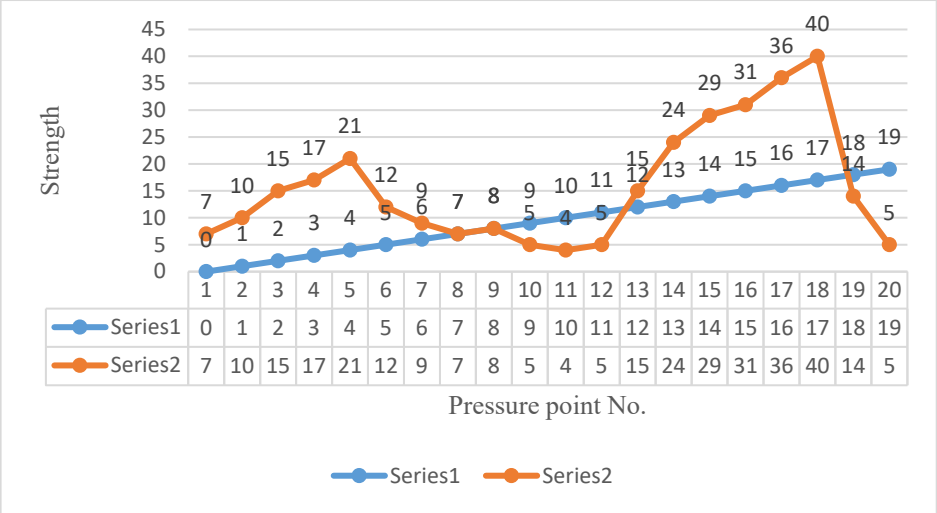


Figure 16 Strength indicators of pasta with the addition of millet 7.7% (sample No. 6).

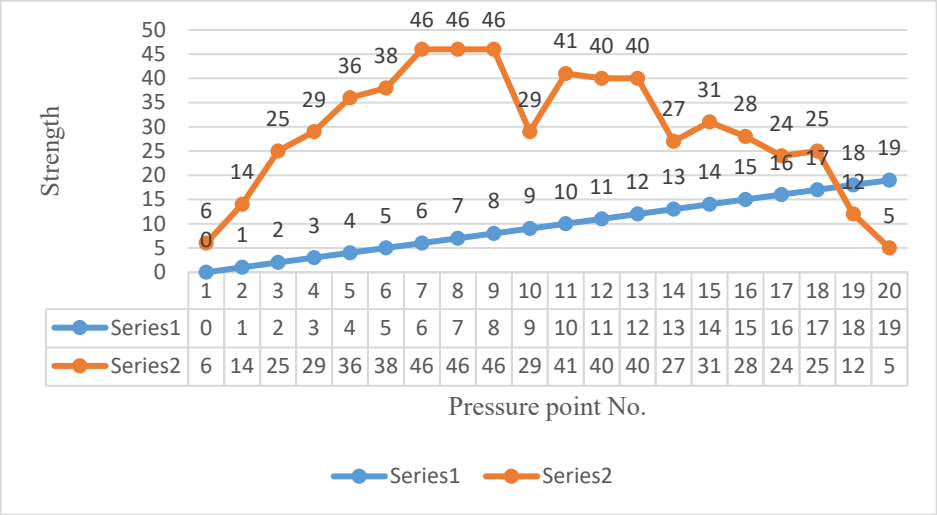


Figure 17 Strength indicators of pasta with the addition of millet 15.5% (sample No. 7).

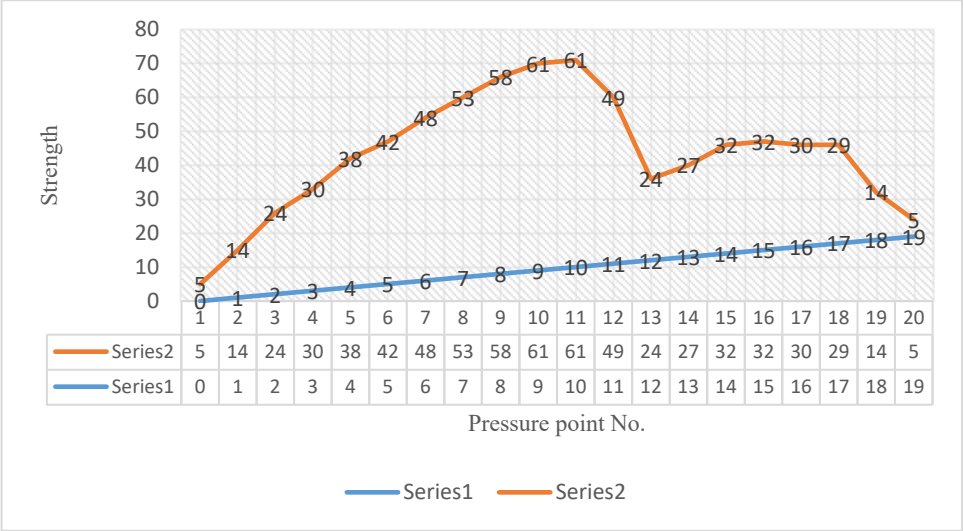


Figure 18 Strength indicators of pasta with the addition of millet 15.5% (sample No. 8).

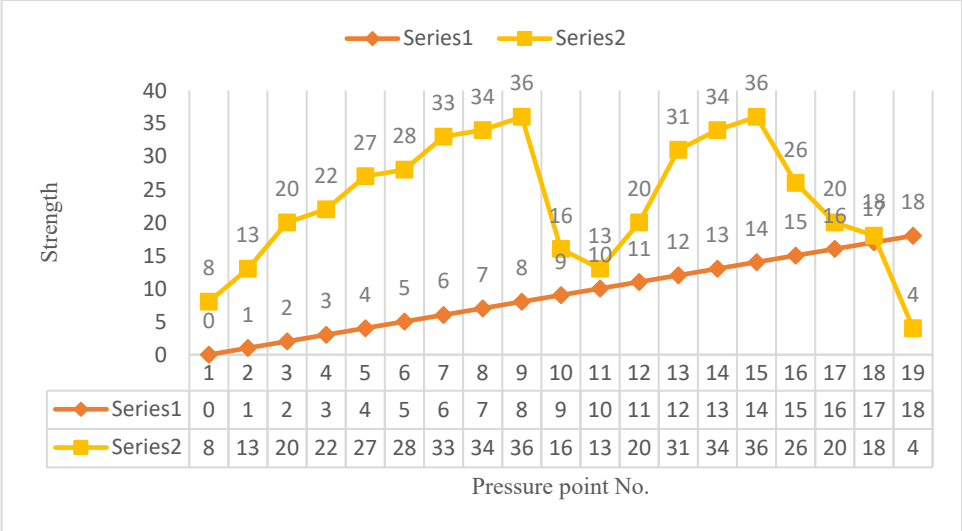


Figure 19 Strength indicators of pasta with the addition of millet 15.5% (sample No. 9).

The tests were carried out from pasta (50 g), and preparation for the analysis was carried out based on clauses 7.7.1 and 7.7.2 of GOST 31964-2012. The pasta was removed from the vessel in an arbitrary order and was subjected to examination on the ST-1M structure meter (Figure 20).

Figure 20 Strength determination device "Structurometer ST-1M", designed to determine the rheological characteristics of raw materials, semi-finished products and finished products, Russian Federation, Moscow, manufacturer "Ochakov Combine of Food Ingredients".

Studies have been conducted according to the methods that are established in interstate standards. Organoleptic indicators were determined according to the results of studies, it was found that with an increase in the amount of millet, the color of pasta becomes more saturated (bright) (Table 6). The taste and smell of pasta with an increase in millet becomes richer.

Table 6 Organoleptic indicators of pasta quality.

The name of the indicator	Characteristics according to GOST 31743-2017	Pasta with the addition of millet, characteristics	
		7.7%	15.5%
Color	Corresponding to the flour grade The color of products using additional raw materials varies depending on the type of this raw material	Yellow	Yellow
Form	Corresponding to the type of products		
Taste	Characteristic of this product, without extraneous taste	There is a certain taste of millet	The taste of millet is observed.
Smell	Characteristic of this product, odorless	A certain smell of millet	A slight smell of millet

According to physicochemical indicators (humidity) with the addition of millet, 7.7% for 3 indicators (except for the method of drying to a constant mass) they amounted to 28% at the pressing stage, with the addition of millet 15.5% according to 2 indicators (with the exception of the express method and drying to a constant mass) amounted to 28% at the pressing stage. Also, according to the physicochemical parameters (strength), studies of pasta were carried out (after the cooking process), and the data obtained were compared with a control sample (pasta made from high-grade grits). The conducted studies show that the strength indicators are close to the control.

CONCLUSION

According to the findings of the experiments, the quality indicators in pasta with millet additions of 7.7% and 15.5% correspond to the control sample. The humidity index at the pressing stage is 28%, the color is yellow, the shape is appropriate for the product, the taste is pleasant when ingested, the scent is typical of the product, and the strength indicators are comparable to the control sample. Additionally, millet pasta recipes have been developed. According to the study's findings, all quality indicators are within acceptable levels, with the exception of pasta with millet, which accounts for 23.3% of the total. Furthermore, investigations of pasta, after cooking, were conducted based on the physicochemical parameters (strength), and the results were compared to a control sample (pasta made from high-grade grits). Based to the results of the investigations, the strength indicators are close to the control.

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

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The potential of non-traditional walnut shells waste for the production of antioxidant reach extracts intended for the food industry

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ABSTRACT

Phenolic compounds extracted from walnut shells are potentially good natural sources of antioxidants for the food industry and have numerous health benefits. Walnuts have more antioxidant capacity than any other nut because the shell is primarily composed of lignin, a strong source of phenols. Studies demonstrated that lignin characterizes the shell strength level and is a source of antioxidants due to its chemical composition. In the current study, an extract obtained by extraction with a hydroalcoholic solvent of various concentrations from a walnut shell was investigated. The results of this study have proven that walnut shell extract contains the main sources of mineral elements and vitamins, which are of great importance. According to the biological value, this extract contains essential amino acids for the body. The high content of quercetin and catechin shows the antioxidant activity of the extract. In the present article, the authors disclose methods for obtaining an experimental batch of a prophylactic product based on walnut shells and give the product a technological characteristic. Consequently, a product was developed for prophylactic usage of 10 ml per 100 ml of water and must be taken 1-2 times a day for 21 days. The required product amount was calculated from the daily intake of vitamins, minerals, and flavonoids.

Keywords: walnut shell, vitamin, mineral, antioxidant, phenolic compound

INTRODUCTION

Currently, in the development of food production technology, natural food additives based on plant raw materials are of particular importance, enhancing the organoleptic characteristics of food products and enriching them with valuable biologically active components **[1]**, **[2]**. Nutrition optimization, as it is known, promotes the introduction of biologically active food supplements and specialized products enriched with biologically active substances into the diet. A special place among them is occupied by supplements enriched with components with high antioxidant activity. The usage of such additives is most relevant in conditions of a significant spread of cardiovascular and oncological diseases caused by the action of free radicals on the cells and tissues of the human body **[3]**. The characterization of polyphenol content and evaluation of the antioxidant activity of diverse plant materials have received much interest recently because frequent consumption of these foods is linked to a lower risk of certain diseases, such as cancer and cardiovascular disorders. They are also relevant for raising immunity **[4]**. The walnut is recognized as a rich source of various valuable chemicals because the kernel, fresh green fruit, husk, shell, peel, bark, leaves, and root have been extensively studied for use in the food, cosmetic, and pharmaceutical industries. In this regard, all parts of the walnut tree can be utilized as an excellent source of various compounds expressing antioxidant and antimicrobial potential, as well as an antihistamine, antiulcer, antiasthma, antidiabetic, immunomodulatory, hepatoprotective, central nervous system stimulant, anti-

inflammatory, wound healing, lipolytic, and many other properties that have positive effects on human health **[5]**. The walnut is classified as a strategic species for human nutrition, as the Food and Agriculture Organization (FAO) has included it in the group of priority plants **[6]**. Meanwhile, inedible parts, including leaves, shells, rinds, green shells, and bark, have been used in traditional medicine for treating various ailments. For instance, walnut leaf infusion is used in some countries for its antioxidant and antimicrobial properties. Moreover, green peel extract has been utilized for treating skin conditions and inflammation **[7]**. The population of those regions where it grows has been using its therapeutic and prophylactic properties for a long time (Moldova, the North Caucasus, Romania, Tibet, Greece, Japan, China, France, etc.). The composition of plant raw materials, extracts, and preparations based on walnut comprises essential oils, organic acids, alkaloids, glycosides, saponins, coumarins, carotenoids, water-soluble vitamins, phytoncides, phenolic compounds, tannins, microelements. Such natural unique complexes determine both the therapeutic and prophylactic effect and the possibility of using raw walnut materials as technological food additives since they have various flavouring, tannic, antioxidant, antimicrobial, and other properties. The chemical composition of all walnut parts is based on the variety, place, and environmental conditions of growth **[8]**. The industrialization of fruits leads to the formation of many plant residues. In this regard, 70% of walnut fruits are estimated to turn into residues, mainly peel, bagasse, green peel, peel, and leaves (Figure 1), containing many biologically active compounds valuable for their use and exploitation. These residues are usually thrown into landfills, incinerated, or used for composting. However, the most effective use of this waste would be a circular economy strategy that would reduce the environmental impact and, at the same time, stimulate the economic sector. In this sense, the agricultural remains of the walnut have been extensively researched in search of natural products. Evidence shows that all parts of the walnut tree can be utilized as a source of compounds with major antioxidant, antimicrobial, antidiabetic, immunomodulatory, hepatoprotective, and anti-inflammatory potential **[9]**.

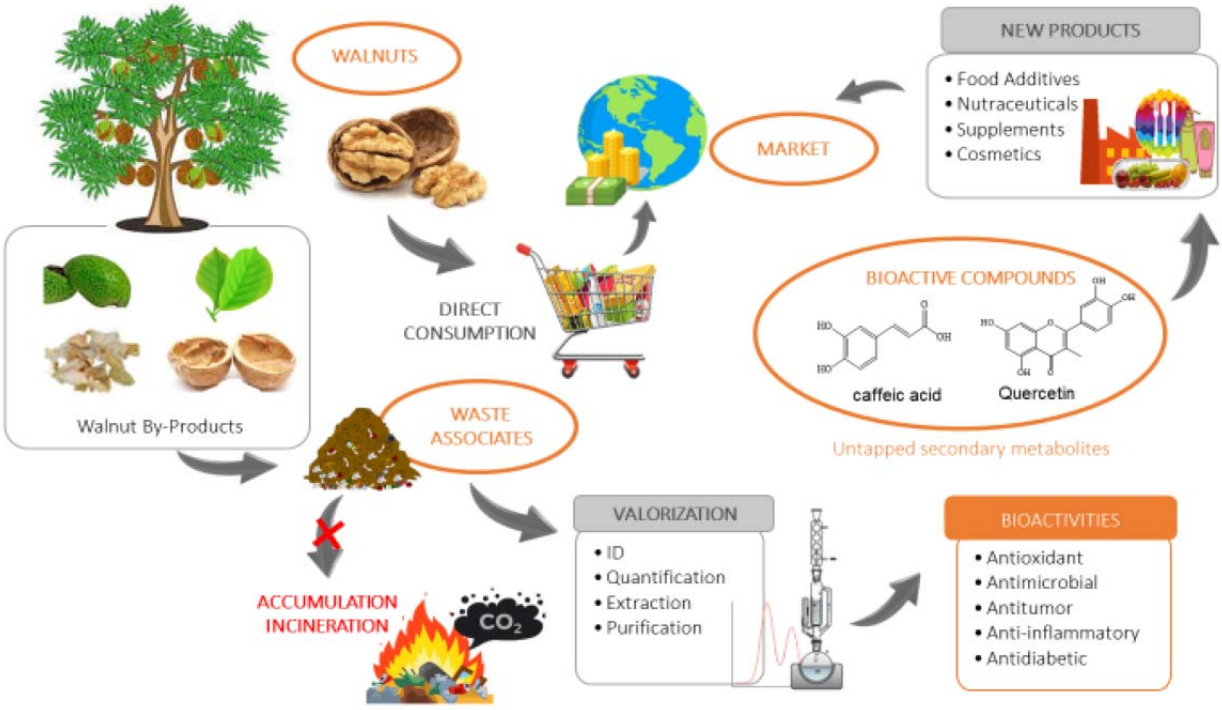

Figure 1 Evaporation of by-products from walnuts according to circular economy approaches and extraction of compounds with biological properties for bio-based products in various industries **[9]**.

The collection of fruits is conducted at a time when they are rich in biologically active healing substances. The most valuable is the walnut fruit, the core of which has not yet hardened and is in a gelatinous state, and the shell is still soft, juicy, and easily cut with a knife; that is, a strong shell has not formed. If such fruit is pierced, the blade easily passes through, and milky juice flows abundantly from the incision. In this state, the fruits of wax ripeness are a natural vitamin concentrate, and it is advisable to use them for processing during this period **[10]**. The walnut has more antioxidant capacity than any other nut because the shell is mainly composed of lignin, a strong source of phenol. Natural antioxidants such as phenolic compounds are gaining importance due to their positive effects on human health, such as reducing the risk of degenerative diseases by reducing oxidative stress and inhibiting macromolecular oxidation **[11]**. In Russia, walnut leaves are utilized only in traditional medicine, and when used in homeopathic practice, reference is made to the regulatory documents of foreign countries. In

order to standardize the quantity of tannins (measured in terms of gallic acid), flavonoids (measured in terms of hyperoside), and naphthoquinones (measured in terms of juglone), researchers have produced a homeopathic matrix tincture from dried and fresh walnut leaves **[12]**. Information about the study of the composition of the walnut leaves (*Juglans regia* L.) growing in the vicinity of the Caucasian Mineral Waters and the development of a dry extract based on it is given. It has been established that the dry extract from the leaves of this plant has antioxidant activity and is classified as a practically non-toxic substance **[13]**. The green peel of nuts includes pentacyclic triterpenes, sesquiterpenes, tetralones, naphthoquinones, phenolic acids, diarylheptanoid, neo-lignans, flavonoids, phenylethanoids, and tannin. The walnut pericarp is superior to rose hips in vitamin C content **[14]**. The walnut is recognized as a crop that produces more waste containing heavy materials. ~70% of the fruit weight is estimated to be made up of shells and husks, low-value waste products rich in different chemicals, mainly phenolic compounds **[15]**. As agricultural waste from walnut processing, walnut shells are available in large quantities. Walnut shells are also beneficial due to their availability as renewable resources. They are utilized as an abrasive for cleaning and polishing soft metals, stone, fibreglass, plastics, and wood. Walnut shell provides an effective way to grind and polish jewellery, gun cases, metal parts, and ink pens. In recent years, some studies have described the production of antioxidant and antimicrobial pyroacids from walnut shells **[16]**, **[17]**. The walnut shell contains antioxidant compounds, including flavonoids, isolated by extraction. Solvent extraction is often utilized for isolating plant antioxidant compounds. However, due to the chemical properties of the extracts and their solubility in a particular solvent, the yields of the extracts and their antioxidant activity vary greatly. Methanol **[18]**, ethanol **[19]**, chloroform **[20]**, water **[21]**, N-butanol **[22]**, and ethyl acetate **[23]** are often utilized for extracting antioxidant compounds from plant matrix.

Scientific hypothesis

Improving a functional product's quality depends on the walnut's quality, the mode, and the production technology. The quality and composition of the extract significantly affect the content of flavonoids and polyphenols (antioxidants).

MATERIAL AND METHODOLOGY

Samples

Walnut shells were taken for research. Walnuts collected in the fall of 2023 were provided for research by a farm (Turkestan region, Zhetysay, Kazakhstan).

Chemicals

All reagents were of analytical grade and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

Instruments

Extraction was performed on a semi-automatic Soxhlet apparatus Vilitek "ASV-6" (Vilitek, Russia), while grinding was carried out on a laboratory mill "MShL-1P" (OJSC Promstroymash, Russia).

Laboratory Methods

The following methods and GOSTs were utilized to achieve the goals and objectives: GOST 32874-2014 “Walnuts. Specifications"; GOST 5962-2013. Ethyl alcohol is rectified from food raw materials. Specifications. GOST EN 12822-2014 Food products. Determination of vitamin E by high-performance liquid chromatography. MUK 4.1.1090-02 "Method for determining the mass concentration of iodine." GOST 26573-2014 "Method for the determination of iron". GOST 26573.2-2014 "Method for determination of zinc". MVI MN 1363-2000 "Method for determining amino acids using high-performance liquid chromatography". GOST R 57990-2017 "Method for determination of quercetin". GOST ISO 14502-2-2015 "Method for determining the content of catechins" **[24]**, **[25]**, **[26]**.

Total phenols were estimated using the Folin-Ciocalteu colourimetric method, and the results were expressed in milligrams of gallic acid equivalents (mg GAE/extract).

The antioxidant activity of nutshell extracts was determined by using the procedures detailed in a study by Sartori et al. The determination using 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) was expressed as the amount of extract. Walnut shell extracts were added to 1.5 ml of DPPH solution (4.02 mg/100 ml in ethanol), and the mixture was kept in the dark for 30 minutes at room temperature. The absorbance at 517 nm was utilized for determining the concentration of the remaining DPPH through a UV-VIS spectrophotometer. The assay was performed three times, and the radical scavenging activity of DPPH was expressed as percent (%) inhibition using the following equation:

$$\text{DPPH scavenging effect \%} = \frac{AD-AS}{AD} * 100 \quad (1)$$

Where: AD is the absorption value at 517 nm of the control type DPPH and AS is the absorption value at 517 nm for the sample.

Description of the Experiment

Sample preparation: The walnut shell was washed and dried, then it was crushed in a Novital Magnum 4V crusher, then the crushed shell was crushed with steel balls in a MSHL-1P mill. The crushed shell was extracted on a semi-automatic Soxhlet extraction apparatus "ASV-6"

Grinding and Laboratory Mill "MShL-1P": Mill "MShL-1P" is a batch device equipped with steel balls. After preparing raw materials, wetting and drying, before feeding to the mill, the walnut shell is crushed using the crusher "Novital Magnum 4V." Then, the shell is crushed using steel balls on the mill "MShL-1P." When the drum rotates, the material is crushed due to the abrasive and impact action of the balls. Grinding time depends on the fineness of grinding and varies from 1 to 3 hours.

Extraction on a Semi-Automatic Soxhlet Extraction Apparatus "ASV-6": Samples are prepared for extraction to start the analysis. Sleeves are made from filter paper, where crushed walnut shells are placed in 5 g. 45 ml of a solvent (water, ethanol) is poured into the extraction flask and placed in a water bath, raising the appropriate glass refrigerator and the sample installed. After reaching the set temperature, the sample is transferred to the solvent, where the sample is processed for 30 min. After that, the sample is transferred to the position for washing with pure solvent. The process of washing with a pure solvent is the main extraction stage, which takes 60-180 minutes. After the extraction end, within 30 minutes, the solvent passes to the top of the refrigerator, and the extracted substance remains in the extraction flask. The extraction method is most often used to isolate antioxidant substances. In order to obtain a prophylactic product and identify antioxidant substances, a technology has been developed for obtaining an extract from walnut shells. To obtain an extract from the walnut shell, certain technological operations are utilized as follows: preparation of raw materials, washing of the sorted batch of the shell and drying it. Only after this is the main technological process of extraction carried out. The resulting extract is filtered, dried, and packaged. The flow diagram of the process is shown in Figure 2.

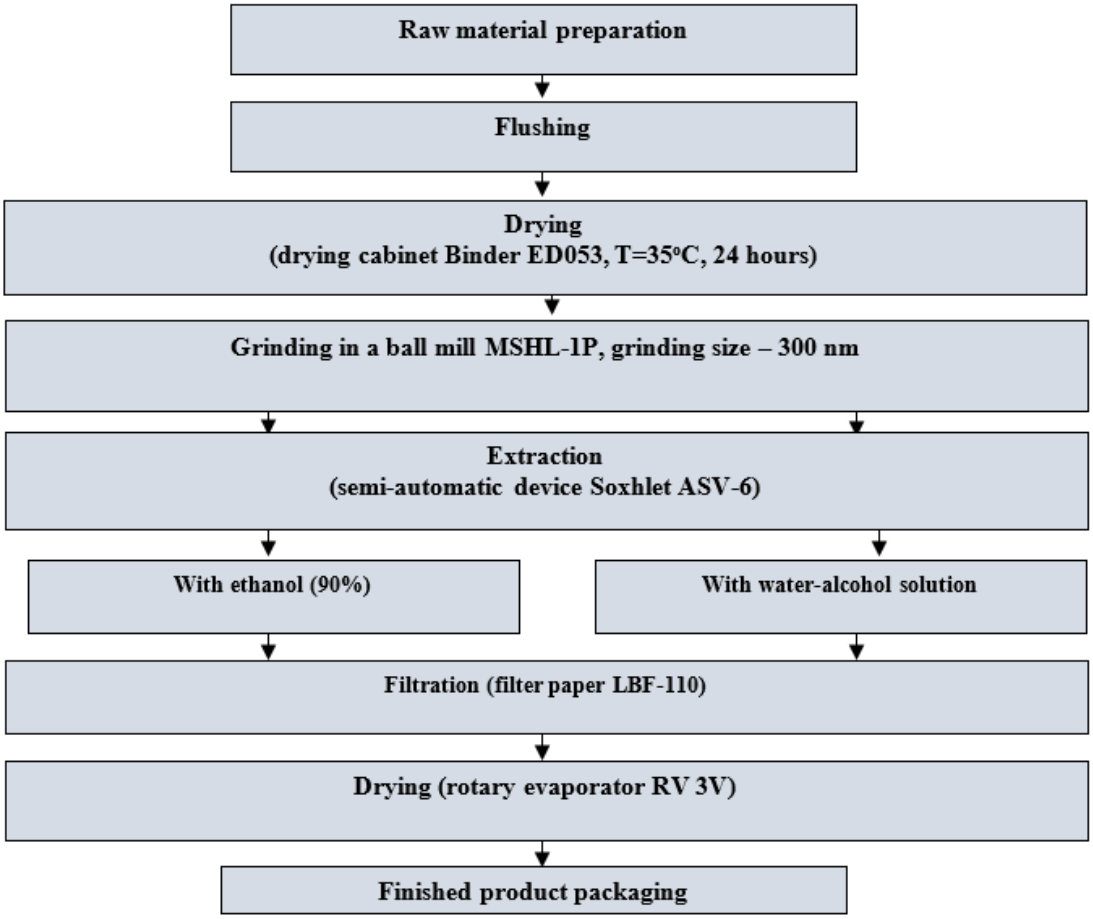

Figure 2 Scheme of the technological process for obtaining an extract from the walnut shell.

To determine the complete extraction of the antioxidant properties from the walnut shell, the walnut shell extraction was conducted in 3 different modes, as shown in Table 1.

Table 1 Modes for obtaining an extract from walnut waste.

Used raw materials	Mass of raw materials, g	Water, %	Ethanol, %	Size, microns	Extraction time, min
Chopped walnut shell	5	30	70	300	120
	5	20	80	300	120
	5	-	90	300	150

According to the relevant regimes, an experimental batch of walnut shell extract was obtained in 100 mg of each sample (Figure 3).

a b c

Figure 3 An experimental batch of walnut shell extract was obtained a) with an alcohol concentration of 70%; b) with an alcohol concentration of 80%; c) with an alcohol concentration of 90%.

Number of samples analyzed: Two samples were analyzed.
Number of repeated analyses: All tests were performed in triplicate.
Number of experiment replication: Replications were conducted 2 times.
Design of the experiment: First, sample preparation was carried out: by washing, drying, and grinding the walnut shell. Next, an extract was obtained using solvents (water, ethanol) on a semi-automatic Soxhlet apparatus ASV-6. Thirdly, the obtained extract was filtered and dried on a rotary evaporator. Next, the packaging of the resulting product was carried out.

Statistical Analysis

The data obtained during the experiments were processed using the mathematical method of variation statistics using the Statistica 10.0 developer: StatSoft, USA. Also, the data were analyzed using MS Excel for Windows version 10 Pro, 2010. One-way analysis of variance ANOVA was used to analyze the data and determine if there were significant differences between samples. The data collected during the study were subjected to independent testing. 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.

RESULTS AND DISCUSSION

The antioxidant activity of the walnut extract was compared with that of three commonly used synthetic antioxidants, BHT, BHA, and TBHQ, as presented in Figure 4. Since DPPH is a stable organic nitrogen-free radical, its scavenging capacity has been widely utilized for evaluating the antioxidant capacity of the extracts from vegetable matter **[27]**, **[28]**, **[29]**, **[30]**.

The uptake activity was the same at lower concentrations of walnut shell extract. In the concentration range from 100 to 500 µg/ml, the absorbing activity of walnut shell extracts was higher than that of TBHQ, BHA, and BHT. These data proved that walnut shell extract could be utilized as a natural replacement for synthetic antioxidants, negatively affecting human health **[31]**, **[32]**, **[33]**.

Furthermore, in the samples of walnut shell extract, the flavonoid composition was investigated, comprising quercetin and catechin, antioxidants that are very useful for the heart, help protect brain functions, support connective tissue, enhance blood circulation, and have an antibacterial effect. Dietary intake of flavonoids ranges from 50 to 800 mg/day, depending on the consumption of food sources containing various flavonoids **[34]**, **[35]**.

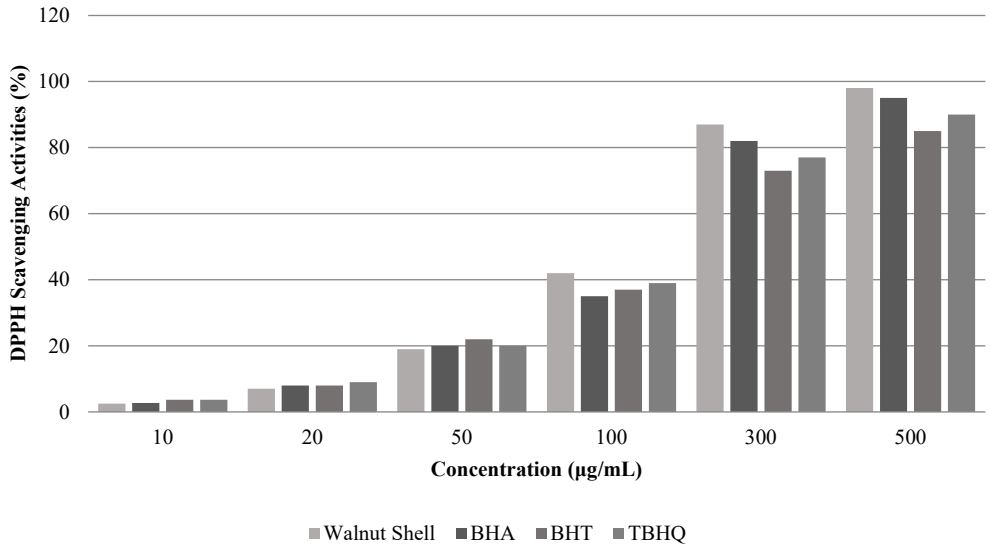

Figure 4 Comparison of antioxidant activities of walnut shell extract with BHA, BHT, and TBHQ by DPPH analysis.

The level of quercetin and catechin is displayed in Figure 5.

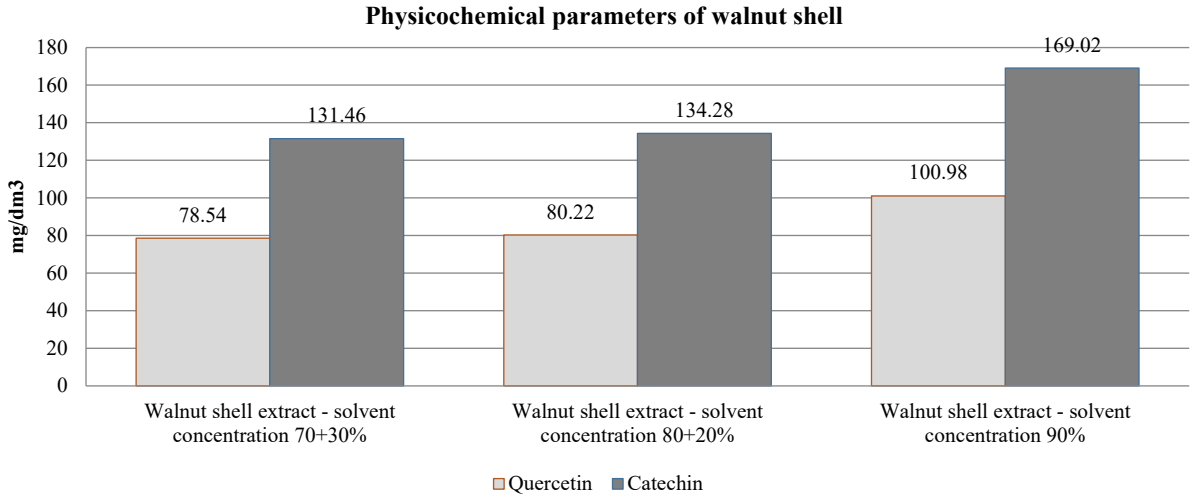

Figure 5 Content of catechin and quercetin in walnut shell extract, mg/dm^3.

As illustrated in Figure 5, as the solvent concentration increases, the quercetin and catechin content increases. The maximum value was in the extract obtained with 90% solvent. According to the maximum value, the catechin content is 169.92 mg/dm^{3,} and that of quercetin is 100.98 mg/dm^3. Given that all antioxidants protect the body from damage by harmful free radicals – toxins that enter from the environment and damage healthy cells, leading to inflammatory processes, it is worth noting the significant role of the flavonoid composition of the walnut shell as one of the components **[36]**, **[37]** in identifying the further direction of research.

The analysis in Figure 6 displays the content of vitamins in the extract obtained with various concentrations of solvent from the walnut shell.

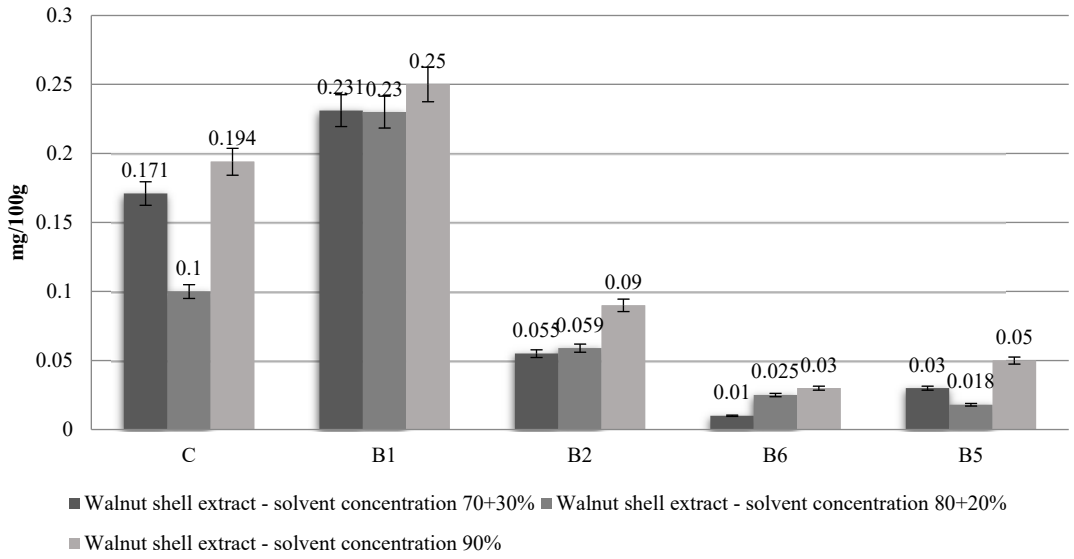

Figure 6 The vitamin content in the walnut shell extract (mg/100g).

Based on this analysis, the amount of vitamins increases as the solvent concentration increases. The content of vitamin C ranges from 0.1 mg/100g to 0.171 mg/100g. Vitamin B1 is stabilized within 0.25 ±0.05mg/100g. Vitamin B2 is found in the range of 0.05-0.09 mg/100g, B6 is 0.03 mg/100g, and B5 is 0.05 mg/100g.

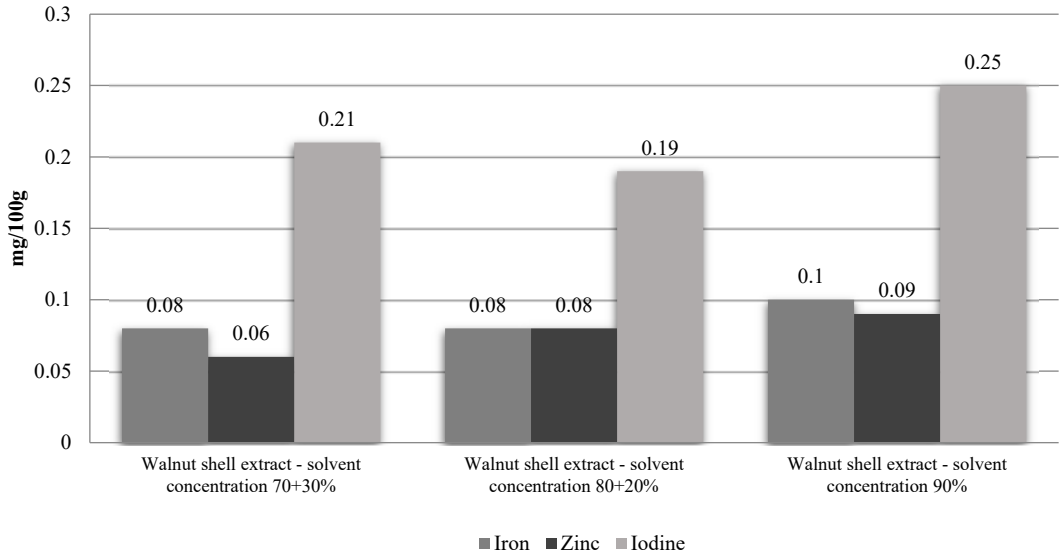

Figure 7 The content of mineral substances in the extract from the walnut shell.

The study of the parameters of the physicochemical properties of the extract obtained from the walnut shell (Figure 7) with various extractants indicated the following:

- iodine content up to 0.1-0.25 mg/100g;
- iron content up to 0.08-0.1 mg/100g;
- zinc content up to 0.06-0.09 mg/100g.

The study results proved that the most optimal extraction mode was the one in which the antioxidant properties were maximally extracted from the walnut shell, where the ethanol content was 90% and the extraction time was 120 minutes.

Studies have also demonstrated the walnut shell extract's rather rich amino acid composition (Figure 8). In this case, the amino acid composition parameter indicates a high level of the nutritional value of the extract **[38]**, **[39]**. One of the most important functions of amino acids is their participation in the synthesis of proteins that perform catalytic, regulatory, reserve, structural, transport, protective and other functions **[40]**, **[41]**, **[42]**.

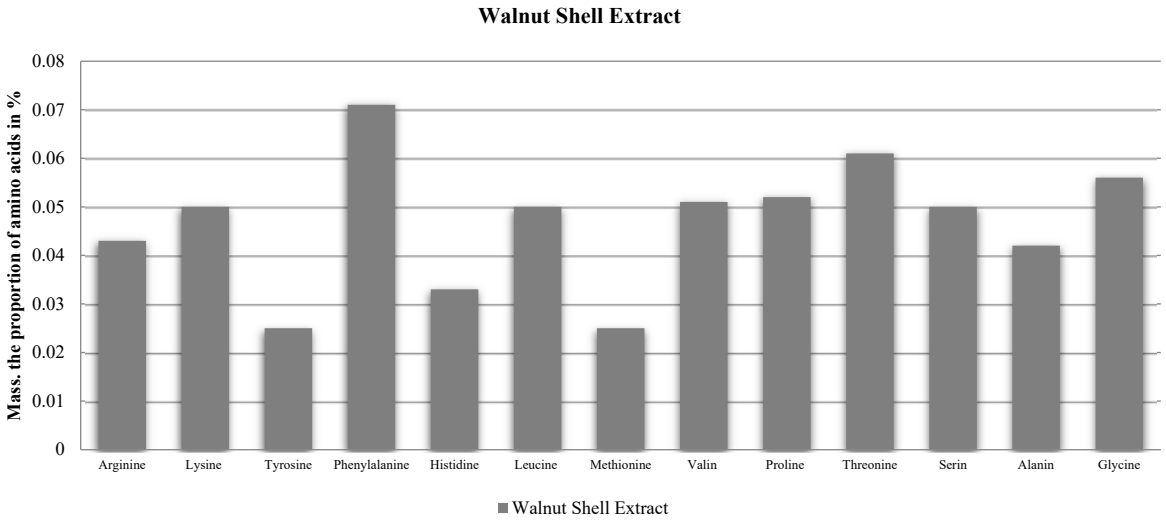

Figure 8 The content of amino acids in the walnut shell extract.

As can be noticed from the diagram, walnut shell extract shows a high proportion of amino acids, indicating their importance in food production. They are rich in essential amino acids not synthesized in the human body **[43]**, **[44]**, **[45]**. The proportion of fat-soluble and water-soluble antioxidants in the walnut shell extract was tested (Figure 9).

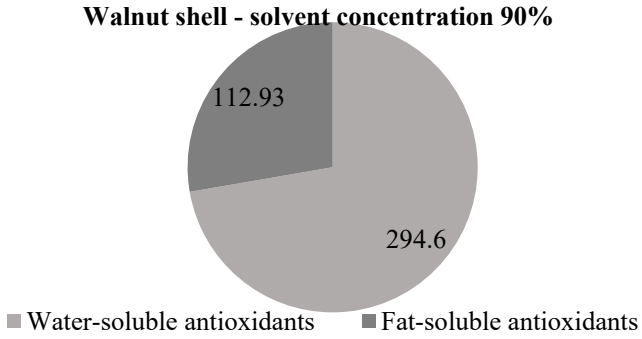

Figure 9 The content of water-soluble and fat-soluble antioxidants.

In terms of the proportion of fat-soluble and water-soluble antioxidants in the walnut shell extract, it was found that it contains 2 times more water-soluble antioxidants. Regarding the fat-soluble antioxidants, our extract contains a small amount of vitamin E.

The walnut shell extract contains vitamins C and E, minerals like iodine, iron, zinc, amino acids, flavonoids, catechin and quercetin **[46]**, **[47]**, **[48]**.

Table 2 presents the recipe for preparing a prophylactic product using walnut extract.

Table 2 Recipe for the preparation of a prophylactic product using walnut shell extract.

Compound	Weight, g
Active substances	
Walnut shell extract	77
Vitamin A	0.225
Iodine	0.005
Fructose (syrup)	20
Excipients	
Lemon acid	2.5
Preservative (sodium benzoate)	0.27
Total	100

The developed prophylactic product is a plant antioxidant whose components increase the body's immunity and defences, protect against the dangerous effects of environmental pollution, give strength, cleanse the body of cholesterol, and prolong youth. This is primarily due to its high content of flavonoids and polyphenols (antioxidants), which, when applied, immediately begin to actively neutralize free radicals, the number of which can be very large in the human body. Even at the initial application stage, the liver begins to be cleansed of toxins and poisons. In this case, the immune system is strongly affected, as if shaken up, allowing you to turn on the body's defences to the maximum.

In alternative medicine, it can be utilized to strengthen the immune system and treat various viral, allergic, and inflammatory diseases. The extract is essential for liver detoxification, strengthening blood vessels, atherosclerosis, and lowering cholesterol. It reduces the risk of developing malignant tumours and stops the reproduction of bacteria harmful to the body **[49]**, **[50]**.

Thus, the active use of the developed complex contributes to the following:

- prevention and replenishment of deficiency of vitamins and macro- and microelements;
- increased immunity and body defences;
- stimulation of the body's resistance to harmful environmental influences and infections;
- reduction of the fragility of capillaries;
- stimulation of the regenerating activity of the body **[51]**, **[52]**.

Organoleptic properties and tasting tests were evaluated following a 6-point system. They demonstrated that the resulting product has a balanced mild taste, a pleasant aroma characteristic of raw materials, and astringency. The bright brown colour is due to the content of catechins and quercetin in the raw material (Figure 10).

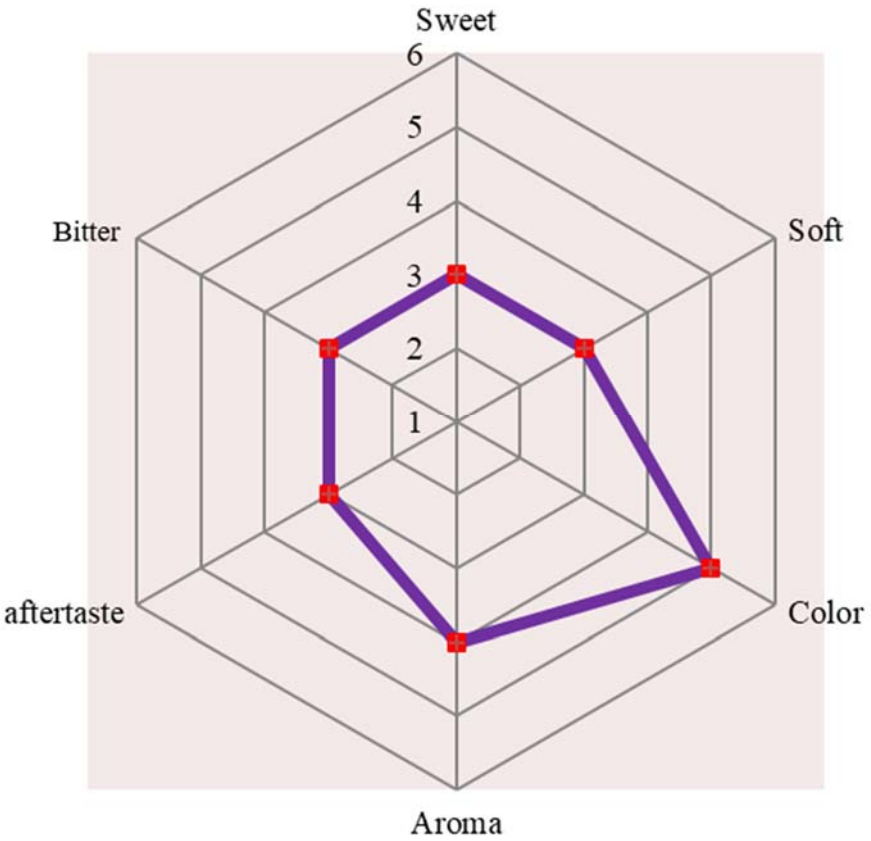

Figure 10 Profilogram of organoleptic and tasting properties of the product.

It is recommended to take the developed product for prophylactic purposes orally in the following amount: 10 ml of the product per 100 ml of water, 1-2 times a day for 21 days. The required product amount was calculated from the daily intake of vitamins, minerals, and flavonoids.

CONCLUSION

As a result of the work, an extract was obtained by extraction with a water-alcohol solvent of various concentrations from a walnut shell. The synthetic antioxidants at concentrations ranging from 100 to 500 μg/ml. Specifically, at a concentration of 100 μg/ml, the walnut shell extract exhibited an antioxidant activity of 75.3%, while the synthetic antioxidants had an activity of 58.2%. At a concentration of 500 μg/ml, the walnut shell extract had an antioxidant activity of 90.1%, while the synthetic antioxidants had an activity of 72.5%. Walnut shell extract has been proven to contain major sources of minerals, vitamins, essential amino acids, quercetin and catechins beneficial to the human body. The resulting product for prophylactic use in the ratio: 10 ml per 100 ml of water and must be taken 1-2 times a day for 21 days. The developed prophylactic product is a plant antioxidant whose components increase the body's immunity and defences, protect against the dangerous effects of environmental pollution, give strength, cleanse the body of cholesterol, and prolong youth. The obtained results demonstrated the potential of walnut shell extract as an economical source of antioxidant agents for the food industry.

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The authors declare no conflict of interest.

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

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Justification and microbiota compositions development for the fermentation of raw meat

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ABSTRACT

In the production of fermented meat products, microorganisms of various taxonomic groups play an extremely important role, namely in the formation of specific taste, aroma, colour, and consistency. Both fermentative and spontaneous microflora take part in the components' transformation of meat raw materials during the maturation of such products, and the course of this process depends on the metabolic activity of the strains. In accordance, this article's purpose is to select microbiota compositions (lactic acid bacteria and coagulase-negative cocci) for the fermentation of meat raw materials. So, as a result of the research, 4 compositions were selected, two of which are lactic acid bacteria with micrococci (No. 2, 3) and two lactic acid bacteria with staphylococci (No. 1, 6). They were characterized by the high productivity of each of the components of the leavening composition, in particular, it was established that the number of MKB increased – by 4.3-6.5 times, and micrococci and staphylococci – by 7.7-28.6 times, respectively. For these compositions, mutual stimulation of the components was observed, contributing to the active microorganisms' development and their biochemical activity. Fermentation compositions No. 1, 6, 4, and 6 had the highest nitrite-reducing activity, and a high level of proteolysis characterized compositions No. 1, 2, 3, and 4. According to the results of determining the antagonistic activity against opportunistic and pathogenic microorganisms, it was established that the investigated compositions exhibit antagonistic activity against both gram-negative and gram-positive microorganisms.

Keywords: microbiota, fermentation, raw meat, leaven cultures, nitrite-reducing activity

INTRODUCTION

Traditional fermented meat products are very popular and have been consumed for many years because of their unique taste **[1]**. These products are usually fermented by spontaneous microbiota, usually do not pose a health hazard, and are currently carried out under controlled conditions with the addition of starter cultures. It is known that meat starters consist of a combination of lactic acid bacteria and coagulase-negative staphylococci. Thanks to such combinations, the technological efficiency increases and the range of desired properties of bacterial preparations expands **[2]**. The participation of lactic acid bacteria is related to acid and aroma formation, under the action of their proteolytic activity, proteins are split into free amino acids which are important components, forming a pleasant taste and aroma of sausages **[3]**. They are responsible for color formation and hygienic safety of meat products **[4]**. An important role is also given to micrococci or staphylococci, which provide a stable color, reduce the content of residual sodium nitrite, slow down the oxidation and rancidity of fats and lead to the formation of aromatic compounds **[5]**, **[6]**. To create leaven for the production of dry sausages, the authors **[7]** recommend using *Lactobacillus sakei*, *Pediococcus acidilactici*, and **[8]** such types of staphylococci

Staphylococcus equorum, Staphylococcus saprophyticus Staphylococcus xylosus and *Staphylococcus carnosus.* Landeta et al. **[12]** recommend two strains of *Staphylococcus carnosus* and *Staphylococcus equorum* with the highest nitrate reductase and proteolytic activity to create potential leaven for the production of meat products **[9]**. A coagulase-negative *Kocuria rhizophila* culture was selected from Nuodeng ham, which is recommended as a potential leaven culture for faster and safer meat fermentation **[10]**. Researchers **[11]** showed the effectiveness of using microbiota *Lactobacillus plantarum* MSZ2 and *Staphylococcus xylosus* YCC3 to improve the taste, quality and duration of storage of fermented sausages. To suppress the formation of biogenic amines, for example, *Lactobacillus* spp. which reduces nitrite residues and suppresses the accumulation of biogenic amines. It is shown that the L. curvatus strain demonstrates optimal performance in suppressing the formation of N-nitrosamine in the production of Harbin dry sausages **[12]**. Lipolytic catalase-negative cocci play an important role in releasing fatty acid precursors and improving the sensory profile of meat **[13]**. The authors **[14]** confirm that the use of a mixture of the *Lactobacillus sakei* strain and the *Staphylococcus equorum, Staphylococcus epidermidis* or *Staphylococcus saprophyticus* strain in the production of sausages makes it possible to obtain homogeneous products without giving up the desired typical characteristics obtained in non-industrial production. During meat fermentation, leaven cultures, mainly lactic acid bacteria and coagulase-negative staphylococci, are often used to standardize product properties, reduce ripening time, and improve product safety nitrate reductase activity of catalazoneative cocci is responsible for the typical stabilized color of dried meat due to the formation of nitroso myoglobin. The activity of nitrate reductase and catalase of cocci also provides protection against severe oxidation of lipids and proteins, which leads to the deterioration of color, texture, taste and nutritional value of meat products **[15]**. Alfaia and others showed the prospects of using selected isolates of coagulase-negative staphylococci *Staphylococcus xylosus*, *Staphylococcus equorum* and *Staphylococcus carnosus*, and lactic acid bacteria *Lactobacillus curvatus*, *Lactobacillus plantarum* and *Lactobacillus sakei*, based on their production, to avoid the formation of biogenic amines in meat products, as well as to ensure special organoleptic characteristics of meat products and bioprotection against pathogen **[16]**. *K. varians (Micrococcus varians)* is used as a leaven to improve the sensory profile of fermented meat and reduce the formation of biogenic amines **[17]**. The relationship between the presence of interaction between beneficial strains of *Lactobacillus sakei* and coagulase-negative cocci *Staphylococcus xylosus* and *Kocuria varians* and the strength of technical characteristics such as proteolysis has been determined. In work **[18]**, it was found that proteolytic *K. varians* affects the amino acid profile, thus potentially enhancing the sensory properties of the meat product, and the mixture of *K. varians* and *Lactobacillus acidophilus* strains brought desirable changes in the amino acid profile and sensory characteristics.

Therefore, the use of leaven cultures in the production of fermented meat products brings desirable biochemical changes, resulting in improved sensory properties through flavour development and softening of texture, as well as proteolysis and lipolysis of added cultures, causing a significant improvement in the organoleptic qualities of fermented meat products. To improve the organoleptic, high-quality colour-forming characteristics, lactic acid bacteria alone are not enough, and the contribution of nitrate/nitrite-reducing organisms becomes significant. The natural leavens development is very promising, as it allows to obtain meat products with high sanitary and sensory qualities.

Scientific Hypothesis

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. It is recommended two leavening compositions are based on productivity, nitrite-reducing, proteolytic and antagonistic activities, which include high-tech strains of *L. rhamnosus Kocuria rosea L. casei* and *L. plantarum.*

MATERIAL AND METHODOLOGY

Samples

The test cultures of *E. coli* HISK 240111, *S. aureus* HISK 049065, *P. vulgaris* HISK 160209, *P. aeruginosa* ATCC 27853, and *L. monocytogenes* NCTC 5105 were used in the work.

Chemicals

Distilled water, H_2O (TOV Novokhim, Ukraine).
Sodium chloride, NaCl (TOV Khimlaborreaktiv, Ukraine).
Hydrochloric acid, HCl (TOV Khimlaborreaktiv, Ukraine).
N-(1-naphthyl)-ethylenediamine-dihydrochloride (TOV Khimlaborreaktiv, Ukraine).
Nitrous oxide, NO_2 (AT ZPD, Denmark).
Sodium nitrite, $NaNO_2$ (ATK Ukraine, Ukraine).

Animals, Plants and Biological Materials

From meat products of non-commercial production, strains of the cocci form were extracted and identified, which belonged to the species *S. simulans, S. carnosus, S. xylosus, M. varians, M. roseus* and lactic acid bacilli of the species *L. casei, L. plantarum, L. rhamnosus, L. sakei and L. curvatus*.

Instruments

Ph meter MP 512 ("Ulab").
Petri dishes.
Unico S 2100 spectrophotometer.
Bunsen beaker, (TOV SkyLab).
Conical flask, (TOV SkyLab).
Glass rods, (TOV SkyLab).

Laboratory Methods

The number of cells was determined by the Koch plate method – counting colonies after sowing appropriate dilutions on Petri dishes for catalase-positive cocci with MPA with 6.5% sodium chloride and growing for 72 ±2 h at a temperature of 30 ±1 °C; of lactic acid bacteria from MRS – 72 ±2 h at a temperature of 30 ±1 °C.

Active acidity (pH) was measured potentiometrically using a pH meter MP 512 ("Ulab", Nitrite-reducing activity of the compositions was assessed by the intensity of the color, which was formed by the interaction of nitrite with sulfonamide and N-(1-naphthyl)-ethylenediamine-dihydrochloride in the protein-free filtrate.

The antagonistic activity of the strains was studied by the method of wells on a solid nutrient medium and by co-cultivation with test cultures.

Description of the Experiment

Sample preparation: The most promising strains for starters are those isolated from the local microbiota. At the preliminary stages of the work, from 29 types of meat products of non-commercial production, strains of the cocci form were extracted and identified, which belonged to the species *S. simulans, S. carnosus, S. xylosus, M. varians, M. roseus* and lactic acid bacilli of the species *L. casei, L. plantarum, L. rhamnosus, L. sakei* and *L. Curvatus.*

From these strains, 7 compositions were composed, in particular, 3 compositions with micrococci and 4 with staphylococci (Table 1).

Number of samples analyzed: 7 compositions were composed, in particular, 3 compositions with micrococci and 4 with staphylococci.

Number of repeated analyses: All measurements were performed 3 times.

Number of experiment replication: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: Cultures of staphylococci and micrococci were maintained on mysopeptone agar (MPA), and lactic acid bacteria on MRS medium, kept at a temperature of 4 ±2 °C between cultures. Before the experiments, the cultures were activated by several successive transplants on appropriate nutrient media (MPA, MPB, MRS), with incubation at optimal temperatures for 14-18 hours.

The ability to grow together in the compositions was checked by the indicator of the accumulation of viable cells of each of the composition components under the conditions of cultivation in meat-peptone broth with the addition of 1% glucose (pH 7.0). The amount of introduced inoculum was 5% of the volume of the nutrient medium. The fermentation composition "Lakmik", which included a four-component combination of strains *L. rhamnosus, L. casei, L. plantarum,* and *M. varians*, was taken as a control (Cl). The decomposition of sodium nitrite dynamics by the created compositions in the medium of MPB with the addition of 1% glucose, 3% NaCl and with the initial salt content of $NaNO_2$ (Merck) 60 mg.100 cm^{-3} was studied. A 5% inoculum of microorganisms was added to the medium and cultivated for 17 days at a temperature of 30 °C. Measurements were performed on the 1st, 3rd, 4th, 6th, 7th, 10th, and 17th day of the study.

It was determined by taking a 0.5 cm^3 aliquot of the medium with the composition and treating this sample with 0.5 cm^3 (10 $g.dm^{-3}$) of sulfonamide in 3 $mol.dm^{-3}$ HCl and 0.5 cm^3 (0.2 $g.dm^{-3}$) N-(1-naphthyl)-ethylenediamine dihydrochloride. After 20 minutes, the solution was diluted to 4.5 cm^3 with deionized water and the absorbance (540 nm) was measured using a Unico S 2100 spectrophotometer. To calculate the amount of NO_2- contained in the sample, the standard curve was prepared in the same way as for the sample, but using 0.5 cm^3 aliquots of $NaNO_2$ standard solutions (containing 0 to 140 $mmol.dm^{-3}$ NO_2).

The control was MPB medium with 1% glucose, 3% NaCl and 60 mg.100 cm^{-3} $NaNO_2$ (K). The proteolytic activity of the compositions was evaluated by the increase of free amino acids in the culture medium according to the modified ninhydrin method 5% of the inoculum was added to the meat peptone broth with 1% glucose and 3% NaCl. After incubation at 30 °C for 4 and 7 days, the compositions were centrifuged to remove bacterial cells before analysis. The total amount of free amino acids were measured. Amino acid content was determined by the

ninhydrin colourimetric method using a Unico S 2100 spectrophotometer, using glutamic acid as a standard. The medium without the addition of bacteria was used as a control.

Table 1 Characteristics of the created leavening compositions.

			The relationship between the strains	Active acidity, units pH
1	*L. coryniformis* 3401+ *L. casei* 3322+ *L. plantarum* 3201+ *S. saprophyticus* 5302	4	1:1:1:1	3.0 ±0.07
2	*L. casei* 3302+ *L. rhamnosus* 3303+ *L. rhamnosus* 3305+ *M. roseus* 5401	5	1:1:1:1	3.80 ±0.05
3	*L. casei* 3321+ *L. casei* 3322+ *L. plantarum* 3201+ *K. roseus* 5400	6	1:1:1:1	3.55 ±0.07
4	*L. coryniformis* 3401+ *L. casei* 3322+ *S. saprophyticus* 5302	7	1:1:1	3.60 ±0.05
5	*L. rhamnosus* 3308+ *L. tolerans* 3340+ *L. rhamnosus* 3305+ *S. simulans* 5301	8	1:1:1:1	3.60 ±0.05
6	*L. rhamnosus* 3308+ *L. tolerans* 3340+ *S. simulans* 5301	9	1:1:1	3.75 ±0.06
7	*L. rhamnosus* 3308+ *L. tolerans* 3340+ *L. plantarum* 3201+ *K. varians* 5200	10	1:1:1:1	3.80 ±0.07
Kl	*L. casei* 3302+ *L. rhamnosus* 3303+ *L. plantarum* 3200+ *M. varians* 5200	14	1:1:1:1	3.80 ±0.06

Statistical Analysis

The STATISTICA Microsoft Excel editor processed experimental data using mathematical statistics methods. The accuracyof 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

The main criteria for evaluating the prospects of leavening compositions for fermentation of meat raw materials were the number of cells of each of the components of the composition, nitrite-reducing and proteolytic activity. It was established that after 18 hours of cultivation, all created leavening compositions reduced active acidity by 45-50% relative to the medium's initial value (pH 7.0) (Table 1). An active decrease in acidity to pH 3.55-3.60 in the culture liquid negatively affected the viability of micrococci (MC), while staphylococci (ST) were insensitive to this factor and had an increase in numbers in the range of 8.9-10.9 times (Figure 1).

In all created compositions, lactobacilli increased for 24 hours of growth, and at the end of cultivation in MPB, the increase continued in compositions No. 1, 2, 3, 7, by 4.3-6.5 times. Composition No. 5 was characterized by a decrease in the number of lactic acid bacteria by 1.3 times the initial content. In composition No. 2, despite the low pH level, an increase of micrococci by 3.7-28.6 times the initial content was observed. The growth of micrococci for 72 hours of cultivation in the remaining compositions decreased by 1.2-2 times.

In compositions with staphylococci #5, 6 their increase by 3 times to the initial content was observed. And in compositions No. 1, and 7, suppression of staphylococci by lactobacilli was observed, their number remained at the level of the initial number or decreased by 1.5 times (Figure 1).

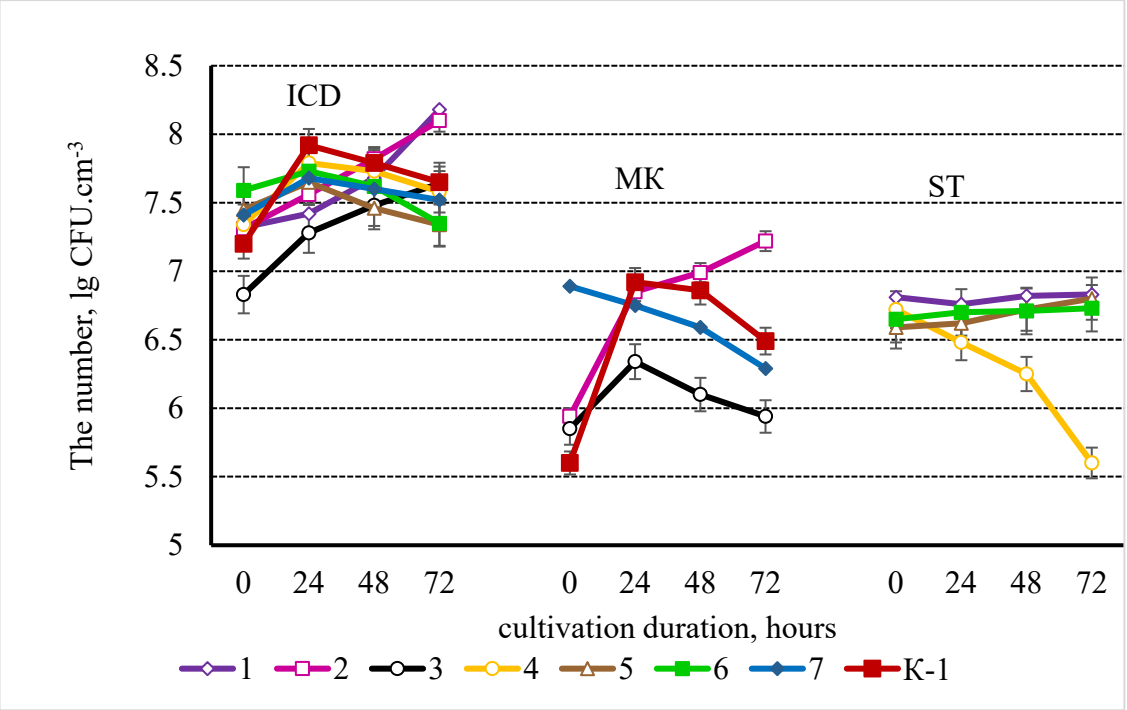
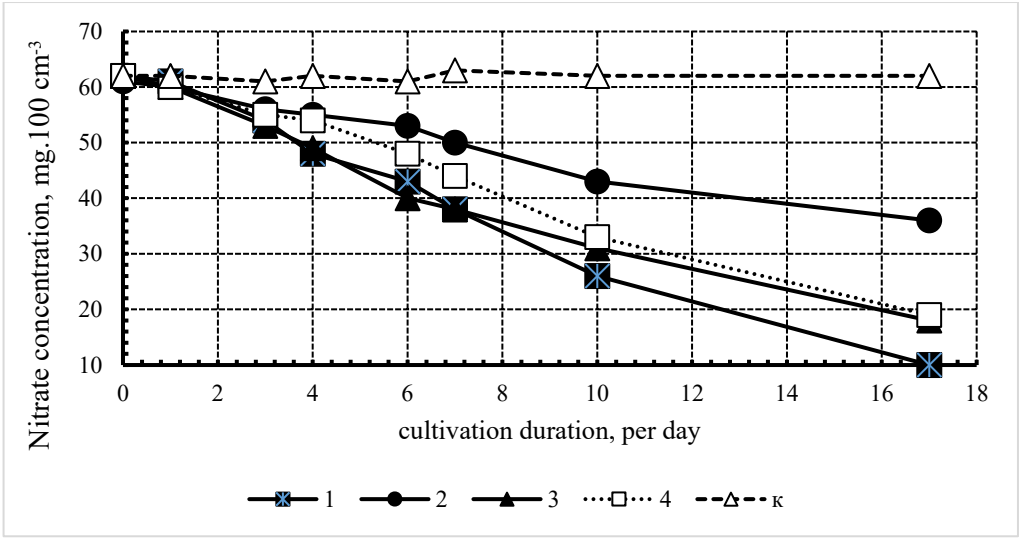


Figure 1 Dynamics of the number of leavening compositions during joint cultivation (ICD – lactic acid bacteria, MK – micrococci, ST – staphylococci).

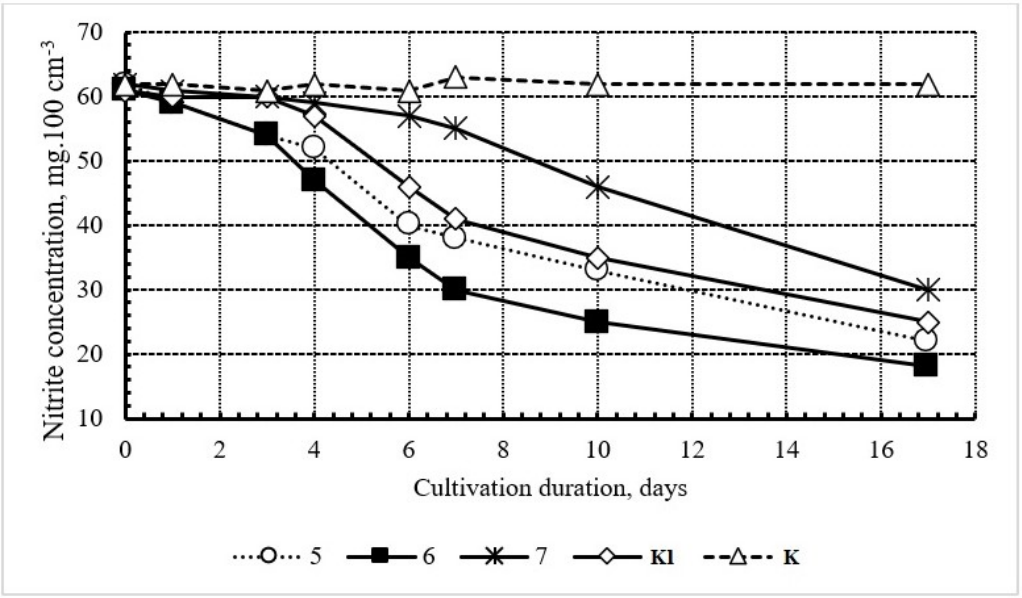
Thus, the compositions of lactic acid bacteria with micrococci (No. 2, 3,) and with staphylococci (No. 1, 6) were the most stable in terms of number, in which the number of microorganisms increased: ICD – by 4.3-6.5 times and micrococci and staphylococci – by 7.7-28.6 times. For these compositions, mutual stimulation of the components was observed, which contributed to the active microorganisms' development. Similar results were obtained and described in many subsequent scientific works, the use of different types of compositions of lactic bacteria **[19]**, research on the development of microorganisms **[20]** and the use of different compositions of lactic acid bacteria **[21]**.

Nitrite-reducing activity: The dynamics of the decomposition of sodium nitrite by the created compositions were studied. It was established that the studied compositions actively reduced the content of nitrites in the culture medium – by 41-83% from the initial. The most active of them were compositions No. 1-6 (Figure 2 a, b). Compositions of lactic acid bacteria with staphylococci (No. 1, 4, 5, 6) more intensively reduced the nitrite content by 67-83%, and compositions with micrococci (No. 2, 3) by 70-71% compared to control K1 (60%). Fermenting composition No. 7 – by 50% was characterized by the lowest nitrite-reducing activity.

In the control, the nitrite content remained constant throughout the experiment (curve K). At the end of fermentation, the residual concentration of sodium nitrite in the culture liquid inoculated with the compositions was 10.5-36.3) mg.100 cm^{-3}.



a)



b)

Figure 2 Dynamics of changes in the content of sodium nitrite during the cultivation of leavening compositions No. 1-4 (a), No. 5-7 (b). K is a medium with sodium nitrite.

The composition of the compositions is given in Table 1. Fermentation compositions No. 1, 3, 4, and 6, which reduced nitrite in the culture medium by 70-83%, are promising for the fermentation of meat raw materials.

Proteolytic activity: The proteolytic activity of the compositions was assessed by the level of increase of free amino acids in the culture medium (Figures 3 and 4). On the 4th day of cultivation in the presence of leavening compositions No. 1, 3, and 4, the dynamics of an increase in the level of cyclic amino acids (C) by 0.6-9.6% compared to the control, at an initial level of 486.1 μg.cm^{-3}, can be observed in the rest of the compositions the consumption of these amino acids is up to 4.2%. Further, on the 7th day of fermentation, an intense decrease in cyclic amino acids was observed in all compositions ranging from 3.5 to 26.1%, except for No. 7, where there was an increase of 22% compared to the initial content of cyclic amino acids (Figure. 3).

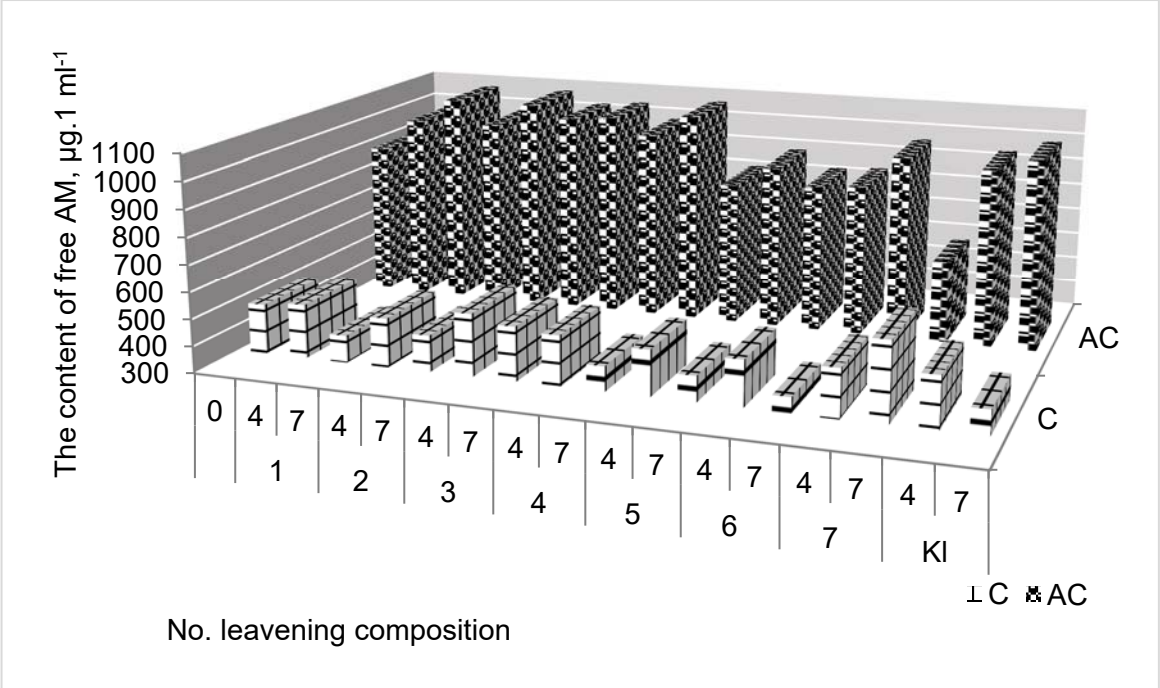


Figure 3 Dynamics of the content of free amino acids during the cultivation of leavening compositions No. 1-7 and Kl. The composition of the compositions is indicated in Table 1. 0, 4, 7 – duration of cultivation, day; C – cyclic amino acids, AC — acyclic amino acids; K is the initial amount of free amino acids.

At the same time, in the environment in the presence of compositions No. 1-5, the dynamics of the accumulation of acyclic amino acids (AC) by 4.2-29.7% was observed throughout the entire fermentation period, compared to the initial content AC = 882.2 µg.cm^{-3}.

On the 4th day, in the medium with all compositions, there was an increase in acyclic amino acids from 9.8 to 19.6%, except for No. 5 and 6, where there was a decrease in AC amino acids by 4.5% and 2.8%, respectively. Whereas on the 7th day in the medium with compositions No. 6, and 7, these amino acids decreased more by 2.35-29.7%, compared to the 4th day of cultivation (Figure 3).

Among the leavening compositions, No. 1-4 were characterized by a high level of proteolysis compared to control KL. The total amount of free amino acids increased the most in variants with micrococci (No. 2, 3) by 15%. In the rest of the compositions, this indicator ranged from 8 to 10% (see Figure 4).

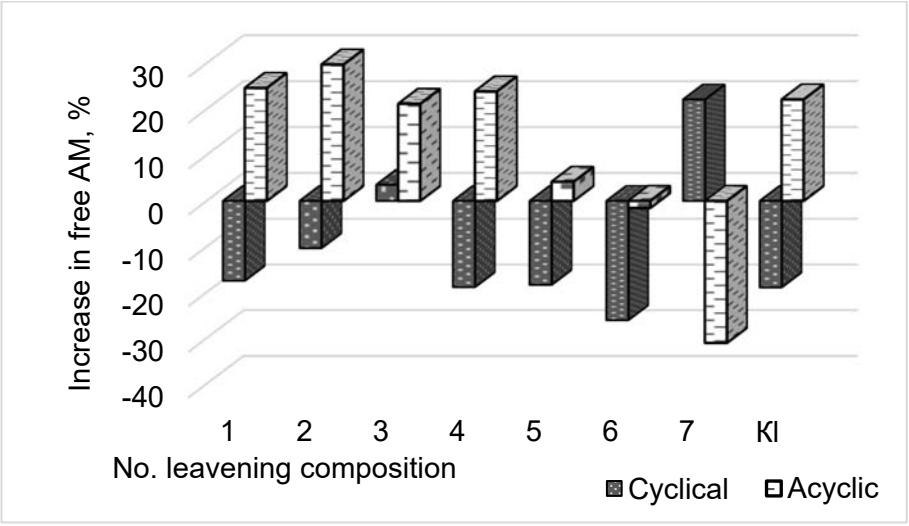


Figure 4 Increase in the content of free amino acids (cyclic (C) and acyclic (AC)) during cultivation of leavening compositions. The numbers of the compositions are indicated in the Table. 1.

Therefore, on the 7th day of cultivation, the composition (No. 2) reduced the level of cyclic amino acids (C) by 10%, relative to the control. Also, in the medium inoculated with this composition, the accumulation of acyclic

amino acids (AC) was 30% higher compared to the control. In the presence of composition No. 3, the amount of both cyclic and acyclic amino acids increased by 3.5% and 22%, respectively. For composition No. 7, the level of acyclic amino acids decreased by 31%, while cyclic amino acids increased by 22% (Figure 4).

So, as a result of the research, 4 compositions were selected, two of which are lactic acid bacteria with micrococci (No. 2, 3) and two lactic acid bacteria with staphylococci (No. 1, 6). They were characterized by the high productivity of each of the components of the leavening composition, in particular, it was established that the number of MKB increased – by 4.3-6.5 times, and micrococci and staphylococci – by 7.7-28.6 times, respectively. For these compositions, mutual stimulation of the components was observed, which contributed to the active microorganism development and the manifestation of their biochemical activity. Fermentation compositions No. 1, 6, 4, and 6 had the highest nitrite-reducing activity, and compositions No. 1, 2, 3, and 4 were characterized by a high level of proteolysis.

Antagonistic activity of compositions: The study of the antagonistic activity of compositions of strains of lactic acid bacteria and cocci about the spontaneous microbiota of meat is a mandatory condition for the selection of cultures of bacterial preparations for the production of fermented meat products, as evidenced by the results described in scientific works **[22]**, **[23]**. The results of the study of antagonistic activity against pathogenic and opportunistic microbiota are presented in Table 2.

Table 2 The composition of antagonistic activity is based on microorganisms of different taxonomic groups.

No. of composition	Types of microorganisms in the composition	Test-cultures				
		P. vulgaris **HISK 160209**	***E. coli*** **HISK 240111**	***P. aeruginosa*** **ATCC 27853**	***L. monocytogenes*** **NCTC 5105**	***S. aureus*** **HISK 049065**
1	*L. coryniformis* 3401+ *L. casei* 3322+ *L. plantarum* 3201 + *S. saprophyticus* 5302	14 ±2	10 ±2	5 ±1	12±1	0
2	*L. casei* 3302+ *L. rhamnosus* 3303+ *L. rhamnosus* 3305+ *M. roseus* 5401	11 ±1	9 ±2	11 ±2	0	12 ±2
3	*L. casei* 3321+ *L. casei 3322*+ *L. plantarum* 3201+ *K. roseus* 5400	16 ±1	14 ±1	9 ±1	0	18 ±2
4	*L. coryniformis* 3401+ *L. casei* 3322+ *S. saprophyticus* 5302	12 ±1	0	0	2 ±1	14 ±2
5	*L. rhamnosus* 3308+ *L. tolerans* 3340 + *L. rhamnosus* 3305+ *S. simulans* 5301	14 ±2	0	12 ±1	18 ±1	0
6	*L. rhamnosus* 3308+ *L. tolerans* 3340+ *S. simulans* 5301	25 ±1	12 ±2	14 ±1	0	16 ±1
7	*L. rhamnosus* 3308+ *L. tolerans* 3340 + *L. plantarum* 3201+ *M. varians* 5200	10 ±1	12 ±1	16 ±1	12 ±1	0
Kl	*L. casei* 3302+ *L. rhamnosus* 3303+ *L. plantarum* 3200+ *M. varians* 5200	11 ±2	13 ±1	5 ±1	0	12 ±2

The investigated compositions have a different degree of antagonistic activity, as evidenced by the size of the growth retardation zones of the test cultures, the size of which varied from 2 to 25 mm.

Of the 7 fermentation compositions studied, antagonistic activity against *P. vulgaris* HISK 160209 was found in all created compositions, *S. aureus* HISK 049065 – 58%, *E. coli* HISK 240111 – 72%, *P. aeruginosa* ATCC 27853 – 86%, *Listeria monocytogenes* NCTC 5105 – 29%.

For compositions No. 1, 3, 5, 7, the maximum growth retardation zone for *L. monocytogenes* reached 12-18 mm, *P. vulgaris* 10-14 mm, *E. coli* 12-14 mm, *S. aureus* 14-18 mm, and *P. aeruginosa* 5-16 mm.

Thus, according to technological parameters the productivity, nitrite-reducing, proteolytic and antagonistic activities, of two leavening compositions No. 3 and No. 7 were selected as promising for fermentation of meat raw materials, which include strains of microorganisms of the species *Kocuria rosea, K. varians, Lactobacillus rhamnosus*, *L. casei, L. plantarum* and *L. tolerans.*

In the production of fermented meat products, microorganisms of various taxonomic groups play an extremely important role, namely in the formation of specific taste, aroma, color, and consistency **[24]**. Both fermentative and spontaneous microflora take part in the components' transformation of meat raw materials during the maturation of such products, and the course of this process depends on the metabolic activity of the strains **[25]**, **[26]**.

According to their composition, leavening preparations can be single- or multi-component. The latter contains several strains of one or more genera, particularly *Lactobacillus, Staphylococcus, Pediococcus, Kocuria*, etc.

A necessary condition for creating stable symbiotic compositions is the combination of lactic acid bacteria, which produce organic acids and bacteriocins, and coagulase-negative cocci, which form specific taste-aromatic compounds and shape the color of the finished product **[27]**.

The studied compositions were characterized by high productivity of each of the components of the leavening composition, in particular, it was established that the number of ICD increased by 4.3-6.5 times, and micrococci and staphylococci by 7.7-28.6 times, respectively. For these compositions, mutual stimulation of the components was observed, which contributed to the microorganism's active development and the manifestation of their biochemical activity.

Expressed nitrite-reducing activity is characteristic of certain strains of staphylococci. Nitrites and nitrates have long been used in producing fermented meat products: on the one hand, they have a positive effect on color, taste and aroma, stability during storage, on the other – in an acidic environment, they can be precursors to the formation of nitrosamines. The lack of substances that are functionally able to replace the use of these compounds prompts the search for cultures with high nitrite reductase activity **[28]**, **[29]**, **[30]**.

The dynamics of the decomposition of sodium nitrite at its initial content of 60 mg.100 cm^{-3} was studied. The concentration of sodium nitrite used in this study was 6 times higher than recommended by the recipe for fermented sausages.

Under such conditions, high nitrite-reducing activity was characteristic of leavening compositions No. 1, 4, and 6, on the 17^{th} day of cultivation, the compositions actively reduced the content of nitrites in the culture medium – by 41-83% from the initial. These compositions had higher nitrite-reducing activity than is known from the literature data **[31]**, **[32]**, **[33]**. The microorganism's participation in the process of forming an aromatic bouquet is associated with the formation of certain amino acids, volatile fatty acids, and aromatic compounds during their vital activity **[34]**, **[35]**, **[36]**.

Evaluation of proteolytic activity made it possible to select the most active compositions, which were tested by the level of increase in free amino acids after 7 days of cultivation in MPB enriched with glucose and salt **[37]**, **[38]**. It was determined that compositions No. 1, 2, 3, and 4 were characterized by a high level of proteolysis, in the medium, there was an increase in the content of acyclic amino acids and an intensive decrease in cyclic amino acids, respectively, by 4.2-29.7% and 3.5 to 26.1%. Undoubtedly, antagonistic activity against opportunistic and pathogenic microorganisms is a desirable trait for the selection of cultures for the fermentation of meat raw materials **[39]**, **[40]**. *L. plantarum* synthesizes some compounds: 3-hydroxy-fatty acids, antifungal cyclic peptides, phenyl-lactic acid, and a mixture of substances with a low molecular weight similar to lactic acid. Most of these substances are active against moulds, and yeasts, and some also against bacteria, including genera *Listeria* and *Salmonella* **[41]**, **[42]**. According to the results of determining the antagonistic activity against opportunistic and pathogenic microorganisms, it was established that the investigated compositions exhibit antagonistic activity against both gram-negative and gram-positive microorganisms. Thus, all compositions suppressed the growth of test cultures *P. vulgaris*, *E. coli*, *S. aureus*, *P. aeruginosa*, and compositions No. were also characterized by the ability to suppress listeria: the zone of inhibition of *L. monocytogenes* growth was (12 ±1) mm. Our data are consistent with the publications of other researchers **[43]**, **[44]**.

CONCLUSION

Fermentation compositions No. 1, 3, 4, and 6, which reduced nitrite in the culture medium by 70-83%, are promising for the fermentation of meat raw materials. So, as a result of the research, 4 compositions were selected, two of which are lactic acid bacteria with micrococci (No. 2, 3) and two lactic acid bacteria with staphylococci (No. 1, 6). They were characterized by the high productivity of each of the components of the leavening composition, in particular, it was established that the number of MKB increased – by 4.3-6.5 times, and micrococci and staphylococci – by 7.7-28.6 times, respectively. For these compositions, mutual stimulation of the components was observed, contributing to the active microorganisms' development and their biochemical activity. Fermentation compositions No. 1, 6, 4, and 6 had the highest nitrite-reducing activity, and a high level of proteolysis characterized compositions No. 1, 2, 3, and 4. Thus, according to technological parameters the productivity, nitrite-reducing, proteolytic and antagonistic activities, of two leavening compositions No. 3 and No. 7 were selected as promising for fermentation of meat raw materials, which include strains of microorganisms of the species *Kocuria rosea, K. varians, Lactobacillus rhamnosus*, *L. casei, L. plantarum* and *L. tolerans.*

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Effects of using composite flour containing wheat flour with different levels of green banana pulp flour on the quality of saj flatbread

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ABSTRACT

There is an increasing trend in formulating food to contain dietary fibers and particularly resistant starch. Saj bread (a type of flatbread baked on a plate placed directly on fire) is a potential candidate to act as a vehicle for delivering resistant starch. This study aimed to investigate the effects of using composite flour containing wheat flour substituted with different levels (0, 5, 10, 15, and 20%) of green banana pulp flour "GBPF" on some physicochemical properties of flour (moisture, ash, wet and dry gluten content, gluten index, falling number, and farinograph parameters) and the quality of saj bread as measured by CIELAB color space, texture (stretchability and texture profile analysis "TPA"), and sensory properties. The texture of the saj bread was monitored during three days of storage. Composite flour moisture content and falling number were unaffected by wheat flour substitution with GBPF, while dry gluten content decreased significantly for composite flour containing 15% or more GBPF. With increasing wheat flour substitution level with GBPF, dough stability decreased. For saj bread color, the L^* and b^* values decreased with increasing substitution levels, while a^* and ΔE^*ab values increased. With increasing substitution levels, the stretchability of bread decreased, and all tested TPA parameters increased. With increasing saj bread storage time, the stretchability of bread decreased, and all TPA parameters increased except cohesiveness which decreased. Using composite flour improved bread taste and odor scores and decreased color acceptability scores. Texture and overall acceptability scores were not affected. In conclusion, GBPF can potentially substitute up to 20% wheat flour without negatively affecting saj bread quality.

Keywords: saj flatbread, green banana pulp flour, texture, color, sensory properties

INTRODUCTION

Dietary fibers increasingly attract researchers' interest **[1]** due to their believed role in the prevention and treatment of several chronic diseases, particularly obesity **[2]**, diabetes **[3]**, and cardiovascular diseases **[4]**. The effectiveness of fibers in promoting health depends on the type of fiber and the amount consumed **[4]**. Resistance starch received great attention among different dietary fibers **[5]**. Resistant starch is the non-digestible fraction of starch that is fermented and converted into short-chain fatty acids in the colon **[6]**. With the worldwide daily consumption of dietary fibers below the recommended dietary allowances **[7]**, there is an increased demand for food to be formulated to contain high dietary fibers and particularly resistant starch. There are five types of resistant starch: physically inaccessible starch (RS1), crystalline starch (RS2), retrograded starch (RS3), modified starch (RS4), and long amylose chain combined with free fatty acids (RS5) **[6]**.

One of the important sources of resistant starch is green (unripe) bananas, particularly RS2 starch **[8]**. However, the hard texture and astringency of the green banana limit consumption, which makes the utilization of its flour (green banana pulp flour (GBPF)) a better approach to increase its consumption through the addition of it to

different foods **[9]**. Depending on the cultivar, the GBPF contains between 80.83-85.5% resistant starch; for instance, the GBPF from Grande Nine cultivar – the primary cultivar in Jordan – contains 80.38% resistant starch **[10]**. Banana is a staple, one of the most cultivated tropical crops **[11]**, **[12]**, **[13]**, which ranked globally fourth after rice, wheat, and corn. The nutraceutical properties of GBPF were recently reviewed in **[14]**. Despite this importance, many bananas are lost as postharvest defects such as miss-shape, off-size, and inappropriate ripening **[5]**, **[12]**. In New Zealand, the waste of bananas represents 3% of total food waste and is ranked second after bread waste **[5]**. A study reported that a third of the produced banana is lost as postharvest waste **[13]**. To solve the waste problem of banana, there is a need to find a processing method to convert this waste into stable food, and this is the third reason after astringency and health properties – that highlight the importance of utilizing the GBPF. The final fourth reason for utilizing GBPF is its functional physicochemical properties, particularly water-holding capacity **[10]**. The addition of GBPF was investigated in different food such as noodles, pasta, cookies, and doughnut **[15]**.

For properly utilizing GBPF, selecting a food product that will serve as a vehicle for delivering it is important. Some researchers highlighted the importance of fortifying high-starch staple food with resistance starch, particularly flatbread **[16]**, **[17]**. The nomination of flatbread comes from its importance; it is the oldest, most consumed stable food worldwide, and its consumption is increasing **[18]**. Flatbread was reported to satisfy the increasing need for increasing food systems sustainability for different reasons discussed in **[19]**, **[20]**. Worldwide, there are 143 types of flatbread, 6 of which are located in Jordan. The classification and processing of flatbreads have been recently reviewed in **[21]**. Saj bread is a type of flatbread baked on a plate placed directly on the fire; this plate may be flat or curved and named "saj," from which the name saj bread is taken **[21]**. Saj flatbread is prepared from simple basic ingredients, using a simple process that does not require ovens, and is popular in Jordan **[21]**, **[22]**. Despite the importance of flatbreads, great attention in literature was given to volume or pan bread in investigating the addition of bioactive ingredients compared to flatbread **[18]**. The addition of GBPF to bread formulation was investigated in pan (sliced) bread **[23]**, **[20]**, **[12]**, **[24]**, **[25]**, **[26]**, **[27]**, **[28]**, chinse steamed bread **[29]**, flatbread **[8]**, and pita flatbread **[30]**. Incorporating GBPF into bread is challenging **[28]** due to the dilution of gluten upon adding it, and from the previous studies, fortifying bread with GBPF will affect its color, flavor, and texture. Therefore, there is a need to investigate the effects of GBPF addition to new types of flatbread. This research aimed to investigate the effects of using composite flour containing wheat flour containing different levels of GBPF on some physical properties, color, texture, and sensory properties of saj flatbread. Additionally, to study the impact of storage for three days on the texture of saj bread.

Scientific Hypothesis

This research aimed to investigate the effects of using composite flour containing wheat flour with different levels of GBPF (0, 5, 10, 15, and 20%) on some quality aspects of the composite flour and saj flatbread. Additionally, the research aimed to determine the maximum substitution level of wheat flour with GBPF without negatively affecting saj bread quality during storage for up to three days. It is expected that substituting wheat flour with different GBPF will at least not negatively affect flour and bread quality. It is expected to replace 20% of wheat flour with GBPF without compromising saj flatbread quality. It is expected that storage time has a negative impact on saj bread texture.

MATERIAL AND METHODOLOGY

Samples

Samples of GBPF were packed in polyethylene plastic bags and kept at room temperature until use. Samples of composite flour were prepared and tested immediately. For the preparation of flat saj bread, composite flour samples were prepared before the preparation process immediately. Samples of flat saj bread were cooled and packed in polyethylene plastic bags and stored at room temperature (25 °C).

Animals, Plants and Biological Materials

GBPF were obtained from low-grade (small size and miss-shaped) green (stage-1 maturity stage) Grand Nain banana. Wheat flour was obtained from the local market (Mawahad, southern Amman mills, Jordan).

Instruments

The following instruments were used: halogen moisture analyzer (Mettler-Toledo, USA), Glutomatic system (Perten, Sweden), Falling number apparatus (Perten, Sweden), muffle furnace (Thermo Scientific, USA), texture analyzer (Perten, Sweden), farinograph system (Perten, Sweden), and non-contact spectrophotometer (X-rite 450, UK), saj bread gas oven (locally made in Jordan).

Laboratory Methods

Composite flour: Composite flours made from wheat flour substituted with different proportions of GBPF were evaluated by measuring moisture, gluten (wet and dry gluten and gluten index), falling number, ash, and

farinograph parameters. Moisture content was determined using a halogen moisture analyzer according to manufacturer recommendations. Wet gluten, dry gluten, and gluten index were determined according to the American Association of Cereal Chemists (AACC) number 38-12A **[31]**. The falling number was determined according to AACC method number 56-8104 **[32]**. Ash was determined according to AACC method number 08-01.01 **[33]**. Faringraph-tested parameters were determined using the AACC method 54-21.02 **[34]**. According to the manufacturer's recommendation, the color was measured using a non-contact spectrophotometer.

Saj bread: Saj bread prepared from different types of composite flour was evaluated by measuring texture (stretchability and TPA), color, and sensory properties. Saj bread stretchability was measured using a texture analyzer using the Perten instruments method 08-05.02 **[35]**. This method uses a stainless steel cylinder probe (18 mm in diameter) and a heavy-duty stand equipped with two 50 mm hole inserts. Using TexCalc software (Perten, Sweden), the texture analyzer was programmed according to the following setting parameters: single-cycle compression, 3.0 mm for sample height, 8.0 mm for starting distance from the sample, 30 mm for compression, 6 mm/s for initial speed, 1.7 mm/s for test speed, and 20 g for trigger force. The texture analyzer was equipped with a 5 kg load cell. After the test initiation, the software drew a curve between the force and distance/time, from which two parameters were determined: breakpoint and stretchability (Figure 1). The breakpoint is the force (g) needed to puncture the sample, while stretchability is the distance (mm) to the maximum force.

Bread Texture Profile Analysis (TPA) was measured using the Perten instruments method 03-04.02 **[36]**. This method used a stainless steel cylinder probe with a 35 mm diameter. The texture analyzer was operated using the following program: double-cycle compression, 3.0 mm for sample height, 50% for compression, 12 s for a pause between cycles, 5 mm/s for initial, test, and retract speed, and 20g for trigger force. The texture analyzer was equipped with a 5 kg load cell. The software (TexCalc, Perten, Sweden) drew a curve between force and distance/time from which the following parameters were determined: hardness, resilience, springiness, cohesiveness, and chewiness. From Figure 2, hardness is force A; resilience is Area a_2/ Area a_1; springiness distance y/ distance x; cohesiveness is Area B/ Area A; chewiness is calculated by multiplying hardness, springiness, and cohesiveness together.

Sensory analysis of saj flatbread samples was performed using a 9-point hedonic scale, where nine denotes like extremely, and 1 denotes dislike extremely **[23]**. Twenty-five untrained panelists evaluated bread samples.

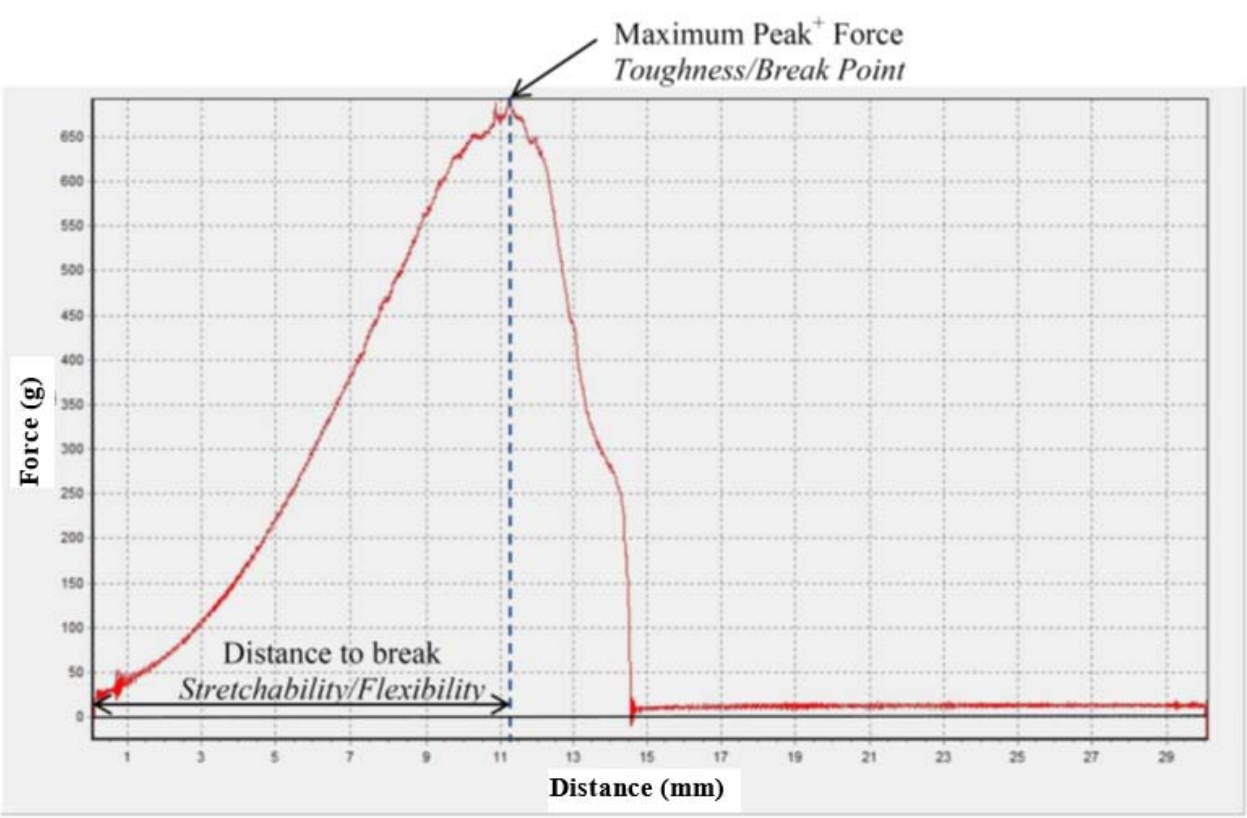

Figure 1 Sample stretchability curve. Source: Perten methods.

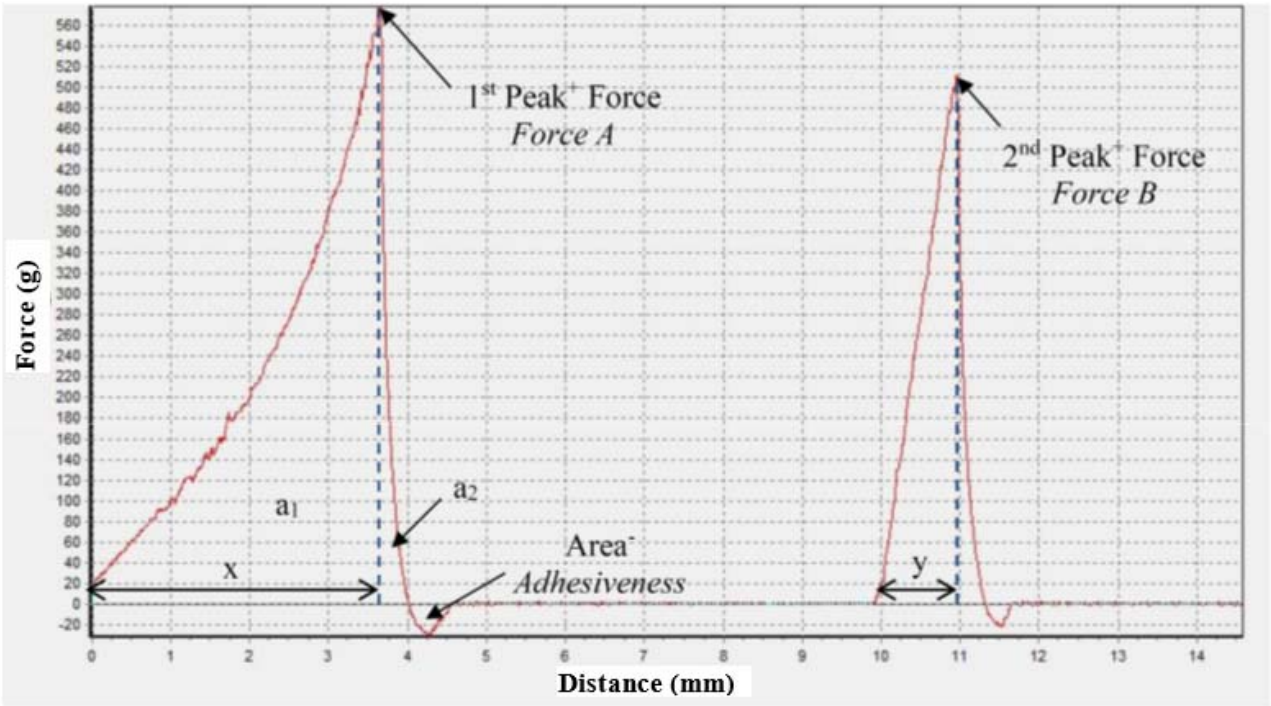

Figure 2 Sample TPA curve. Source: Perten methods

Description of the Experiment

Sample preparation: No special sample preparation was used for tests to evaluate the composite flour. For bread evaluation, each treatment run gave eight loaves. Four were assigned to measure TBA and color, and the others were assigned to measure stretchability. Each bread loaf was quartered into four equal parts; each part was assigned to be tested at zero, one, two, and three days of storage. A stainless steel cutter was used to cut three circular discs from each bread quart for TPA. The three circular bread discs were stacked together and tested for color first and then for TPA. As a total, four readings were made for stretchability, TPA, and color CIELAB values. These readings were averaged for statical analysis. The whole experiment was repeated three times. The color and sensory evaluation were performed at zero storage time, while texture analysis was performed throughout three days of storage.

Number of samples analyzed: The number of samples differed according to the test performed. For composite flour tests, 15 samples (five types of composite flour with three replicates) were analyzed. 60 samples (five composite flour*four storage times*three replicates) were analyzed for bread texture measurement tests. For bread color measurement, 15 samples were analyzed. For sensory analysis. Five samples (five types of composite flour) were analyzed

Number of repeated analyses: Sample measurements were duplicated for composite flour tests. For texture and color measurements, each sample was tested four times. For sensory analysis, each sample was tested by 25 panelists.

Number of experiment replication: Three.

Design of the experiment: The experiment had five main phases. In the first phase, GBPF was prepared according to the steps described in **[30]**. In the second phase, five composite flour were prepared by substituting wheat flour with different levels of GBPF: 0, 5, 10, 50, and 20%. The composite flour was evaluated in the third phase by measuring moisture content, ash, gluten, falling number, and farinograph parameters. In the fourth phase, five types of saj bread were prepared. The formulas used to prepare saj bread are shown in Table 1. All dry ingredients were weighed and mixed together first, then worm water (35 °C) and oil were added. The mixture was kneaded for about 7 mins. The dough was covered with plastic film and proofed for 1 hr at 35 °C. After that, the dough was divided and shaped into balls (125 g each). The dough balls were flattened using a rolling pin to reach the desired diameter (30 cm). The flattened dough was baked using a flat metal plate heated with a gas stove to reach 200 °C. The plate was preheated for 15 mins before baking to equilibrate the temperature. Each side of the dough was heated to about 0.5 min. The backed bread was allowed to cool, then packed in plastic bags and stored at room temperature. In the fifth phase, bread made with different types of composite flour was evaluated; bread color and sensory properties were evaluated directly after preparation, while texture measurements were performed directly after backing and after 1, 2, and 3 days of storage.

Statistical Analysis

Data were analyzed using Minitab software (19.2020.1, Minitab Inc., USA). A completely randomized design was used to analyze the results of the composite flour evaluation tests and bread color and sensory evaluation results. A factorial design was used to investigate the main effects of different types of composite flour, storage time, and their interaction effect on flatbread texture (stretchability and TPA). Means separation was performed using Tukey's test with $p \leq 0.05$.

Table 1 Composite flour formula used in the preparation of saj flatbread.

Ingredients	Weight of ingredients (g)				
	Wheat flour substitution level with GBPF (%)				
	0 "Control"	5	10	15	20
Wheat flour	500	475	450	425	400
GBPF	0	25	50	75	100
Yeast	10	10	10	10	10
Salt	9	9	9	9	9
Sugar	20	20	20	20	20
Oil	20	20	20	20	20
Water	330	330	330	330	330

Note: GBPF means green banana pulp flour.

RESULTS AND DISCUSSION

Composite Flour: Testing the physical properties of composite flour is of great importance because of the role of gluten content, gluten index, and falling number in predicting final product characteristics. Table 2 shows the moisture, ash, gluten, and falling number results. Substitution of wheat flour with different levels of GBPF did not significantly affect moisture content (between 12.11 and 12.39%) and falling number (between 385 and 411.5 sec). These falling numbers are considered high and indicate low α-amylase activity, which will negatively impact bread qualities in terms of volume and dryness due to the increased water-holding capacity of starch **[32]**. In literature, the falling number values of different types of composite flour depend on the material used to substitute wheat flour. Adding buckwheat and millet flour to wheat flour significantly increased the falling number **[37]**. Similarly, adding cassava flour to wheat flour significantly increased the falling number **[38]**. In contrast, substituting wheat flour with millet flour **[39]**, grape seed flour **[40]**, and bamboo shoot and cassava flour **[41]** significantly decreased the falling number. Similar to our results, **[42]** found that substituting wheat flour with barley flour did not affect the falling number. Freen banana flour had falling number values between 1666.8-2376.6 sec, and adding it to wheat flour significantly increased the falling number in values depending on the amount added, which contradicts our result **[43]**. It is worth to be mentioned that the banana used in **[43]** study was pretreated by chemical and physical methods, which may inhibit the α-amylase activity in banana flour, according to the author's interpretation. Ash content significantly increased with every increment of GBPF addition to composite flour, where control wheat flour had the lowest value of 0.7% and composite flour containing 20% GBPF had the highest value of 1.25%; these results are in agreement with the result of **[24]**. Wet gluten and dry gluten significantly decreased with increasing wheat flour substitution levels with GBPF, where the highest values were for control wheat flour, and the lowest values were for composite flour containing 20% GBPF. There were no significant differences in wet and dry gluten content between composite flour containing 5, 10, and 15% GBPF. The gluten content decrease in composite flour was mentioned in **[44]**, who reported that gluten content in composite flours decreased when using flour devoid of gluten in substituting wheat flour, which is the case in GBPF **[43]**. Not only is the decrease in gluten content important, but the rate of reduction corresponding to each substitution level is also essential. The values of dry gluten content decreased significantly when the wheat flour substitution level with GBPF reached 15 and 20%, with a 9.79 and 15.23% reduction in dry gluten content, respectively. Interestingly, the substitution of wheat flour with GBPF significantly increased the gluten index compared to control wheat flour, with no significant differences between different types of composite flour. The small decrease in dry gluten content and the increae in gluten index that ocuured when sustituting wheat flour with GBPF suggested a complex formation between resistant starch in banana flour and wheat gluten proteins **[45]**.

Table 2 Falling number and contents of moisture, ash, and gluten in composite flour containing wheat flour substituted with different levels of GBPF.

Composite flour	Moisture (%)	Ash (%)	Wet gluten (%)	Dry gluten (%)	Gluten index (%)	Falling number (sec.)
0% GBPF	12.37 ±0.06^{a}	0.70 ±0.00^{e}	27.79 ±0.26^{a}	9.19 ±0.01^{a}	90.05 ±1.48^{b}	385.0 ±14.1^{a}
5% GBPF	12.39 ±0.01^{a}	0.82 ±0.00^{d}	25.47 ±0.13^{b}	8.73 ±0.06^{ab}	97.4 ±1.13^{a}	392.5 ±3.45^{a}
10% GBPF	12.29 ±0.10^{a}	0.99 ±0.01^{c}	24.36 ±0.17^{bc}	8.37 ±0.12^{abc}	96.35 ±0.07^{a}	410.0 ±14.1^{a}
15% GBPF	12.11 ±0.13^{a}	1.15 ±0.00^{b}	24.09 ±0.47^{bc}	8.29 ±0.17^{bc}	97.01 ±0.7^{a}	410.5 ±7.78^{a}
20% GBPF	12.38 ±0.18^{a}	1.25 ±0.00^{a}	22.90 ±0.98^{c}	7.79 ±0.45^{c}	97.3 ±0.85^{a}	411.50 ±10.61^{a}

Note: GBPF means green banana pulp flour. Values are expressed as means ±standard deviation, and values sharing the same letter in the same column are not significantly different (p >0.05) as tested by Tukey's test.

For farinograph-tested parameters (Table 3-A and 3-B), development time (ranging between 95.5-128 sec) and consistency (ranging between 495 and 512 FE) were not significantly affected by wheat flour substitution with GBPF (Table 3-A). However, other tested parameters (water absorption, stability time, degree of softening, and farinograph quality number) were significantly affected (Table 3-A and 3-B). Water absorption (ranging between 58.65 and 59.90%) increased with the substitution of wheat flour with GBPF; however, this increase was significant only at the substitution level of 15% when compared to the control. It is evident that there were no significant differences between control wheat flour and composite flour containing 5% GBPF in terms of stability time, degree of softening, and farinograph quality number, whereas other substitution levels differed significantly. The stability time decreased significantly when substitution levels were above 5%, with no significant differences between those composite flour. The highest stability time was for control wheat flour with a value of 502.0 ±48.1 sec, and the lowest was for flour with a 15% substitution level with a value of 274 sec. The degree of softening increased significantly in composite flour with substitution levels above 5% compared to the control wheat flour. The lowest degree of softening was for control wheat flour (34 ±7.07 FE), and the highest value was for composite flour with a 20% substitution level (96 ±2.83%). The farinograph quality number significantly decreased when using composite flour with substitution levels above 5% compared to the control wheat flour. The highest farinograph quality number was for control wheat flour (94.50 ±9.19 mm), and the lowest was for flour containing 15 and 20% GBPF (46.50 ±3.54 mm). These results are in parallel with the results of **[46]**, who related the increase in water absorption in composite flour to the numerous hydroxy groups in resistant starch that are capable of forming hydrogen bonds with water **[45]**. Mohebbi et al. **[45]** found that adding resistant starch to wheat flour increased water absorption of the composite flour due to the high amount of amylose in resistant starch. There is a direct relationship between dough development time and protein content in different composite flour, which decrease the dough development time due to the decrease in protein content. However, this depends on the material used to substitute flour; adding some types of fibers and RS may improve the farinograph properties **[45]** due to the formation of a complex with wheat gluten. This may explain why the dough development time did not change in composite flours in our results. The decrease in dough stability in composite flour may be related to the dilution of gluten content in composite flour or the interaction between resistant starch and wheat gluten **[45]**. The decrease in the dough stability time and farinograph quality number and the increase in the degree of softening indicated that the composite flours – in this study – could not tolerate extended kneading times **[47]**.

Table 3-A Farinograph tested parameters for composite flours containing wheat flour substituted with different levels of GBPF.

Composite flour	Water absorption (%)	Development time (sec)	Consistency (FE)
0% GBPF	58.65 ±0.21^{b}	128 ±8.49^{a}	506 ±5.66^{a}
5% GBPF	59.65 ±0.35^{ab}	119 ±15.6^{a}	495 ±17^{a}
10% GBPF	59.65 ±0.21^{ab}	120 ±4.24^{a}	495.5 ±24.7^{a}
15% GBPF	59.90 ±0.28^{a}	95.5 ±7.78^{a}	493.5 ±4.95^{a}
20% GBPF	59.60 ±0.42^{ab}	102.5 ±13.44^{a}	512 ±4.24^{a}

Note: GBPF means green banana pulp flour. Values are expressed as means ±standard deviation, and values sharing the same letter in the same column are not significantly different (p >0.05) as tested by Tukey's test.

Table 3-B Farinograph tested parameters for composite flours containing wheat flour substituted with different levels of GBPF.

Composite flour	Stability time (sec)	Degree of softening (FE)	Farinograph quality number (mm)
0% GBPF	502.0 ±48.1^{a}	34 ±7.07^{d}	94.50 ±9.19^{a}
5% GBPF	475.5 ±46.0^{a}	41 ±8.49^{cd}	85.50 ±7.78^{ab}
10% GBPF	338.5 ±9.19^{b}	59 ±5.66^{bc}	62.00 ±5.66^{bc}
15% GBPF	274.0 ±0.00^{b}	80 ±4.24^{ab}	49.50 ±0.71^{c}
20% GBPF	280.0 ±0.00^{b}	96 ±2.83^{a}	46.50 ±3.54^{c}

Note: GBPF means green banana pulp flour. Values are expressed as means ±standard deviation, and values sharing the same letter in the same column are not significantly different (p >0.05) as tested by Tukey's test.

Saj Bread: The produced bread was evaluated in color, texture, and sensory properties. For color and sensory properties, only one factor was investigated: wheat substitution with different proportions of GBPF. However, in texture, two factors were investigated: wheat substitution with different proportions of GBPF and storage time.

CIELAB color values: Saj bread CIELAB color values were significantly affected by the level of wheat flour substitution with different levels of GBPF (Table 4). The L^* values of bread significantly decreased in samples made from composite flour compared to the control (81.28 ±0.65), with no significant differences between different types of composite flour. Bread made from composite flour a^* values were significantly higher than the control bread made from wheat flour (0.2 ±0.22), with no significant differences between different types of bread made from composite flour containing different levels of GBPF. Bread made from composite flour containing 5% GBPF b^* value (17.34 ±2.54) did not significantly differ from the control bread (19.98 ±0.64); however, other types of bread made from composite flour significantly differed from the control bread. Bread made from composite flour containing 15% GBPF had the highest ΔE^*ab value (16.66 ±3.09), significantly higher than bread made from composite flour containing 5% GBPF, with no significant differences with other types of bread made from composite flour. The trend in color change found in this study with increased substitution of wheat flour with GBPF was in harmony with the results of steamed Chinese bread fortified with GBPF **[29]** and pita bread made from composite flour containing **[30]**. The changes in composite flour color found in this study are related to the dark color of the GBPF. The dark color of GBPF was reported in previous studies; this dark color negatively affects the utilization of GBPF in different food applications **[26]**. The dark color of GBPF is attributed to the enzymatic browning reactions occurring during the drying process **[23]**. Khoozani et al. **[5]** investigated the effect of different drying conditions on the color and other functional properties of GBPF.

Table 4 CIELAB color values of saj flatbread made from composite flours containing wheat flour substituted with different levels of GBPF.

Composite flour	L^*	a^*	b^*	ΔE^*ab
0% GBPF	81.28 ±0.65^{a}	0.20 ±0.22^{b}	19.98 ±0.64^{a}	-
5% GBPF	73.73 ±1.11^{b}	1.87 ±0.70^{a}	17.34 ±2.54^{ab}	11.88 ±1.84^{b}
10% GBPF	72.30 ±1.61^{b}	2.80 ±0.69^{a}	15.75 ±0.99^{bc}	13.33 ±1.42^{ab}
15% GBPF	69.95 ±3.17^{b}	2.43 ±0.31^{a}	12.80 ±1.26^{c}	16.66 ±3.09^{a}
20% GBPF	73.06 ±2.54^{b}	2.41 ±0.20^{a}	13.27 ±0.45^{c}	13.84 ±1.96^{ab}

Note: GBPF means green banana pulp flour. Values are expressed as means ±standard deviation, and values sharing the same letter in the same column are not significantly different (p >0.05) as tested by Tukey's test.

Saj bread texture: Saj bread texture (stretchability and TPA) was only significantly affected by the main effects (levels of wheat flour substitution and storage time), with no significant interaction between them. Therefore, in the following discussion of texture results, only the results of the main effects will be presented and discussed.

The effects of substituting wheat flour with different levels of GBPF on the stretchability test are presented in Table 5. The stretchability of bread was more sensitive to the use of composite flour in bread making than breakpoint. There was a significant difference between the breakpoint value of bread made from composite flour containing 15% GBPF (925.7 ±156.2 g) and control bread (770.5 ±130.4 g), with no significant differences between control bread and other types of bread made from composite flour. For stretchability, using composite flour containing 15% GBPF or more significantly reduced the stretchability compared to the control bread

(13.39 ±3.44 mm). These results did not agree with the results of **[30]** for pita bread made from composite flour containing different proportions of GBPF. **[30]** reported an increase in the breakpoint of pita bread with using the composite flour; there were no significant differences between different GBPF substitution levels. For stretchability, **[30]** reported an increase in the stretchability of bread with increasing GBPF substitution, which contradicts our results, where with increasing GBPF level, the stretchability decreased. The difference in results may be related to the different types of bread used, and the ingredients added. The results of the effects of storage time on the bread stretchability test parameters are presented in Table 6. Only bread stored for three days had a significantly lower breakpoint value of 716.3 ±147.6 g than bread at zero storage time (873.8 ±150.8 g). Bread stretchability decreased significantly with each storage day, with no significant differences between bread stored for 2 and 3 days. These results agreed with pita bread made from composite flour containing GBPF **[30]**.

Table 7 shows the effects of wheat flour substitution with different levels of GBPF on the TPA of bread. It is evident that the hardness, resilience, and chewiness of bread increased significantly when flour substitution levels were 15% or more compared to the control bread. No significant differences were observed in cohesiveness between control bread and bread made from different types of composite flour. The results were in agreement with the results of **[30]** for pita bread prepared using composite flour containing GBPF. Thakaeng et al. **[26]** reported a decrease in springiness values for bread made by adding unripe banana flour to wheat flour. The increase in TPA parameters in our study may be attributed to the low gluten content of gluten in composite flour, which causes a decrease in elasticity due to a decrease in gas holding capacity **[26]**. In contrast to our results, **[8]** reported an increase in hardness and a decrease in other TPA parameters. Table 8 shows the results of the effects of storage time on TPA of bread. Resilience was not affected by the storage time. Hardness, cohesiveness, and chewiness significantly changed with storage time (hardness and chewiness increased, while cohesiveness decreased), with no significant differences between these parameters between bread stored for 2 and 3 days (Table 8). These results agreed with **[30]**, except for resilience which decreased with storage time for pita bread.

Table 5 Effects of substitution of wheat flour with different levels of GBPF on breakpoint and stretchability of saj flatbread.

Composite flour	Breakpoint (g)	Stretchability (mm)
0% GBPF	770.5 ±130.4^{bc}	13.39 ±3.44^{a}
5% GBPF	811.3 ±160.3^{abc}	12.86 ±3.63^{ab}
10% GBPF	699.5 ±117.8^{c}	12.52 ±3.60^{bc}
15% GBPF	925.7 ±156.2^{a}	11.97 ±3.57^{c}
20% GBPF	852.5 ±159.9^{ab}	11.99 ±3.52^{c}

Note: GBPF means green banana pulp flour. Values are expressed as means ±standard deviation, and values sharing the same letter in the same column are not significantly different (p >0.05) as tested by Tukey's test.

Table 6 Effects of storage time on breakpoint and stretchability of saj flatbread.

Storage time (days)	Breakpoint (g)	Stretchability (mm)
0	873.8 ±150.8^{a}	18.20 ±1.05^{a}
1	832.6 ±153.0^{a}	11.77 ±0.97^{b}
2	824.9 ±162.0^{ab}	10.42 ±0.92^{c}
3	716.3 ±147.6^{b}	9.79 ±1.05^{c}

Note: Values are expressed as means ±standard deviation, and values sharing the same letter in the same column are not significantly different (p >0.05) as tested by Tukey's test.

Table 7 Effects of substitution of wheat flour with different levels of GBPF on TPA of saj flatbread.

Composite flour	Hardness (g)	Resilience	Cohesiveness	Cheweness (g)
0% GBPF	5190 ±1749^{c}	0.47 ±0.03^{b}	0.81 ±0.05^{ab}	4077 ±1278^{b}
5% GBPF	5952 ±2169^{bc}	0.48 ±0.03^{b}	0.79 ±0.05^{b}	4601 ±1487^{b}
10% GBPF	5894 ±2315^{bc}	0.48 ±0.03^{b}	0.79 ±0.06^{b}	4465 ±1570^{b}
15% GBPF	6862 ±2170^{ab}	0.52 ±0.02^{a}	0.82 ±0.04^{a}	5496 ±1528^{a}
20% GBPF	7084 ±2558^{a}	0.51 ±0.03^{a}	0.82 ±0.05^{a}	5650 ±1819^{a}

Note: GBPF means green banana pulp flour. Values are expressed as means ±standard deviation, and values sharing the same letter in the same column are not significantly different (p >0.05) as tested by Tukey's test.

Table 8 Effects of storage time on TPA of saj flatbread.

Storage time (days)	Hardness (g)	Resilience	Cohesiveness	Cheweness (g)
0	3262 ±603^{c}	0.50 ±0.02^{a}	0.88 ±0.02^{a}	2835 ±545^{c}
1	5800 ±1091^{b}	0.48 ±0.03^{a}	0.79 ±0.03^{b}	4576 ±845^{b}
2	7943 ±1585^{a}	0.49 ±0.04^{a}	0.77 ±0.02^{c}	6073 ±1261^{a}
3	7780 ±1456^{a}	0.50 ±0.05^{a}	0.77 ±0.03^{c}	5948 ±1101^{a}

Note: Values are expressed as means ±standard deviation, and values sharing the same letter in the same column are not significantly different (p >0.05) as tested by Tukey's test.

Sensory evaluation: Figure 3 shows the different types of saj bread prepared, and Table 9 shows the sensory evaluation scores of saj bread made from different types of composite flour containing different proportions of GBPF. The texture and overall acceptability of saj bread were not affected by wheat flour substitution by GBPF, whereas other parameters were significantly affected. Color scores significantly decreased in bread made from composite flour containing 10% GBPF or more, with no significant differences between control bread and bread made from composite flour containing 5% GBPF. Incorporating GBPF improved taste and odor scores significantly, with no significant differences between different substitution levels.

The results indicate that GBPF can be used to substitute up to 20% of wheat flour without negatively affecting the overall acceptability of bread. The perceived improvement in taste and odor of saj bread prepared from composite flour may be related to the increased concentration of Maillard reaction products **[20]**. These results were parallel to previous results. For instance, Viana et al. **[23]** reported that bread formulated with 15 or 20% GBPF had higher than 90% acceptance for all sensory parameters investigated. Khalil et al. **[8]** suggested that GBPF can be used up to 30% in flatbread with acceptable sensory and physical properties. Ebabhamiegbebho et al. **[12]** concluded that GBPF could be used to substitute flour in a ratio of up to 20% for the preparation of bread with good sensory properties. On the other hand, **[24]** found that bread with the highest overall acceptability scores contains 5% GBPF.

Table 9 Sensory evaluation scores of saj flatbread.

Composite flour	Color	Texture	Taste	Odor	Overall acceptability
0% GBPF	7.72 ±0.52^{a}	7.30 ±1.33^{a}	7.01 ±0.78^{b}	6.95 ±0.38^{b}	8.23 ±1.38^{a}
5% GBPF	7.66 ±1.25^{a}	7.00 ±1.72^{a}	8.10 ±0.98^{a}	8.37 ±0.56^{a}	7.95 ±1.79^{a}
10% GBPF	6.25 ±0.89^{b}	7.15 ±1.17^{a}	8.44 ±0.77^{a}	8.92 ±0.57^{a}	8.22 ±1.17^{a}
15% GBPF	6.34 ±1.01^{b}	7.26 ±1.40^{a}	8.21 ±0.57^{a}	8.08 ±0.54^{a}	8.31 ±1.49^{a}
20% GBPF	6.48 ±1.93^{b}	7.24 ±1.34^{a}	8.32 ±0.55^{a}	8.43 ±0.38^{a}	7.29 ±2.05^{a}
0% GBPF	6.37 ±1.86^{b}	7.17 ±1.38^{a}	8.40 ±0.64^{a}	8.08 ±0.57^{a}	8.37 ±1.99^{a}

Note: Values are expressed as means ±standard deviation, and values sharing the same letter in the same column are not significantly different (p >0.05) as tested by Tukey's test.

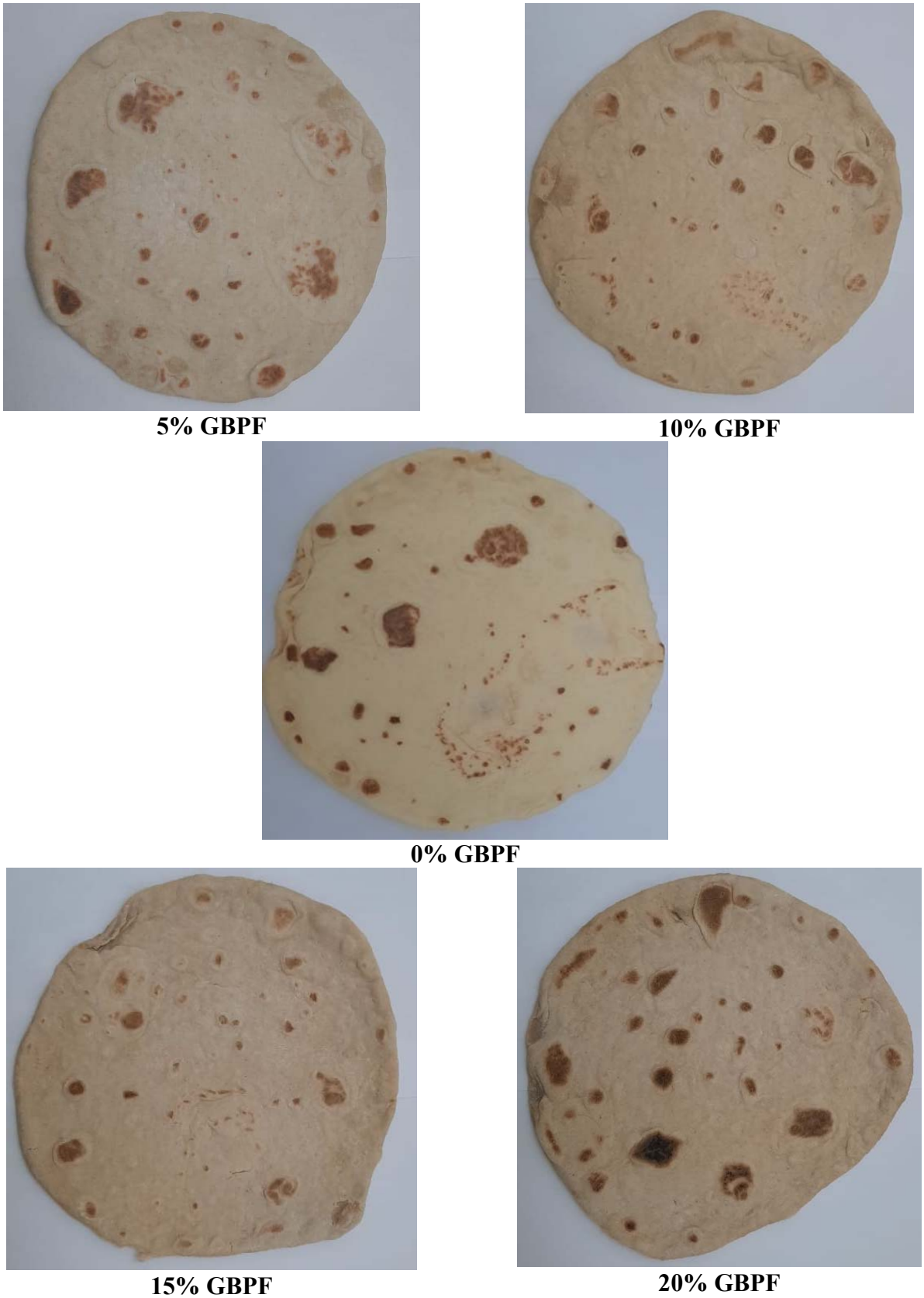

Figure 3 Saj flatbread made from composite flours containing wheat flour with different substitution levels with GBPF.

CONCLUSION

The possibility of making saj flatbread from composite flour containing wheat flour with different levels of GBPF was investigated. Considering the little scientific work on flatbread, to the best of our knowledge, this is the first research that investigated the potential use of GBPF in saj bread. Saj flatbreads were successfully produced from all types of composite flour used. Results indicated the possibility of using composite flour containing up to 20% GBPF without compromising sensory acceptability, except for the color, which made the bread look like that produced from whole wheat flour. Using composite flour affected the objective physical parameters tested in this study, dependent on the GBPF substitution level and storage period (for texture measurement of saj bread). The results of this research are of great importance to banana producers; it gives them a sustainable solution to reduce the postharvest waste of bananas due to rejects from miss-shape and small sizes. Additionally, the results introduce a new functional food made from saj bread formulated with the incorporation of GBPF in their preparation, which is in line with consumer requirements in providing new functional food by replacing traditional foods with healthier alternatives. Further studies are needed to improve the color of GBPF, and another study with a larger panelist number is needed to confirm the sensory evaluation results.

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The author declares no conflict of interest.

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

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Improving milk quality to prevent microelement deficiencies: a socio-hygienic perspective on adding bioavailable trace elements

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ABSTRACT

Based on the study of actual nutrition and the availability of macro- and microelements, it was found that the adult population of the North Caucasus Federal District (NCFD) of Russia belongs to the risk group for the development of micronutrient insufficiency associated with a low content in the diet of several essential elements (copper, zinc, calcium, selenium), which are a priority for correction. This is because 89% of the population in the NCFD has a diet that is significantly out of balance both quantitatively and qualitatively, negatively impacting nutritional status and the dispersion of trace elements. It was found that a significant part of the population of the NCFD is characterized by a lack of dairy products in the diet (59.8%), as well as insufficient intake of vitamins B2, B6, C, PP, folic acid, I, Se, Cu, Zn, Mg, Ca, fiber, polyunsaturated fatty acids, tryptophan. Volunteers were selected for the experiment – adult men living in the NCFD. The volunteers took 200 ml of "Voznesenovsky Ecoprodukt" milk (2.5% fatness) for 60 days, produced by a local enterprise using the technology proposed by the authors. Significant violations of mineral metabolism were found in 68.3% of the population at the start of the trial, according to the findings of screening examinations conducted on the hair of the experiment's volunteer participants. Among the priorities for the correction of essential elements are: Se (deficiency in 88.2% of the examined), J (82.2%), Cu (59.1%), Zn (66.7%), Ca (29.8%). The proportion of people with calcium deficiency decreased from 29.8 to 21.5%, copper from 59.1 to 36.2%, selenium from 88.2 to 72.4%, zinc from 66.7 to 38.4%, and iodine from 82.2 to 68.4% when "Voznsenovsky Ecoproduct" was added to the milk diet. At the end of the preventive course, an increase in the concentration in the hair was noted: calcium (by 26.6%), zinc (by 11.0%), copper (by 10.1%), iodine (by 32.5%) and selenium (by 38.9%). Regular consumption of "Voznesenovsky Ecoprodukt" milk allowed to increase the consumption of dairy products among the study participants, to receive a rapid physiological response of the body in the form of an increase in the content of the studied micro- and macroelements in the hair, reducing the number of people with calcium, zinc and selenium deficiency.

Keywords: milk, microelementosis, fortification, rational nutrition

INTRODUCTION

To minimize the prevalence of hypovitaminosis and microelementosis, which have become pervasive, it is currently of special importance to research the reasons and devise strategies for improving population nutrition **[1]**. World experience shows that the most effective and economical way to improve the supply of micronutrients to the population is the regular inclusion of specialized mass-consumption foods enriched with vitamins and trace elements **[2]**, **[3]**.

Studies of the actual nutrition of the population in various countries have shown the presence of both general and specific problems depending on socioeconomic, environmental and industrial factors, as well as on the dietary traditions of a particular population group **[4]**, **[5]**. This negative process is expressed by the chronization of human diseases, an increase in mortality in the employable population, an increase in mortality in childhood, and a decrease in the birth rate and average life expectancy of a person. The state of health is not least determined by

malnutrition. A special role in the normal functioning of all physiological systems of the body is assigned to trace elements, which are part of at least 2000 enzymes that catalyze a variety of biochemical reactions **[6]**, **[7]**.

Trace elements enter plants from the soil, and animals and humans receive them with food **[8]**. It has been established that higher mortality from cardiovascular diseases is observed with a general deficiency of trace elements in the soil. Thus, in Europe, the highest mortality from coronary artery disease is observed in the northern regions of Great Britain and the northeastern region of Finland, where podzol soils with a deficiency of trace elements predominate **[9]**. Stomach and lung cancer is more common among residents of settlements located on soils poor in selenium, gedez, cobalt, and zinc **[10]**, **[11]**, **[12]**. 80% of the Russian population has an inadequate selenium supply (less than 70 µg/l) **[13]**.

Violation of trace element metabolism and imbalance of trace elements involved in maintaining homeostasis and normal functioning of the human body at the cell level must be considered in treating a variety of diseases **[14]**. The main micronutrient risks have been identified in the North Caucasus Federal District of Russia, and the leading directions for the prevention of alimentary-dependent diseases, primarily oncological, cardiovascular, and endocrine diseases, have been identified **[15]**. One of the most effective solutions to the problem of deficiency of essential trace elements can be the development of daily diet foods enriched with essential trace elements and bioavailable form following the established indicators of deficiency of specific trace elements in a particular region. This study aimed to solve the problem in the direction of preventive nutrition on the territory of Russia's North Caucasus Federal District (NCFD).

Scientific Hypothesis

Enriching milk with essential trace elements in bioavailable form is an effective solution in the fight against microelementosis. In particular, daily consumption of milk enriched with calcium, zinc, copper and iodine will correct the deficiency of these elements in the body.

MATERIAL AND METHODOLOGY

Samples

For the experiment, Ecoprodukt Voznesenovsky (Voznesenovskoye, Russia) produced specialized milk (2.5% fatness) enriched with trace elements. To study the effect of milk on the microelement balance of the experimental participants, the hair of adult volunteers living in the NCFD was used as the experimental samples.

Chemicals

We used reagents of recognized analytical purity and distilled water. For atomic emission spectroscopy and mass spectrometry with inductively coupled argon plasma, state standard samples of Calcium, Copper, Iron, Iodine, Magnesium, Selenium, and Zinc, were purchased in LenReactive LLC (Sants Petersburg, Russia).

Animals and Biological Material

The work used biological material - the hair of adult volunteers living in the NCFD.

Instruments

Laboratory Spray Dryer BIORUS BIO-8000 (BIORUS, Moscow, Russia), Muffle furnace UED-7-10D (UED, Saint Petersburg, Russia), atomic emission spectrometer with microwave plasma Agilent 4210 (Agilent, Santa Clara, CA, USA), inductively coupled argon plasma mass spectrometer NexION 350 IPS-MS (PerkinElmer, Akron, Ohio, USA).

Laboratory Methods

The study of the actual nutrition of the adult population of the NCFD (n = 1000) was carried out by the method of 24-hour (daily) reproduction of nutrition recommended for these purposes by the Federal State Budgetary Research Institute of Nutrition (Moscow, Russia) **[16]**. Additionally, a specially designed questionnaire was used.

The macro-, and microelement composition of “Voznesenovsky Ecoprodukt” milk was studied by atomic emission and mass spectrometry with inductively coupled argon plasma.

To assess the preventive effect of milk enriched with trace elements, a before–after" study was conducted on 200 adult volunteers aged 16-59 years living in the territory of the NCFD and selected following the inclusion–exclusion criteria (informed consent to participate in the study, absence of acute, diseases, chronic diseases in the stage of exacerbation or decompensation) who took 200 ml of the product daily for 60 days. The change in the amount of macro- and microelements in the body served as the yardstick for measuring the study's effectiveness (according to the hair mineralograms at the points "before" and "after" the course of taking the product).

In parallel, the actual nutrition was monitored by analyzing the frequency of food consumption.

Determination of the content of mineral elements in the hair was carried out by atomic emission and mass spectrometry with inductively coupled argon plasma. The selection of persons for the study (n = 50) was carried

out among the participants of the previous stage of the study, subject to their informed consent. The sample was representative.

Description of the Experiment

Sample preparation: "Voznesenovsky Ecoprodukt" milk was dried on a BIO RU BIO-8000 laboratory spray dryer (BIO RUS, Moscow, Russia). Milk powder was mineralized in a muffle furnace UID-7-10D (UED, Saint Petersburg, Russia) and sent to atomic emission spectroscopy and mass spectrometry with inductively coupled argon plasma to determine the mineral composition.

The hair was taken from adult volunteers naturally using tweezers (3 hairs per selection). The selected hair was placed in a sterile bag with a tag containing the person's contact details, the date and the time of selection. Before studying the trace element composition, the hair was mineralized in a muffle furnace UID-7-10D (UED, Saint Petersburg, Russia) and sent to atomic emission spectroscopy and mass spectrometry with inductively coupled argon plasma.

Number of samples analyzed: 301

Number of repeated analyses: 903

Number of experiment replication: 1

Design of the experiment: The subject of the study was the adult population of the NCFD, the subject of the study was the structure of nutrition of the population, and the object of the study was the content of trace elements in biosubstrates (hair). In the course of the work, the preventive effectiveness of "Voznesenovsky Ecoprodukt" milk was established. At the first stage of the study, the study of the actual nutrition of the adult population of the NCFD (n = 1000) was carried out by the method of 24-hour (daily) reproduction of nutrition recommended for these purposes by the FSBI Research Institute of Nutrition (Moscow, Russia) **[16]**. Additionally, a specially designed questionnaire was used.

A "before-after" study was conducted on 200 adult volunteers aged 16-59 living in the North Caucasus Federal District to assess the preventive efficacy of milk enriched with microelements. Volunteers were selected using online survey for adults living in the suding region. Volunteers participating in the experiment were daily given "Voznesenovsky Ecoproduct" milk enriched with macro- and microelements. The milk required for the experiment was provided by Ecoproduct Voznesenovsky (Voznesenovskoye, Russia). The elemental composition of milk was studied at the beginning of the experiment by atomic emission spectrometry and mass spectrometry with inductively coupled argon plasma. Standardized milk (2.5% fatness) "Molochnaya legenda" (Nalchik Dairy Plant, Nalchik, Russia) was used as a control sample for comparison. Conclusions on the preventive efficacy of milk were made based on the analysis of the elemental composition of hair.

Determination of the content of mineral elements in the hair was carried out using atomic emission and mass spectrometry with inductively coupled argon plasma. The selection of individuals for the study (n = 50) was carried out from among the participants of the previous stage of the study, provided that their informed consent was obtained. The sample was representative.

Statistical Analysis

The normality of the distribution of quantitative traits was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests, and the hypotheses about the equality of general variances were tested using the Levene test. To compare the numerical data of two independent groups, the Mann-Whitney U test was used; to compare the qualitative data of two or more independent groups, the Fisher test was used.

RESULTS AND DISCUSSION

An assessment of the structure of actual nutrition and consumption of basic nutrients by the population of the NCFD showed a relatively low amount of fish products in the diet (377.2 ±5.3 g/day), as well as dairy products (296.1 ± 7.4 g/day) which are significantly less than the recommended values. Insufficient consumption of dairy products (including cottage cheese, sour cream, cheese, etc.) was observed in 59.8% of the population. The importance of the consumption of milk and dairy products as a part of rational nutrition was mentioned in several works **[17]**, **[18]**, **[19]**. Moreover, recent studies have found a correlation between insufficient consumption of dairy products and the risks of cardiovascular disease **[20]**, diabetes **[21]**, lymphoma **[22]** and even bones fracture **[23]**.

When analyzing the balance of the diet, it was found that only 11% of the population had the content of basic nutrients within the recommended values. The optimal ratio between energy consumption and the energy value of the diet was 14% of the population. An unbalanced diet in terms of the ratio of proteins: fats: carbohydrates was observed in 89% of the population.

Dietary protein consumed by the population of the NCFD contained the only limiting amino acid, tryptophan (75.7%). The main sources of protein, providing a third of its amount, were meat and meat products, 21% of the

protein came from bakery products, 14% from dairy products. The data obtained are consistent with those presented by Chmyrev et al. **[16]**.

In general, the daily intake profile of the most important nutrients by the population of the NCFD was characterized by a significant lack of vitamins B2, B6, C, PP, folic acid, a number of macro- and microelements, fiber, polyunsaturated fatty acids (PUFA) with excessive consumption of salt, cholesterol, triglycerides and alcohol. This is in line with the trend declared by Gerasimov et al. **[24]**.

The values of the daily intake of a number of the most important micronutrients are given in Table 1. The largest proportion of people with insufficient consumption was noted among women, mainly in the age groups of 30–39 years old (zinc, copper) and 18–29 years old (calcium, selenium, iodine). The median daily intake of selenium was 38.3 μcg for women and 46.0 μg for men, about half of the recommended intake. The diet's low calcium content (655.9 mg in men and 566.1 mg in women) also deserves close attention, consistent with the above data on insufficient amounts of milk and dairy products, vegetables and fruits (Table 1).

Table 1 Values of the average daily intake of the most important macro- and microelements by the population of the NCFD by sex and age.

Index	Age, years	Man		Women	
		median	rate of consumption below RDI*	median	rate of consumption below RDI
Calcium, mg	18-29	566.5	66.7	476.6	99.9
	30-39	549.0	70.1	517.2	81.3
	≥40	652.5	65.4	585.4	78.3
	≥18	655.9	63.9	566.1	79.1
Copper, μg	18-29	1450.1	42.3	1118.9	42.1
	30-39	1473.8	44.5	1181.2	46.5
	≥40	1488.8	41.6	1193.0	42.3
	≥18	1455.3	41.3	1133.7	42.8
Zinc, μg	18-29	8628.1	51.2	7707.2	65.1
	30-39	9129.2	47.8	7816.3	67.6
	≥40	9207.7	46.2	7833.9	64.9
	≥18	9044.2	46.0	7819.1	65.2
Selenium, μg	18-29	55.2	49.7	27.9	77.1
	30-39	47.7	49.1	28.8	76.5
	≥40	46.3	50.2	36.6	65.2
	≥18	46.0	51.8	38.3	68.9
Iodime, μg	18-29	77.3	76.6	58.1	65.3
	30-39	77.8	78.2	48.7	56.6
	≥40	66.5	70.5	45.3	55.1
	≥18	66.9	71.3	49.7	58.6

Note: * RDI – recommended daily intake.

As a result, the NCFD adult population's diet was characterized by a quantitative and qualitative imbalance, which contributed to negative changes in nutritional status and the proliferation of microelementoses **[25]**, **[26]**.

In a study of the provision of the body of residents of the NCFD with chemical bioelements, it was found that significant violations of mineral metabolism (moderate and pronounced) were present in 70.5% of the population **[16]**. The priority essential elements for correction were: Se (deficiency in 88.2%), I (82.2%), Cu (59.1%), Zn (66.7%) and Ca (29.8%). Thus, based both on the data of the assessment of actual nutrition and on the results of the assessment of the provision of the population with macro- and microelements, micronutrients were identified that are a priority in terms of correction for the adult population of the NCFD: folic acid, vitamins PP, B2, B6 and C, minerals: I, Se, Cu, Zn, Mg, Ca. One of the most effective areas of population prevention of pathology associated with micronutrient deficiencies is the enrichment of essential foods with them **[27]**, **[28]**.

Considering the range of micronutrients identified as priorities for correction, we have developed the composition of new preventive milk, considering the enrichment principles given in several works **[29]**, **[30]**, **[31]**. As enrichers, it is proposed to use a mineral premix (Ca, Zn, Cu) and an additive containing selenopyran. The content of introduced micronutrients (Ca, Zn, Cu, Se) in one serving ranged from 15 to 25% of the RDI, and from the upper safe intake level – from 7.6% (copper) to 19.3% (zinc). The presence of additives did not change the organoleptic properties of the enriched product, as in other works **[32]**, **[33]**. Following the developed technology, regulatory and technical documentation was developed, permits were obtained for the production of innovative milk in the conditions of Ecoproduct Voznesenovsky (Voznesenovskoye, Russia). Milk "Voznesenovsky Ecoproduct" is a source of the organic form of selenium, which is best absorbed and does not have toxicity even at very high concentrations **[13]**, **[34]**. The introduction of this form of selenium into a dairy product containing tryptophan contributes to the rapid inclusion of both components in metabolism **[35]**. To exclude antagonism in the process of assimilation, the compatibility and interaction of the introduced mineral substances were taken into account, based on the works **[36]**, **[37]**.

Before starting the experiment with volunteers, we studied the mineral composition of "Voznesenovsky Ecoproduct" milk. The research results are presented in Table 2.

Table 2 Mineral composition of "Voznesenovsky Ecoproduct" milk.

Element	Content in "Molochnaya legenda" milk (2.5% fatness)	Content in "Voznesenovsky Ecoproduct" milk (2.5% fatness)
Calcium, mg/l	24.7 ±1.2	35.6 ±1.7
Copper, µg/l	14.9 ±0.8	48.8 ±2.3
Iron, µg/l	76.6 ±3.1	214.3 ±8.1
Iodine, µg/l	9.5 ±0.4	20.2 ±0.9
Magnesium, µg/l	22.7 ±0.9	43.8 ±1.6
Selenium, µg/l	2.2 ±0.1	8.1 ±0.6
Zinc, µg/l	0.4 ±0.1	2.3 ±0.2

To assess the preventive efficacy of the bioproduct in relation to the provision of the body with micronutrients, a before-after" study was conducted with the involvement of a group of volunteers (n = 200) **[38]**. At the first point (starting stage), 88.2% of the hair examined for the content of microelements had an insufficient supply of selenium, 82.2% – iodine, 66.7% – zinc, 59.1% – copper, 29.8% – calcium (Figure 1). The inclusion of "Voznsenovsky Ecoproduct" milk in the daily diet resulted in a decrease in the proportion of persons with calcium deficiency (from 29.8 to 21.5%, $p = 0.017$), copper (from 59.1 to 36.2%, $p < 0.001$), selenium (from 88.2 to 72.4%, $p = 0.022$), zinc (from 66.7 to 38.4%, $p < 0.001$) and iodine (from 82.2 to 68.4%, $p < 0.001$). As a result of the intervention (inclusion of "Voznesenovsky Ecoproduct" milk in he daily diet), the concentrations of elements in biosubstrates increased (Table 3). On average, the increase in hair concentrations for calcium was 26.6% ($p = 0.032$), zinc – 11.0% ($p = 0.002$), copper – 10.1% ($p < 0.001$), iodine – 32.5% ($p < 0.001$) and selenium – 38.9% ($p < 0.001$). At the same time, the proportion of individuals with a deficiency of the studied elements decreased **[39]**.

Table 3 The content of elements in the hair of the subjects at the points "before" and "after" a 2-month prophylactic course with "Voznesenovsky Ecoproduct" milk (in µg/g; n = 50; Wilcoxon paired test).

Element	P25	P50	P75	*p* (before-after)
Ca before	863.72	1615.49	2692.11	
Ca after	853.51	1998.45	2591.07	0.032
Cu before	7.85	8.54	10.20	
Cu after	8.13	9.68	11.61	<0.001
Se before	0.21	0.51	0.66	
Se after	0.28	0.60	0.68	<0.001
I before	0.16	0.47	0.72	
I after	0.19	0.53	0.71	<0.001
Zn before	122.17	153.08	169.79	
Zn after	140.22	178.15	195.77	0.002

The normal content of selenium in hair is 0.7-1.5 μg/g. The level of 0.3-0.7 μg/g corresponds to suboptimal provision **[13]**.

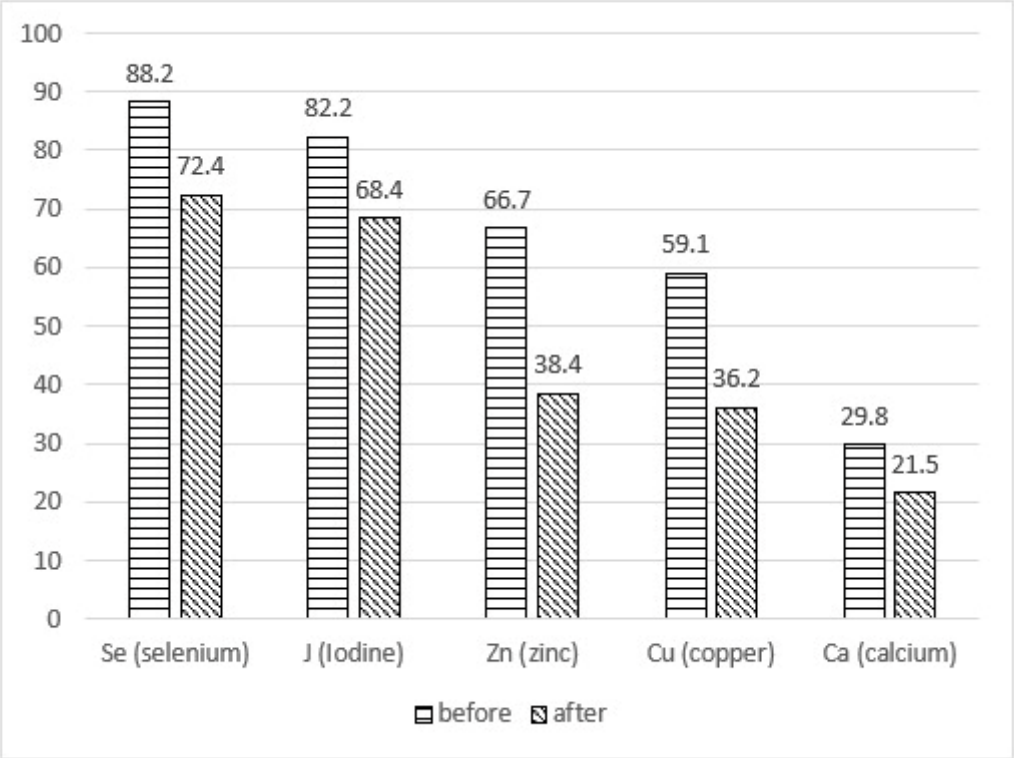

Figure 1 The share of persons with insufficient provision of macro- and microelements at the points "before" and "after" a 2-month prophylactic course with “Voznsenovsky Ecoproduct” milk.

Evaluation of the effectiveness of this intervention showed the possibility of correction and prevention of conditions caused by absolute and relative deficiency of calcium, copper, zinc, iodine and selenium **[40]**, **[41]**. Given that, according to the analysis of actual nutrition, some vitamins are classified as deficient nutrients, it is important to research to study the provision of the population with water-soluble vitamins and consider the possibility of creating products enriched with a complex of micronutrients.

Several works are worth considering regarding enriching milk and dairy products with essential micronutrients. Pfrimer et al. obtained milk richer in vitamin E, polyunsaturated fatty acids and Se produced by cows fed a diet supplemented with these nutrients. Immunologic analysis revealed a positive effect of the consumption of biofortified milk on inflammation in institutionalized older people **[42]**. Adegboye et al. in their study **[43]** prepared Vitamin D and calcium-fortified milk for pregnant women with periodontitis. Clinical trials showed that fortified milk helps women to deal with issues related to metabolic disorders and inflammation associated with periodontitis, which may have important health consequences for the pregnant woman and her offspring. With a similar product, Khadgawat et al. carried out a study on the effect of milk reached with vitamin D on the status of healthy school children aged 10-14 years (300 boys and 413 girls) **[44]**. The authors found that fortifying milk for 12 weeks is a safe and effective strategy for dealing with widespread vitamin D deficiency in school children. Sharifan et al. found that intake of fortified dairy products containing nano-encapsulated vitamin D_3 was associated with improved anthropometric indices, glucose homeostasis, and lipid profiles, particularly in individuals receiving fortified milk **[45]**. The authors declared that along with other benefits, fortifying dairy products with vitamin D may be a practical approach to improve some cardiometabolic indicators, such as insulin resistance. All these results prove that milk can be used for biocorrection and balance of the physiological status of people of different genders, ages and activities. However, the most promising direction is the correction of microelementosis by diagnosing the regional characteristics of the microelement status of the population and the development of enriched milk and dairy products of regional significance. In the future, we plan to develop this project in other regions and various population groups.

CONCLUSION

Nutrition of the North Caucasus Federal District population is characterized by a significant imbalance in the diet in quantitative and qualitative terms (89% of the population), contributing to adverse changes in the nutritional status and the spread of microelementoses. A significant part of the population is characterized by a lack of dairy products in the diet (59.8%), as well as insufficient intake of vitamins B2, B6, C, PP, folic acid, I, Se, Cu, Zn, Mg, Ca, fiber, PUFA, tryptophan. According to the results of screening studies of hair, significant disorders of mineral metabolism were present in 68.3% of the population. Among the priorities for the correction of essential elements are: Se (deficiency in 88.2% of the examined), J (82.2%), Cu (59.1%), Zn (66.7%), Ca (29.8%). The inclusion of "Voznsenovsky Ecoproduct" in the diet of milk resulted in a decrease in the proportion of persons with calcium deficiency (from 29.8 to 21.5%), copper (from 59.1 to 36.2%), selenium (from 88.2 to 72.4%), zinc (from 66.7 to 38.4%) and iodine (from 82.2 to 68.4%). At the end of the preventive course, an increase in the concentration in the hair was noted: calcium (by 26.6%), zinc (by 11.0%), copper (by 10.1%), iodine (by 32.5%) and selenium (by 38.9%). Regular consumption of "Voznesenovsky Ecoprodukt" milk allowed to increase in the consumption of dairy products among the study participants to receive a rapid physiological response of the body in the form of an increase in the content of the studied micro- and macroelements in the hair, reducing the number of people with calcium, zinc and selenium deficiency. Thus, it is necessary to recommend using this enriched product in the nutrition of the North Caucasus Federal District population, as well as other regions and countries, and to continue studying the long-term preventive effects of the systematic use of the product under study.

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Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

To study the effect of milk on the microelement balance of the experimental participants, the hair of adult volunteers living in the NCFD was used as the experimental samples. A corresponding agreement was concluded with all participants of the experiment. The experiment was approved by ethics commission of North Ossetian State Medical Academy (Protocol #SOGMA_1/22). Additional information is available upon request from the author for correspondence.

Limitations:

The study does not consider the reasons for the population's inadequate nutrition, such as cultural or economic factors, which could have an impact on the design of interventions to address the nutritional issues. Additionally, the article does not explore the association between the population's nutritional status and their health outcomes. Further research will explore the underlying causes of the inadequate nutrition and the health consequences of the population's poor nutritional status.

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Study of indicators of quality and safety of sour cream with vegetable oils

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ABSTRACT

The article covers the quality and safety indicators of sour cream with vegetable oils, in the composition of which blended oil (sunflower oil + linseed oil) is used as a fat phase in the form of a food emulsion stabilized by an emulsifying complex containing sodium caseinate. According to the chemical composition, sour cream with vegetable oils is characterized by an increased content of the mass fraction of proteins by 0.2% and a reduced content of the mass fraction of carbohydrates by 1.7% compared to classic sour cream, which is connected with the use of food emulsion in its composition. Due to the content of food emulsion, sour cream with vegetable oils has the content of polyunsaturated fatty acids increased by 24 times and the content of saturated and monounsaturated fatty acids decreased by 82.11% and 41.2%, respectively, compared to classic sour cream. The indicator of water activity in sour cream with vegetable oils is 0.983 aw, which is lower than classic sour cream (0.988 aw). According to the results of the study of microbiological parameters, on the fifth day of storage in sour cream with vegetable oils, the titrated acidity index was 86 °T, the number of lactic acid bacteria was 107 CFU/g, and no bacteria of the *Escherichia coli* group, mould and yeast were detected; it corresponds to the normalized indicators as for classic sour cream. At the end of the storage period, the value of syneresis in sour cream with vegetable oils is 23% lower than the value of syneresis in the control sample. In sour cream with vegetable oils, during five days of storage, the value of peroxide 3.0 – 4.0 ½ O mmol/k and acid value 2.5 – 2.6 mg acid number/g are within the normalized values for blended oil (sunflower oil + linseed oil).

Keywords: sour cream, dairy product, blended oil, water activity, microbiological quality indicator, nutritional value, storage

INTRODUCTION

Currently, the technologies of milk-containing products are rapidly developing, especially those in the recipe compositions in which fats of non-dairy origin replace milk fat, usually by oils and substitutes for milk fat **[1]**, **[2]**. This tendency is relevant due to the issue of resource conservation in the milk processing industry, which arose against the background of a reduction in the volume of cow milk as a raw material **[3]**. Considering the economic crisis of recent years, such an extremely expedient technological solution allows, first of all, to reduce the cost of finished products and, as a result, the retail price to meet the needs of consumers of all social categories **[4]**. However, scientists and the price reduction pay great attention to increasing the nutritional and biological value of developed and improved milk-containing products **[5]**. Improvement in the technologies of milk-containing fermented milk products is promising since including such products in the diet of the population of countries allows to solve the issue of bacterial balance in the human body **[6]**. It should be noted that using vegetable oils as fats of non-dairy origin in the technology of milk-containing products makes it possible to increase the content of polyunsaturated fatty acids while balancing the fatty acid composition of finished products

[7]. This makes it possible to improve the nutrition structure of the population, the analysis of which indicates a deficiency of polyunsaturated fatty acids against the background of consumption of an excess number of saturated ones **[8]**. The production of fermented milk products and the type of sour cream is relevant today **[9]** since sour cream as a classic dairy product is popular among consumers as a sauce for dishes, a basis for preparing desserts, etc. **[10]**. Thus, the technology of a milk-containing fermented milk product - sour cream with vegetable oils as an analogue of classic sour cream – has been developed. This technology involves using finely dispersed and aggregate-resistant food emulsion with a fat content of 50% based on blended oil (sunflower oil + linseed oil) and xanthan gum stabilizer. The use of food emulsion made it possible to exclude the traditional high-cost technological operation from the technological process such as dispersing the entire milk-vegetable mixture. The technology of sour cream with vegetable oils aims not only to expand the range of milk-containing products but also to use this milk-containing product as a semi-finished product to produce snack and dessert products **[11]**.

However, the developed sour cream with vegetable oils should be characterized by quality and safety indicators, similar or improved chemical composition to products obtained by classical technology.

Scientific Hypothesis

The use of food emulsion in the technology of sour cream with vegetable oils improves the chemical composition, fatty acid composition, does not affect the safety indicators during storage.

MATERIAL AND METHODOLOGY

Samples

The study was conducted with two samples:

- 20% fat sour cream with vegetable oils using food emulsion;
- sour cream with a fat content of 20% as a control sample, obtained by classical technology using cream obtained from cow milk. The recipe compositions of the studied samples are given in Table 1.

Table 1 Recipe compositions of the control sample and sour cream with vegetable oils.

Components	Mass fraction, %
Control sample*	
Cream obtained from cow's milk (with a fat mass fraction of 20%)	100.0
Total	100.0
Sour cream with vegetable oils	
Fat-Containing Fermented-Milk Base	99.85
Xanthan gum	0.15
Total	100.0

Note: Direct application bacterial preparation. It is not indicated in the formulated composition.

Chemicals

Distilled water, H_2O (TOV Novokhim, Ukraine).
Phenolphthalein alcoholic solution, $C_{20}H_{14}O_4$, 1.0% (Shostka Chemical Reagents Plant, Ukraine).
Sodium hydroxide, NaOH, 0.1 N (TOV Khimlaborreaktiv, Ukraine).
Cobalt sulphate solution, $CoSO_4$, 2.5% (TOV Khimlaborreaktiv, Ukraine).
Sodium methylate, CH_3ONa (ATK Ukraine, Ukraine).
Sodium sulphate, Na2SO4 (AT ZPD, Denmark).
MRS-agar (Conda, Ukraine).
Concentrated hydrochloric acid H_2SO_4 (Shostka Chemical Reagents Plant, Ukraine).
Boric acid H_3BO_3, 2.0 % (ATK Ukraine, Ukraine).
Sodium hydroxide NaOH, 33.0 % (TOV Khimlaborreaktiv, Ukraine).

Animals, Plants and Biological Materials

Iprovit SSK bacterial preparation (Institute of Food Resources NAAS of Ukraine, Ukraine) containing *Lactococcus lactis* ssp. *lactis; Lactococcus lactis* ssp. *cremoris; Lactococcus lactis* ssp. *diacetilactis; Streptococcus salivarius* ssp. *thermophilus*.

Instruments

Laboratory thermometer (TOV Standard-Lab).

Mohr pipettes, (TOV SkyLab).
Bunsen beaker (TOV SkyLab).
Conical flask (TOV SkyLab).
Glass rods (TOV SkyLab).
Titration assembly (TOV Labour-Technik).
Thermostat TSO-80 (TOV Ukragrotest).
Gas chromatograph (GE LifeSciences BPG 100/500, Germany).
Petri dish (TOV Termolab).
Counter of colonies of microorganisms JL-1C (TOV Spectrolab).
Microscope XS-5520 LED (TOV Micromed).
Analytical scales (Thermoengineering LLC, Ukraine)
Kjeldahl flask (TOV SkyLab).
Water bath (TOV Ukragrotest).
Cylinders with a capacity of 10 and 50 cm^3 (Thermoengineering LLC, Ukraine)
Exhaust fume hood (TOV Simvolt, Ukraine).
A glass funnel with a diameter of 3-4 cm (TOV SkyLab).
Installation for distillation of ammonia (Thermoengineering LLC, Ukraine).
Water activity analyser (TOV Simvolt, Ukraine).
Refrigerator (TOV Axcis, Ukraine).

Laboratory Methods

The titrimetric method determined the titrated acidity, which is based on the neutralisation of acids contained in the investigational product with a sodium hydroxide solution in the presence of an indicator according to **[12]**. Determination of the fatty acid content was carried out by chromatographic according to **[13]**. The mass fraction of fat was determined according to **[14]**, the mass fraction of protein – by Kjeldahl method **[15]**, and the mass fraction of carbohydrates according to **[16]**. The number of viable lactic acid bacteria, *Escherichia coli* bacteria, mold and yeast was determined by the method of sowing serial dilutions in agar nutrient media according to **[17]**. The peroxide number was estimated according to **[18]**, and the acid number – according to **[19]**. Syneresis determined by the centrifugal method. The same amount of product was weighed into two glass tubes with a capacity of 10 cm^3, closed with stoppers and centrifuged for 10 min at a speed of 1000 min^{-1}. The layer of whey, that settled on top of the sample, was determined on a scale in cm^3. Water activity was determined using the water activity analyser Walcom WA-60A, in the measurement range of 0-1 Aw (0-100% rh). Samples at 20 °C were collected in a container and placed in the measuring chamber. A water activity probe is installed from above. The measurement cycle lasts 3-5 minutes, after which the water activity and temperature values for each probe are displayed on display.

Description of the Experiment

Sample preparation: Sour cream with vegetable oils was obtained by fermentation of a mixture consisting of a finely dispersed food emulsion based on blended oil (sunflower oil + linseed oil) with a fat content of 50% and skimmed cow milk in a thermostat with the subsequent addition of xanthan gum (Table 1) **[11]**. Classical sour cream as a control sample was obtained by adding a bacterial preparation to cream with a fat content of 20% obtained from cow milk (Table 1), followed by their fermentation in a thermostat at a temperature of 30 °C until the titrated acidity of the clot reaches 60 °T.

Number of samples analyzed: During the experimental studies, 9 samples were used, the chemical, fatty acid composition, water activity in 6 samples, microbiological quality indicators, titrated acidity, peroxide and acid numbers were determined in 3 samples.

Number of repeated analyses: All measurements were performed 3 times.

Number of experiment replication: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: Fatty acid composition, mass fractions of fat, protein, carbohydrates were determined in the obtained sour cream with vegetable oils and the control item according to the methods **[13]**, **[14]**, **[15]**, **[16]**, respectively, as well as water activity using water activity analyser Walcom WA-60A.

Sour cream with vegetable oils was cooled to a temperature of 0 – 6 °C and stored at this temperature for 5 days in a refrigerator. Every 24 hours, the number of lactic acid bacteria, *Escherichia coli* bacteria, mold fungi, and yeast was determined according to the method **[17]**, peroxide, acid number – according to the methods **[18]**, **[19]**, titrated acidity – according to the method **[12]**, syneresis – determined by the centrifugal method.

Statistical Analysis

The STATISTICA Microsoft Excel editor it in combination with XLSTAT processed experimental data using mathematical statistics methods. The accuracy of the obtained experimental data was determined using the Student's t-test with confidence coefficient ≤0.05 with many parallel definitions of at least 5 (confidence probability $p = 0.95$).

RESULTS AND DISCUSSION

A comparative analysis of the chemical composition of sour cream with vegetable oils and sour cream obtained by classical technology is given in Table 2.

Table 2 Chemical composition of sour cream with vegetable oils in comparison with the control.

Sample	Mass fraction, %		
	Protein	Fat	Carbohydrates
Control	2.6	20.0	3.5
Sour cream with vegetable oils	2.8	20.0	1.8

According to the data in Table 2, it can be seen that the mass fraction of protein in sour cream with vegetable oils is higher by 0.2% compared to sour cream obtained by classical technology. It is explained by the fact that sour cream with vegetable oils consists of 60% skim milk, which chemically has a higher mass fraction of protein **[20]** compared to cream obtained from cow milk **[21]**, which is used in classical technology sour cream.

Also, it should be noted that the protein composition of sour cream with vegetable oils, in comparison with the control parameters, is characterized by the content of sodium caseinate due to the use of finely dispersed food emulsion in which it is included. Therefore, there is an assumption that sour cream with vegetable oils will provide consumers with energy for a long time, since caseinates, compared to other milk proteins, are digested much longer by the human body **[22]**, **[23]**.

As for the content of carbohydrates, their content is lower in sour cream with vegetable oils (by 1.7%) because this product consists of 40% food emulsion, which does not contain carbohydrates, and 60% cow defatted milk, which is a carrier of carbohydrates – lactose, being a growth medium for lactic acid bacteria during the fermentation of a normalized mixture **[24]**, **[25]**. Currently, the reduced lactose content in the food product can positively affect the human body by preventing lactose intolerance **[26]**, **[27]**.

Mass fractions of saturated, monounsaturated and polyunsaturated fatty acids in sour cream with vegetable oils are presented in Table 3.

Table 3 Fatty acid composition of sour cream with vegetable oils in comparison with the control.

Sample	Mass fraction, %		
	Saturated fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids
Control	11.80	7.10	0.52
Sour cream with vegetable oils	2.11	4.17	12.49

Analysis of fatty acid composition (table 3) shows a reduced content of saturated and monounsaturated fatty acids compared to the control by 82.11% and 41.2%, respectively. In addition, a 24-times increased polyunsaturated fatty acids in sour cream with vegetable oils is observed compared to the control.

The content of vegetable oils explains this effect on the fatty acid composition - blended oil (sunflower oil + linseed oil) in the form of a food emulsion within sour cream with vegetable oils, which differ mainly in the unsaturation of fatty acids **[28]** in comparison with the milk fat contained in classic sour cream, which, on the contrary, mainly contains saturated fatty acids **[29]**.

The increased content of polyunsaturated fatty acids in sour cream with vegetable oils is a positive point, as today there is a need to encourage consumers the consumption of such acids against high consumption of saturated ones **[30]**, **[31]** for the prevention of various painful conditions and diseases, in particular, atherosclerosis and ischemic heart disease **[32]**, **[33]**.

The indicator of water activity in sour cream with vegetable oils compared to classic sour cream is given in Table 4.

Table 4 Indicator of water activity in sour cream with vegetable oils and control.

Sample	Water activity indicator, aw
Control	0.988
Sour cream with vegetable oils	0.983

Table 4 shows that the water activity value in sour cream with vegetable oils is lower than in the control. The activity of water in food products is determined by the degree of connection of water with other food ingredients and determines the speed of many chemical and enzymatic reactions occurring in food products **[34]**.

In sour cream, the water phase is bound primarily by milk proteins **[35]**. However, there is a food emulsion in the composition of sour cream with vegetable oils, which contains sodium caseinate forming a gel **[36]** due to better water binding than other milk proteins **[37]**.

According to **[38]**, the shelf life of sour cream obtained by classical technology is 5 days at no higher than 6 °C. That is why the indicated storage conditions of classical sour cream were taken as a standard for experimental determination of the change of titrated acidity, syneresis, microbiological safety indicators, peroxide and an acid number of sour cream with vegetable oils.

The titrated acidity and microbiological indicators of sour cream with vegetable oils in the storage process, presented in Figure 1 and Table 5, respectively, were compared with the normative indicators for classic sour cream.

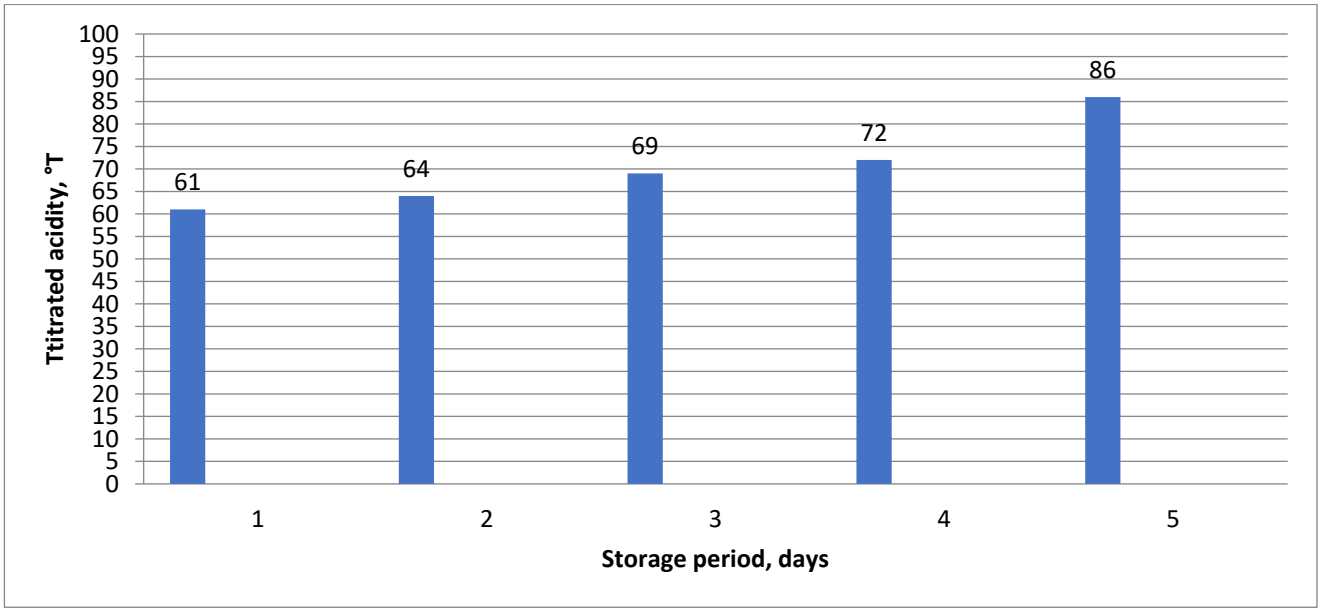

Figure 1 Changes in the titrated acidity of sour cream with vegetable oils during storage.

Table 5 Microbiological parameters of sour cream with vegetable oils during storage.

Indicator	Duration of storage, days					Standard [38]
	1	2	3	4	5	
Number of lactic acid bacteria CFU/g	10^8	10^8	10^7	10^7	10^7	Not less than 1.0×10^7 CFU/g
Escherichia coli bacteria in 0.01 g	Not found					Not allowed in 0.01 g
The number of mold fungi CFU/g						Not more than 50 CFU/g
Amount of yeast, CFU/g						Not more than 50 CFU/g

According to the figure 1, the index of titrated acidity of sour cream with vegetable oils on the fifth day of storage was 86 °T, which meets the regulatory requirements **[38]**, according to which the index of titrated acidity of classic sour cream should be no more than 100 °T.

As seen from the data in Table 5, many viable lactic acid bacteria in sour cream and vegetable oils are within limits at the end of the shelf life.

For the consumer to receive health benefits, fermented milk products shall have viable microorganisms throughout the shelf life **[39]**, **[40]**, **[41]**.

Bacteria and groups of *Escherichia coli*, mould and yeasts were not detected in sour cream with vegetable oils at the end of the shelf life.

The degree of syneresis of sour cream with vegetable oils compared to classic sour cream in the storage process is presented in Figure 2.

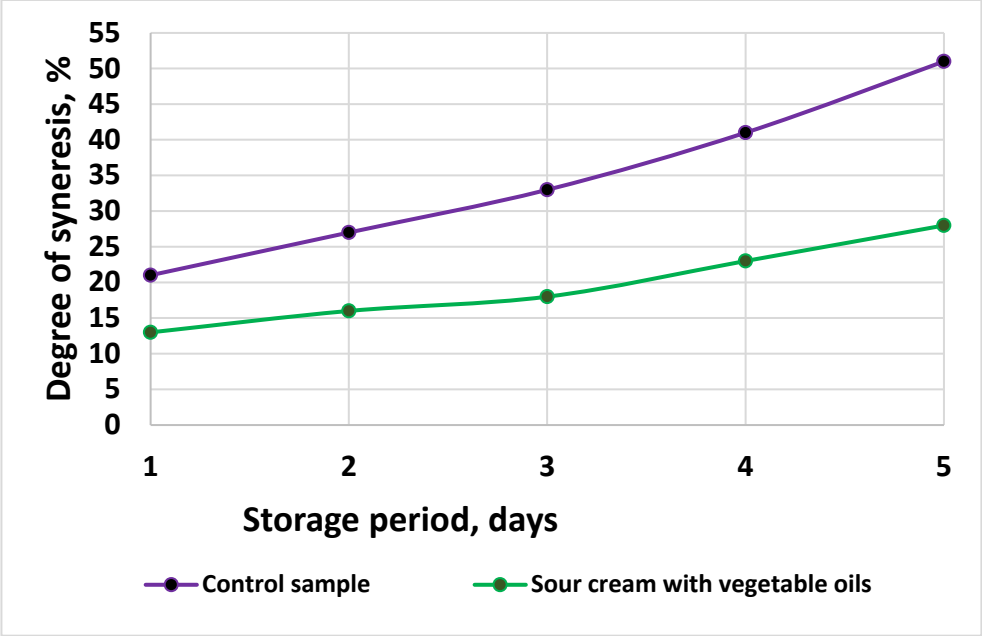

Figure 2 Degree of syneresis of sour cream with vegetable oils compared to classic sour cream during storage.

From the data in Figure 2, it can be seen that the value of syneresis on the first day of storage of sour cream with vegetable oils is 12%, which is lower than the value of syneresis in the control sample (21%). At the end of the storage period, the value of syneresis in sour cream with vegetable oils (28%) is 23% lower than the value of syneresis in the control sample, which is 51%.

The degree of syneresis depends on the gel's ability to hold bound water **[42]**. The decrease in the degree of syneresis occurred due to the moisture-binding capacity of sodium caseinate **[43]**, contained in the food emulsion, and xanthan gum **[44]**, which are components of sour cream with vegetable oils. Considering this, the decrease in syneresis in sour cream with vegetable oils correlates with the decrease in the water activity indicator (Table 4).

The index of peroxide and acid number of sour cream with vegetable oils during the storage process, presented in Table 6, was compared with the normative indicators for blended oil **[45]**, which is the fat phase of this product.

Table 6 Indicator of peroxide and acid number of sour cream with vegetable oils during storage.

Indicator	Duration of storage, days					Standard [42]
	1	2	3	4	5	
Peroxide number, ½ O mmol/k	3.0	3.0	3.0	3.0	4.0	not more than 10 ½ O mmol/k
Acid value, mg acid number/g	2.5	2.5	2.5	2.6	2.6	no more than 4.0 mg acid number/g

According to the data in the Table 6, the peroxide and acid numbers are within the established norms during the standard storage period in accordance with **[38]** in sour cream with vegetable oils.

Vegetable oils are more prone to oxidative deterioration with the formation of compounds of a peroxide nature, due to a significant content of unsaturated fatty acids **[46]** in comparison with animal fats, the composition of which is mainly represented by saturated fatty acids **[47]**, **[48]**.

Therefore, the peroxide number is one of the main safety indicators, which determines the degree of freshness of oils and food products based on them **[49]**.

Therefore, according to the research results, the ingredients of the recipe composition and the temperature regimes for the production of sour cream with vegetable oils do not affect the quality and safety indicators and are within the standard values for sour cream obtained traditionally.

CONCLUSION

There was conducted a study of the chemical composition and water activity of sour cream with vegetable oils, which contains a finely dispersed food emulsion with a fat content of 50% based on blended oil (sunflower oil + linseed oil), with a comparison with the normalized indicators of classic sour cream. The changes in microbiological indicators, peroxide, and acid number values in sour cream with vegetable oils were studied during 5 days of storage at temperatures of 0 – 6 °C as a standard period and mode of storage of classic sour cream. The determined chemical composition of sour cream with vegetable oils showed that the protein content is increased by 0.2%, and the carbohydrate content is reduced by 1.7% compared to classic sour cream, which is explained by the content of skimmed milk and food emulsion in it, which does not contain carbohydrates. According to the study of the fatty acid composition of sour cream with vegetable oils, an increased polyunsaturated fatty acid (12.49%) was revealed compared to classic sour cream (0.52%). The content of saturated and monounsaturated fatty acids in sour cream with vegetable oils compared to classic sour cream is reduced by 82.11% and 41.2%, respectively. A reduced indicator of water activity was established in sour cream with vegetable oils (0.983 aw), compared with classic sour cream (0.988 aw). According to the date of the 5th day of storage, sour cream with vegetable oils has an index of titrated acidity of 86 °T, the number of viable lactic acid bacteria is 10^7 CFU/g, and bacteria of the group of *Escherichia coli*, mold and yeast were not detected, which coincides with the normalized indicators as to sour cream obtained by classical technology. The value of syneresis in sour cream with vegetable oils on the fifth day of storage is 28%, which is 23% less than the value of syneresis in the control sample. It was found that the value of peroxide and acid number of sour cream with vegetable oils during 5 days of storage is within the normalized values for blended oil (sunflower oil + linseed oil) and are (3.0-4.0) ½ O mmol/k and (2.5-2.6) mg acid number/g, respectively. So, the developed sour cream with vegetable oils is characterized by an improved fatty acid composition in terms of the content of polyunsaturated fatty acids, and its quality and safety indicators are within the normative values for classic sour cream.

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Application of the Se NPs-Chitosan molecular complex for the correction of selenium deficiency in rats model

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ABSTRACT

Selenium is an integral component of vital biologically active compounds of the human body. Currently, the population of many countries is characterized by selenium deficiency. In this regard, many preparations of inorganic and organic forms of selenium have been developed. Nevertheless, it is evident that the most effective solution to the problem is to enrich the diet with bioavailable forms of selenium. Thus, this work aimed to synthesize and study the antioxidant and immunomodulatory effects of the molecular complex of selenium nanoparticles (Se NPs) and chitosan in laboratory rats with induced hyposelenosis. During the experiment with animals, we found that as a result of 70-day consumption of food with a low selenium content, rats develop an alimentary selenium deficiency state, as evidenced by a significant decrease in the content of this trace element in control group rats to 48.2 ±6.71 μg/kg versus 149.3 ±21.63 μg/kg in intact animals. Course, administration of the molecular complex Se NPs- Chitosan to rats of the experimental group, contributed to the replenishment of selenium deficiency: its concentration in the blood of animals was 96.6 ±3.57 μg/kg. Thus, in animals of the control group, there was a decrease in the total number of lymphocytes by 2.7 times, T-lymphocytes – by 1.8 times, and B-lymphocytes – by 2.3 times compared with similar data in intact animals. In the context of hyposelenosis, it is worth mentioning that there was a slight increase in the content of T-helper cells and cytotoxic T-lymphocytes. The synthesized Se NPs – Chitosan complex administration during hyposelenosis demonstrated a notable immunomodulatory effect by restoring the body's immune response indicators. Thus, the total number of lymphocytes increased by 3 times, T-lymphocytes – by 1.9 times, and B-lymphocytes – by 2 times. The number of T-helper cells and cytotoxic T-lymphocytes increased by 1.9 times compared to the group of intact animals and 1.6 times compared to selenium-deficient rats. Thus, the course introduction of the molecular complex Se NPs – Chitosan against the background of selenium deficiency was accompanied by inhibition of free radical oxidation processes, activation of the antioxidant system and restoration of the immune status of the organism of laboratory animals.

Keywords: selenium, polysacharides, selenium deficiency, immunity

INTRODUCTION

The organism's most pronounced dependence on biogeochemical factors manifests in the form of endemic diseases caused by a sharp deficiency, excess or imbalance of trace elements in the biogeochemical food chain **[1]**. One of the essential trace elements is selenium. It is an integral component of more than 30 vital biologically active compounds of the human body **[2]**. As part of the antioxidant enzyme glutathione peroxidase, selenium protects cells from excess oxygen, peroxides and free radicals **[3]**, **[4]**. Selenium stimulates the conversion of methionine into cysteine and the synthesis of glutathione **[5]**. The selenium protein complex catalyzes the biosynthesis of thyroid hormones **[6]**. About 75 different pathologies are associated with the deficiency of this trace element. People with hyposelenosis have a low life expectancy due to premature ageing **[7]**. Selenium is

necessary for the normal functioning of the immune system, both cellular and humoral: it stimulates the function of natural killers **[8]**; increases the production of interleukin-1 and interleukin-2 **[9]**; suppresses immediate-type hypersensitivity and delayed-type hypersensitivity **[10]**; modulates the phagocytic function of polymorphonuclear leukocytes **[11]**; potentiates the function of natural killers and antibody genesis **[12]**; has anti-apoptogenic and radioprotective effects **[13]**; blocks the transcription of viruses, including the AIDS virus **[14]**. Selenium has a powerful immunomodulatory activity **[15]**.

Selenium enters the body with food and water. Depending on the type of soil and the underlying rocks, a different amount of it is assimilated by plants and gets into human and animal food **[16]**. Regions with a selenium content in the soil below 50 µg/kg are endemic **[17]**. The low content of trace element in soils is associated with its lack in the underlying soils; the presence of a layer of permafrost prevents its leaching from deep layers into surface ones; intensification of agricultural production **[18]**. An adequate dose of selenium, depending on the region of residence, ranges from 50 to 200 µg/day and is at least 70 µg for adult men and 55 µg for adult women (at least 1 µcg/kg/day) **[19]**. One of the most important advantages of biologically active additives (dietary supplements) with organic selenium compounds is, in addition to low toxicity, their wide possibilities for accumulation and deposition in the body. When an excess of selenomethionine and selenocysteine enter the body, they are easily incorporated into protein molecules instead of methionine and cysteine **[20]**. The capacity of the "protein depot" in the body is quite large. This is due to the low toxicity of selenomethionine compared to sodium selenite **[21]**. Recently, complex dietary supplements based on inorganic and organic selenium compounds have been developed and proposed for practical use **[22]**. At the same time, the list of selenium-containing medicines is not so large, and some of them have several disadvantages, such as toxicity, rapid elimination from the body, and some others **[23]**.

Moreover, a more effective solution to the problem of selenium deficiency is associated with the enrichment of food products in the daily diet instead of numerous offers of selenium-containing medicines. In this regard, the current direction of the modern food industry is the development of new selenium-containing complexes, the most effective and safe for use in food products. Thus, this work aimed to synthesize and study the antioxidant and immunomodulatory effects of the molecular complex of selenium nanoparticles (Se NPs) and chitosan in laboratory rats with induced hyposelenosis.

Scientific Hypothesis

The use of the bioavailable molecular complex Se NPs-Chitosan in the diet is an effective solution to the problem of selen deficiency. The supplementation of feed with molecular complex Se NPs-Chitosan will increase Se content in rats' blood. Also, we are expecting that the course introduction of the molecular complex Se NPs-Chitosan against the background of selenium deficiency will cause inhibition of free radical oxidation processes, activation of the antioxidant system and restoration of the immune status of the organism of rats.

MATERIAL AND METHODOLOGY

Samples

Molecular complex Se NPs-Chitosan.

Chemicals

We used reagents of recognized analytical purity and distilled water. The work used the following chemicals: Ethanol, Sodium hydroxide, Sodium Selenite, Ascorbic acid, and Chitosan. All chemicals above were purchased by LenReactive LLC (Sants Petersburg, Russia) and were of analytical grade quality.

Animals and Biological Material

Experimental work was carried out on 50 white male rats weighing 150-160 g in standard vivarium conditions.

Instruments

Magnetic Stirrer IKA I-MAG (ChimMed, Russia), pipet dispenser Vitlab micropipette (Vitlab, Moscow, Russia), biochemical blood analyzer Olympus AU 400 (Olympus Europa SE & Co. KG, Hamburg, Germany), hematological analyzer MEK 7222 (NihonKohden, Tokio, Japan), flow cytofluorimeter Cytomics FC 500 (Beckman Coulter, New York, USA), the device for the total determination of antioxidants Tsvet Yauza-AA-01 (ChemAutomatika, Moscow, Russia), liquid chromatograph Shimadzu 20-AD (Shimadzy, Tokio, Japan), X-ray diffractometer Shimadzu XRD 7000 (Shimadzy, Tokio, Japan), spectrophotometer Shimadzu UV-2600 (Shimadzy, Tokio, Japan), laser analyzer Shimadzu Sald 2300 (Shimadzy, Tokio, Japan), scanning electron mycroscope Sigma Ziess (Carl Zeiss QEC GmbH, Koln, Germany).

Laboratory Methods

Sodium selenite and ascorbic acid were used to obtain Se NPs in solution. Se NPs were stabilised using chitosan with a degree of deacetylation of about 75%. Transmission electron microscopy was performed using a

scanning electron microscope Sigma Zeiss (Carl Zeiss QEC GmbH, Cologne, Germany). X-ray diffraction analysis was performed using X-ray diffractometer Shimadzu XRD 7000 (Shimazu, Tokyo, Japan). The size distribution was studied using laser analyzer Shimazu Salt 2300 (Shimaji, Tokyo, Japan). Optical properties of the synthesized molecular complex were sudied with spectrophotometer Shimadzu UV-2600 (Shimaji, Tokyo, Japan).

The fluorimetric method determined the selenium content in the blood of experimental animals **[24]**. Indicators of the immune status of the body: the total number of lymphocytes, T-lymphocytes (TL), T-helper (TH) cells $CD^{3+}CD^{4+}$, cytotoxic T-lymphocytes (CTL) $CD^{3+}CD^{8+}$, B-lymphocytes (BL), natural killers (NK), were determined by the method of flow cytofluorimetry **[25]**. To assess the activation of autoimmune processes, the ratio of TH/CTL was calculated **[26]**. The intensity of free radical oxidation processes in blood plasma and erythrocytes was determined by the accumulation of malondialdehyde (MDA) **[27]**. Overall antioxidant activity was determined as the main indicator of antioxidant protection **[28]**.

Description of the Experiment

Sample preparation: The Se NPs-Chitosan molecular complex was prepared as follows: ascorbic acid (0.35 g/l) and sodium selenite (0.15 g/l) were added to 0.25 g/l chitosan solution. The resulting solution was thoroughly mixed on a magnetic stirrer IKA I-MAG (ChimMed, Russia) for 30 minutes. As a result of the redox reaction, a red colloidal solution was formed.

Number of samples analyzed: 3.

Number of repeated analyses: 3.

Number of experiment replication: 1.

Design of the experiment: At the first stage, the molecular complex Se NPs-Chitosan was synthesized. Various research methods were used to characterize the synthesized molecular complex. The obtained samples' structure, shape, morphology, and size were investigated. Hyposelenosis in laboratory animals was modelled by alimentary selenium deficiency, for which the animals were kept on a diet with a low selenium content (14 µg/kg) for 70 days. Animals with hyposelenosis were intragastrically administrated with Se NPs-Chitosan complex at a dose of 0.5 µg/kg in a volume of 10 ml/kg once a day for 10 days. Animals of the control group received an equivalent volume of distilled water according to a similar scheme. For the purity of the experiment, a group of intact rats with the usual daily diet, which did not cause hyposelenosis, was also used in the work.

Statistical Analysis

Statistical processing of the results was performed with elements of nonparametric statistics using the Statistica 12.0 software package (StatSoft, USA) using the Student's T-test (p <0.05).

RESULTS AND DISCUSSION

Figure 1 shows the characteristics of the resulting NPs. The NPs consist of crystalline selenium, have a spherical shape, and have a diameter of 30-40 nm, typical for Se NPs stabilized with polysaccharides **[29]**. The electromagnetic absorption spectrum in the UV/visible region peaks at 400 nm, similar to the results obtained in other works **[30]**, **[31]**. According to previous studies, the effect of the selenium: polysaccharide mass ratio was determined by the saturation region of the adsorption capacity, which affects the formation process and morphological characteristics of nanostructures **[29]**, **[32]**. According to UV spectroscopy, viscometry and pH-metre, this region corresponds to a range of mass ratios (v) from 0.02 to 0.08 **[33]**. In this work, a selenium nanocomposite with a mass ratio of v = 0.04 was used, a selenium nanocomposite with a mass ratio of v = 0.04 was used The X-ray diffraction pattern showed the presence of peaks characteristic of polycrystalline selenium **[34]**. Thus, we have confirmed that nanoscale selenium has been synthesized. During the 10 days of the experiment, the particles did not aggregate, which confirms the stability of the Se NPs-Chitosan molecular complex.

Similar Se NPs-Chitosan complexes have been synthesized and studied by other researchers. Song et al. used TEM to study the cellular uptake and intracellular distribution of Se NPs modified with chitosan in HepG2 cells **[35]**. Se NPs have been localized in intracellular structures such as endosomes and lysosomes. El-Megharbel et al. demonstrated that the novel Se NPs-Chitosan complexes had a potent effect against Diclo-Na-induced testicular toxicity and hormonal disturbance in male rats, particularly high oxidative stress, thus protecting against testicular necrosis and dysfunction **[36]**. Abozaid et al. demonstrated the therapeutic effect of Se NPs-Chitosan against experimentally induced diabetes mellitus in adult male rats **[37]**. This effect is afforded by the antioxidant, hypoglycemic, and hypolipidemic properties of Se NPs-Chitosan, resulting from using selenium and chitosan. All these findings point out the relevance and importance of the study and characterization of synthesized Se NPs-Chitosan complex.

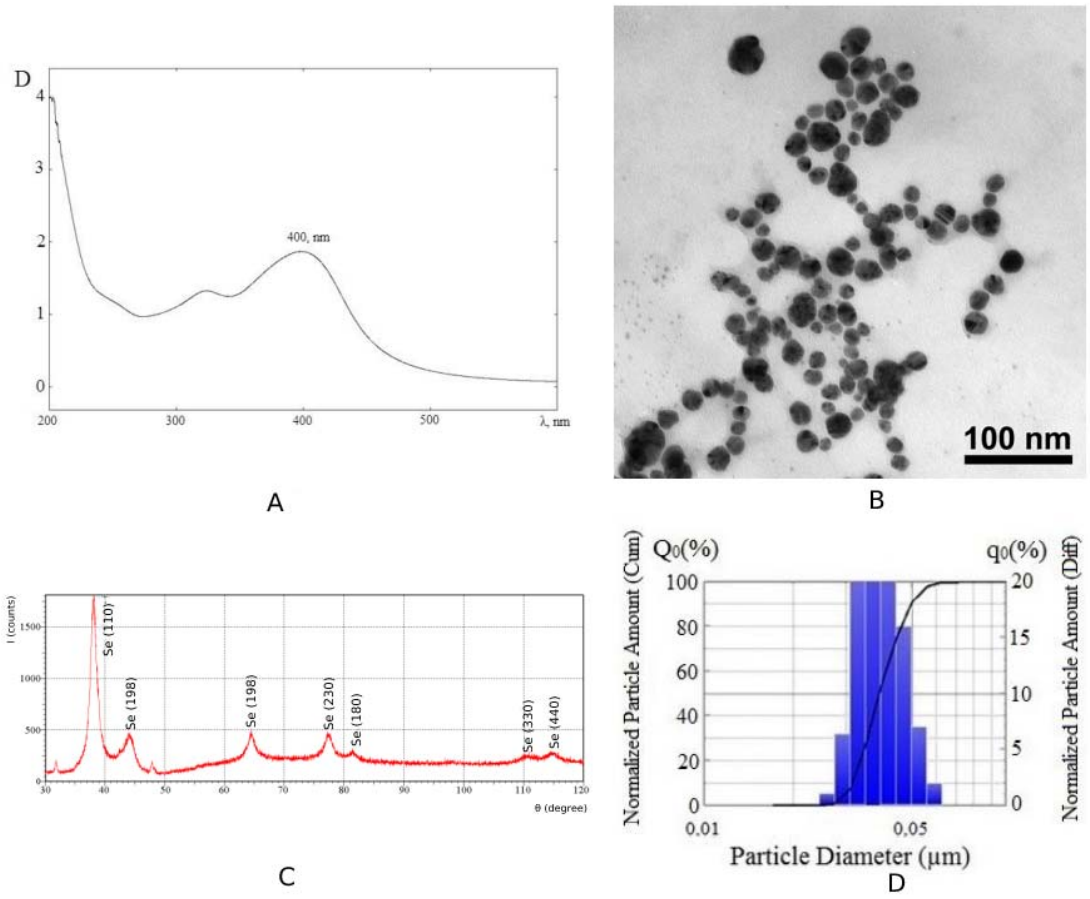

Figure 1 Characteristics of obtained Se NPs stabilized with Chitosan. Note: UV/visible absorption spectrum (A), transmission electron microscopy (B), X-ray pattern (C), size distribution of nanoparticles (D).

During the experiment with animals, we found that as a result of a 70-day intake of food with a low selenium content, rats develop a nutritional selenium-deficient state, as evidenced by a significant decrease in the content of this trace element in rats of the control group to 49.2 ±6.2 μg/kg versus 129.8 ±22.3 μg/kg in intact animals. The course administration of the molecular complex Se NPs-Chitosan to the rats of the experimental group contributed to the replenishment of the deficiency of selenium: its concentration in the blood of the animals was 99.1 ±5.6 μg/kg (differences compared with the data of the rats of the control group are significant, p <0.05). Our results showed that, against the background of selenium deficiency, animals develop oxidative stress, as evidenced by a significant increase in the concentration of MDA in the blood serum and erythrocytes of rats in the control group, as well as a decrease in the total antioxidant activity (Table 1). Other works also confirm this **[38]**, **[39]**.

Table 1 Effect of the molecular complex Se NPs-Chitosan on the parameters of free radical oxidation processes in experimental selenium deficiency.

Index	Group		
	Intact group n **= 10**	**Control group** n **= 10**	**Experimental group** n **= 10**
MDA in blood serum, μmol/mg lipids	3.6 ±0.1	9.7 ±0.6	3.9 ±0.4
MDA in erythrocytes, μmol/mg lipids	42.3 ±1.2	113.8 ±5.5	45.7 ±1.3
Total antioxidant activity, %	4.5 ±0.1	2.1 ±0.1	4.4 ±0.1

Note: Significance of differences (p <0.05) was observed between all groups.

A two-fold average reduction in the concentration of MDA in the blood serum and erythrocytes of the animals in the experimental group, as well as an increase in the total antioxidant activity, show that the course administration of the test agents against the background of selenium deficiency had an antioxidant effect. It should

be highlighted that following the addition of the synthetic compound Se NPs-Chitosan, the levels of MDA and total antioxidant activity were reduced to those of intact laboratory animals, and the levels of these antioxidants were increased.

Moreover, hyposelenosis has been shown to cause immunodeficiency in experimental animals, as shown by a sharp decline in the parameters of the humoral connection to immunity (Table 2) **[40]**. Thus, in animals of the control group, a decrease in the total number of lymphocytes was observed to 2.7 times, TL – by 1.8 times, BL – by 2.3 times compared with similar data in intact animals. It should be noted that against the background of hyposelenosis, the content of TC and CTL slightly increased, but the ratio of TC and CTL practically did not change. The course introduction of the synthesized complex Se NPs-Chitosan against the background of hyposelenosis had a significant immunomodulatory effect, restoring the parameters of the body's immune response. Thus, the total number of lymphocytes increased 3 times, TL – 1.9 times, BL – 2 times. The amount of TC and CTL in such rats only tended to increase: their number increased by 1.9 times compared with the group of intact animals and by 1.6 times compared with selenium-deficient rats. Similar results have been previously reported by Bai et al. **[41]**. At the same time, the content of NK decreased (differences are statistically significant, $p < 0.05$) in the experimental group. Obviously, this phenomenon is explained by the fact that these cells perform their function much earlier than CTL and are the "first line" of the body's defense. Therefore, a decrease in their number is observed against the background of activation of the adaptive link of immunity **[42]**, **[43]**. The data obtained indicate that using the Se NPs-Chitosan molecular complex not only restores the parameters of the immune response, but also causes a pronounced activation of the adaptive link of immunity, which is confirmed in other works **[44]**, **[45]**, **[46]**. The immunity of rats with hyposelenosis in the experimental group was restored to the physiological norm.

Table 2 Influence of the molecular complex Se NPs-Chitosan on indicators of immunity in the selenium-deficient state in animals.

Index	Group		
	Intact group $n = 10$	**Control group** $n = 10$	**Experimental group** $n = 10$
Total number of lymphocytes, %	60.7 ±4.2	22.5 ±3.4	67.7 ±5.2
TL, %	75.5 ±3.6	41.6 ±4.5	79.6 ±6.1
TH ($CD^{3+}CD^{4+}$),%	51.2 ±3.3	56.3 ±5.1	49.9 ±2.8
CTL ($CD^{3+}CD^{8+}$), %	37.2 ±2.1	42.1 ±3.6	70.1 ±6.2
TH/CTL	1.37 ±0.1	1.4 ±0.1	0.7 ±0.1
BL, %	70.1 ±3.2	31.4 ±2.4	62.7 ±5.3
NK, %	12.8 ±0.3	13.9 ±0.7	9.6 ±0.6

Note: TL – T-lymphocytes, TH – T-helpers, CTL – cytotoxic T – lymphocytes, BL – B-lymphocytes, NK – natural killers. The significance of differences ($p < 0.05$) was observed between all groups.

Thus, the course administration of the Se NPs-Chitosan molecular complex against the background of a selenium-deficient state was accompanied by inhibition of free radical oxidation processes, activation of the antioxidant system, and restoration of the immune status of the body of laboratory animals. It is known that selenium is an indispensable component of the immune control system **[47]**. However, the synthesized molecular complex also contained chitosan, an immunomodulator **[48]**, **[49]**, **[50]**. In particular, the antioxidant mechanism of action of the synthesized molecular complex can also be based on the ability to protect capillary walls from the damaging effects of free radicals by neutralizing reactive oxygen species and terminating free radical chain reactions **[51]**, **[52]**.

With selenium deficiency, a lack of deiodinases of various types is formed, the formation of TL decreases, leading to stimulation of the hypothalamic-pituitary axis by the negative feedback system and an increase in the synthesis of thyroid-stimulating hormone (TSH) **[6]**. TSH stimulates the production of thyroid hormones and increases the activity of deiodinases, restoring the level of thyroid hormones. But at the same time, it stimulates the formation of hydrogen peroxide, for the inactivation of which, again, selenoprotein – glutathione peroxidase is required, the activity of which is reduced in conditions of selenium deficiency **[53]**. Hydrogen peroxide accumulates in the thyroid gland, which leads to damage to thyrocytes and the development of fibrosis **[54]**. Increased formation of hydrogen peroxide in the thyrocyte is observed in all cases of excessive thyroid stimulation by TSH, for example, in patients with autoimmune thyroiditis and subclinical hypothyroidism. As a result, there is damage to thyrocytes, the progression of hypothyroidism and the development of fibrosis. In such a situation, selenoproteins, having antioxidant activity, can prevent or at least slow down the destruction of thyrocytes and decrease their functional activity **[55]**.

Selenium-containing enzymes (iodothyronine deiodinase, glutathione peroxidase, and thioredoxin reductase), in addition to influencing thyroid metabolism, also play a significant role in organ-specific immune reactions **[11]**. According to several scientific papers, selenium in chronic inflammatory lesions of the thyroid gland protects follicles from oxidative stress and infiltration by autoreactive cells, reducing the production of pro-inflammatory cytokines **[56]**, **[57]**, **[58]**. Moghaddam et al. showed that selenium deficiency and, accordingly, a decrease or absence of glutathione peroxidase activity contributes to oxidative damage, thyroid damage and the development of fibrosis **[59]**. It can be assumed that even with moderate selenium deficiency, this mechanism is an important environmental factor initiating or supporting autoimmune thyroiditis **[60]**. Glutathione peroxidase can reduce the concentration of hydrogen peroxide and hydroperoxides, thereby reducing the spread of free radicals and reactive oxygen species. A decrease in the concentration of lipid hydroperoxides and phospholipids reduces the production of inflammatory cytokines **[61]**. A possible decrease in the immunomodulatory effects of glutathione peroxidase and thioredoxin reductase in selen deficient conditions switches the cytokine pattern towards a Th-2-dependent immune response, which leads to an intensification of inflammatory reactions in the body against the background of autoimmune processes or infections.

Thus, selenium-dependent enzymes give antioxidant and anti-inflammatory effects. This is because selenoproteins reduce lipid and phospholipid hydroperoxides, reducing the amount of free radicals and reactive oxygen species. A decrease in the concentration of hydroperoxides in tissues inhibits the formation of inflammatory prostaglandins and leukotrienes **[62]**. This mechanism may contribute to a decrease in inflammatory activity in the organ-specific autoimmune response and may serve as an explanation for the data obtained on a decrease in the content of lymphocytes **[63]**. Probably, a significant decrease in the concentration of natural killers in animals of the experimental group was achieved due to the indicated mechanism of selenium exposure. Our studies have shown that even with a slight deficiency of selenium, additional intake of this trace element has a clinically significant effect on the anti-inflammatory activity of the body.

The molecular complex Se NPs-Chitosan has a wide range of potential applications in specialized food products for therapeutic and prophylactic appointments. For example, it can be used as an alternative to the delivery of bioactive peptides with the potential as an emulsion stabilizer for food applications **[64]**, as a composite for active food packaging **[65]**, **[66]**, for extension of shelf life of dairy products **[67]** and minced meat **[68]**. Chen et al. reported that Se NPs-Chitosan complex has anticancer, anti-diabetic, antibacterial, and hepatoprotective activities and can improve the nutraceutical value of animals and crops for human consumption **[69]**. Golmohammadi et al. also confirmed that the nano complex Se NPs-Chitosan might be a development for treating diabetic wound infection at mild stage **[70]**. All this determines future directions in studying and applying the synthesized molecular complex Se NPs-Chitosan.

CONCLUSION

For the experiment, we synthesized the Se NPs-Chitosan molecular complex. It is established that the nanoparticles consisted of crystalline selenium, and had a spherical shape with a diameter of 30-40 nm. The electromagnetic absorption spectrum in the UV/visible region showed a peak of 400 nm. The X-ray showed the presence of peaks characteristic of polycrystalline selenium. Thus, we confirmed that we synthesized nanoscale selenium. During 10 days of the experiment, the particles were not aggregated, which confirms the stability of the Se NPs-Chitosan molecular complex. During the experiment with animals, we found that as a result of 70-day consumption of food with a low selenium content, rats developed an alimentary selenium deficiency state, as evidenced by a significant decrease in the content of selenium in the control group rats to 49.2 ±6.2 μg/kg versus 129.8 ±22.3 μg/kg in intact animals. Course administration of the molecular complex Se NPs-Chitosan to rats of the experimental group contributed to the replenishment of selenium deficiency: its concentration in the blood of animals was 99.1 ±5.6 μg/kg. Thus, in animals of the control group, there was a decrease in the total number of lymphocytes by 2.7 times, T-lymphocytes – by 1.8 times, and B-lymphocytes – by 2.3 times compared with similar data in intact animals. It should be noted that against the background of hyposelenosis, the content of T-helper cells and cytotoxic T-lymphocytes increased slightly. The course administration of the synthesized Se NPs-Chitosan complex against the background of hyposelenosis had a significant immunomodulatory effect, restoring the indicators of the body's immune response. Thus, the total number of lymphocytes increased by 3 times, T-lymphocytes – by 1.9 times, and B-lymphocytes – by 2 times. The number of T-helper cells and cytotoxic T-lymphocytes increased by 1.9 times compared to the group of intact animals and 1.6 times compared to selenium-deficient rats. Thus, the course introduction of the molecular complex Se NPs-Chitosan against the background of selenium deficiency was accompanied by inhibition of free radical oxidation processes, activation of the antioxidant system and restoration of the immune status of the organism of laboratory animals. The obtained results provide a basis for further exploration of the Se NPs-Chitosan molecular complex in developing specialized food products for therapeutic and prophylactic applications.

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The authors declare no conflict of interest.

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The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. Experiment on white male rats was approved by Ethics Comission of Rostov State Medical University (Protocol #17 from 01.04.2022).

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Supply chain management analysis of avocado in south Sumatra province through the Food Supply Chain Network (FSCN) method

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ABSTRACT

One of the agricultural sub-sectors that occupy a strategic position in agricultural development is the horticultural sub-sector, with one of its potential commodities being avocado. Avocado is one of the export-based commodities, especially in South OKU Regency, South Sumatra Province. This study aims to obtain an overview of the Avocado Agribusiness Supply Chain management in South OKU Regency. The study was conducted in Warkuk Selatan District, South OKU Regency in February 2022. The research method used was a qualitative descriptive method with a type of data using primary data and secondary data. The data analysis method uses the Food Supply Chain Network (FSCN) method, which illustrates South Oku Regency's avocado supply chain model. The results showed that the Avocado Management Supply Chain Management model with the FSCN framework consisted of four main components: the supply chain structure, the business chain process, supply chain management, and supply chain resources. Avocado supply chain targets are still dominated to meet the domestic market and products in the form of fresh avocados for consumption. Avocado supply chain management comprises election partners, contractual agreements, transaction systems, government support, and supply chain collaboration. The avocado supply chain structure in South OKU Regency consists of farmers, collecting traders, local and non-local traders, retailers, and consumers with respective roles in the supply chain structure. Business processes in avocado supply chain management consist of procurement, replenishment, and customer order cycles. Based on marketing margin analysis, the lowest total marketing margin is found in channel IV, with a margin value of Rp 1,500 per kg. The four avocado marketing channels in South Warkuk Ranau District have Farmer's Share ≥40%, so it is categorized as an efficient channel.

Keywords: horticulture, avocado, agribusiness, management, FSCN

INTRODUCTION

Agriculture is still believed to be one of the roots of the Indonesian economy. The agricultural sector is one of the sectors which contribute to Indonesia's economy **[1]**. The horticultural sub-sector occupies a strategic position in agricultural development. The contribution of the horticultural subsector to agricultural development continues to increase, as seen in several indicators of economic growth, such as gross domestic product (GDP), export value, and employment.

One of the horticultural commodities that have prospects with growing market potential is the fruit commodity. Fruits are horticultural products that significantly impact Indonesian agriculture **[2]**. In other sectors, fruits also play a role in increasing farmers' income. Commodities that have the potential to be developed so that market needs can be met and benefit from farmers, namely avocados **[3]**. The fruit known as the avocado is sold worldwide and is renowned for providing various health benefits **[4]**.

Avocado (*Persea americana Mill*) is a plant originating from the highlands of Central America and has many varieties that are spread throughout the world. The avocado is an evergreen tree, although the leaves have a

surprisingly short longevity of 12 months. It is characterized by rapid growth in height and spread, reaching heights up to 20 m, its roots are shallow and have poor water uptake and hydraulic conductance. The trees generate many blooms, but less than 0.1% of those flowers often turn into fruit **[5]**. The climacteric fruit avocado (*Persea americana*) is primarily consumed as a vegetable. Avocados, like olives, are abundant in oleic acid, a monounsaturated fatty acid, health-enhancing phytosterols, and phenolic antioxidants **[6]**.

Table 1 Top 10 avocado-producing countries in the world.

Nr.	Country	Production (Tons)
1	Mexico	2,442,944.64
2	Colombia	979,617.72
3	Peru	777,095.96
4	**Indonesia**	**669,260,46**
5	Dominican Republic	634,368.16
6	Kenya	416,802.72
7	Brazil	300,894.00
8	Haiti	248,135.12
9	Vietnam	212,977.00
10	Chile	169,031.26

Note: Source – Food and Agriculture Organization (FAO), 2021.

According to data from the Food and Agricultural Organization (FAO) in 2021, Mexico is the world's center of avocado production, accounting for the first position with a total production value of 2,442,944 tons, while Indonesia ranks fourth with a production value of 669,260 tons. Avocado is an agricultural commodity with a harvest time of approximately 6-7 months from when flowers bloom. If planted through the vegetative system will bear fruit after 5-8 years old, but several effective ways can make avocado plants bear fruit within 3 years, namely by graft and shooting shoots. Avocado-producing areas in Indonesia are West Java, East Java, South Sulawesi, and South Sumatra (Figure 1). In South Sumatra, one of the areas producing avocado is South OKU Regency, with the most production in South Warkuk Ranau (Figure 2).

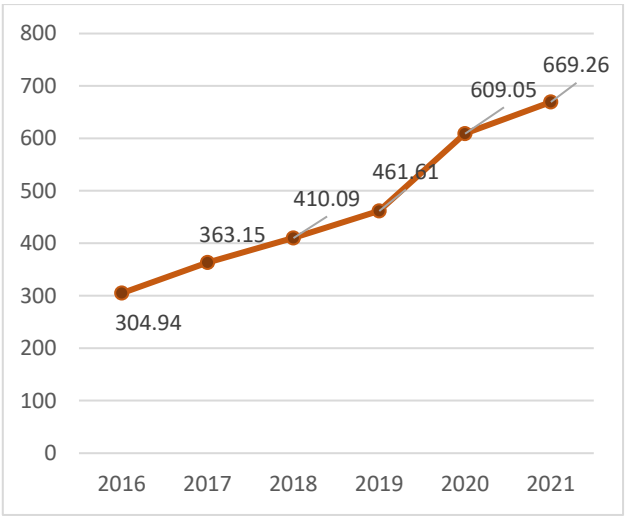

Figure 1 Avocado production in Indonesia.

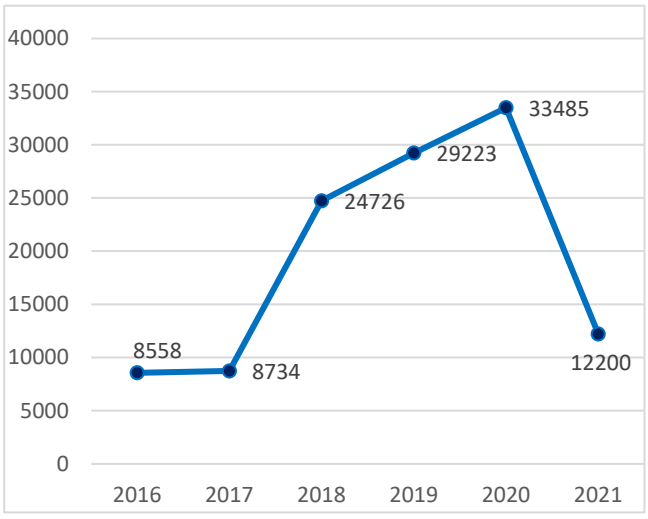

Figure 2 Avocado production in South Oku Regency.

Avocado Fruit Production in South Ogan Komering Ulu (OKU) Regency has increased from 2016-2018 with a very significant increase in 2018, avocados, including one of the commodities from the horticultural sector that is being developed, especially in South OKU Regency. In 2020 Indonesia was hit by the Covid-19 Pandemic which paralyzed the community's economy and required to stop some marketing supply chains that farmers usually dofarmers in their daily habits usually do. The marketing supply chain is an important defence in fighting Covid-19. In the current competition, business actors are required to realize that competition that occurs is competition between supply chain networks **[7]**. As an avocado center area in the Province of South Sumatra, the development of agribusiness fruit applications experiences various problems ranging from upstream subsystem agribusiness, cultivation, downstream agribusiness, institutional, and marketing. Where all of them are interrelated to each other in a supply chain.

The Agricultural Quarantine Agency, working with the Center for Plant Quarantine and Biosafety, has developed an avocado phytosanitary certification system based on in-line inspection in the form of Avocado Phytosanitary Certification Guidelines to support the export of avocados. This directive will act as a reference for all parties in organizing the export of avocados from Indonesia so that the exported avocados meet the requirements of the destination country, the quality of the fruit is properly maintained, the fruit is safe for consumption, and the exported fruit has good traceability. The Agricultural Quarantine Agency believes that a guideline for avocado phytosanitary certification, which will be used as a reference for all parties in implementing avocado exports, is specifically required to assist the export of avocados. Indonesia as well as to guarantee that exported avocados are of high quality, suitable for human consumption, and match the standards of the destination nation. The following constitutes the legal justification for avocado certification **[8]**:

i. Regulation of the Minister of Agriculture of the Republic of Indonesia Number: 44/Permentan/OT.140/10/2009 Concerning Guidelines for Post-Harvest Handling of Yields Agriculture of Good Plant Origin (Good Handling Practices).
ii. Regulation of the Minister of Agriculture Number: 48/Permentan/OT.140/10/2009 concerning About Guidelines for Good Agriculture Practices for Fruit and Vegetables (Good Agriculture Practices For Fruit and vegetables).
iii. Regulation of the Minister of Agriculture Number: 88/Permentan/PP.340/12/2011 concerning Food Safety Supervision on Importation and Exportation of Fresh Food Plant Origin.
iv. Regulation of the Minister of Agriculture Number: 73/Permentan/OT.140/7/2013 concerning Guidelines for Harvesting, Postharvesting, and Management of Horticultural Postharvest Wards The good one.

According to the UNECE standard on avocados **[9]**, based on their quality, Avocados are classified under three classes, as defined below:

1. Class "Extra"

This category only accepts avocados of the highest caliber. They have to be traits exclusive to the variety. They must be devoid of flaws, except minor surface flaws, so long as they don't damage the produce's overall appearance, quality, keeping quality, or presentation in the packaging. The stalk must be intact if it is there.

2. Class 1

Avocados must be of high caliber to qualify. They have to be traits exclusive to the variety. However, suppose the following minor flaws don't damage the produce's overall appearance, quality, keeping quality, or presentation in the packaging. In that case, they may be tolerated: A minor shape flaw, a minor color flaw, a minor skin flaw (corkiness, healed lenticels), and a minor sunburn are all acceptable as long as they don't spread. The overall maximum area shouldn't be larger than 4 cm^2.

3. Class II

Avocados that meet the minimal standards outlined above but do not meet the criteria for participation in the higher classes are included in this class. As long as the avocados maintain their fundamental qualities in terms of quality, keeping quality, and presentation, the following flaws are acceptable: Skin flaws (corkiness, healed lenticels, and sunburn, provided they are not progressing), faults in shape, and color, with a maximum total area of 6 cm^2.

Relationship management is a key component of supply chain management, and each link in the chain is overseen separately. Managers from various corporate functions, including marketing, sales, finance, production, purchasing, logistics, and research and development, make up each process team **[10]**. The management of supplier and customer relationships upstream and downstream to increase value in the ultimate marketplace while incurring fewer costs for the supply chain as a whole is known as supply chain management **[11]**.

Supply Chain Management is a concept or mechanism to increase the company's total productivity in the supply chain through optimization of time, location, and flow of material quantity. Supply chain administration Agriculture represents the overall control of the production process, from processing to distribution and marketing to getting the desired products into consumers' hands **[12]**. Supply chain management integrates management practices and information technology to optimize the flow of information and goods between processes and business partners in a supply chain, which is a network of business processes and relationships between businesses required to create, market, and deliver products to end customers **[13].**

The Supply Chain Network (SCN) is a network that depicts the movement of resources, capital, and information from the point at which raw materials enter the network to the point at which goods are in the hands of consumers. Typically, a network's beginning points are suppliers, manufacturers, distribution centers, and retail, and its ending points are consumers **[14]**. The supply chain is related to the flow and transformation of goods and services, starting from the stages of providing raw materials to the final product in the hands of consumers. Management of agricultural commodity supply chains is different from non -agricultural commodity

supply chain management because it is related to the nature of agricultural products that are easily damaged. The planting process, growth, and harvesting depend on the climate and season, and the harvest has a variety of shapes and sizes **[15]**. All of these variables must be considered for the supply chain management of agricultural commodities to be comprehensive, effective, efficient, responsive, and sustainable.

Supply chain management is needed to meet consumer demand for agrarian industry products for raw materials and fresh and halal products for directly consumed so that they can benefit from both farmers and consumers **[16]**. Based on the problem above, researchers are interested in conducting further studies regarding the analysis of avocado supply chain management in South OKU Regency by using one of the supply chain methods, namely *the food supply chain network* (FSCN) method. FSCN structure consists of multi-farmers, factories (processing), multi-distribution centers (DC), and multi-consumers (retail/customers). This study aims to obtain an overview/mapping of the Avocado Agribusiness Supply Management in the South OKU Regency.

SCIENTIFIC HYPOTHESIS

The study had two hypothesis:

1. Avocado marketing margins are most efficient in the marketing channel with the shortest chain because it has the lowest marketing margin value.
2. The value of farmers' share in each avocado marketing channel in OKU Selatan is greater than 40%, with marketing channel IV having the highest value.

MATERIAL AND METHODOLOGY

Study Area

This research was conducted in South Warkuk Ranau, South OKU Regency (Figure 3). Location selection is made intentionally (*purposive*). This sub-district was chosen because the South Warkuk Ranau District was a pilot area for Avocado Cultivation with the largest area of cultivation and production compared to other regions in South OKU. The research was carried out in February 2022.

Figure 3 Map of research locations in Buay Madang East OKU District.

Data Collection

Types of data use primary and secondary data. Primary data comes from survey results and direct interviews with farmers. The primary data collected is the area of farmer's land, production, farming costs, selling price, marketing of avocado fruit, and other related primary data. Researchers also interviewed collectors, local and non-local traders, and final buyers regarding buying and selling prices in avocado marketing activities. At the same time, secondary data are obtained through documentation studies through relevant literature/literature such as research location maps, previous research as references, and others related to research content.

Samples

Determination of informants in this study were selected purposively and snowball sampling. The population of this study were farmers in Buay Madang, OKU Selatan. Samples were taken from 10 avocado farmer informants and information collection on other supply chain members use the technique of snawball sampling by following the flow of marketing to the final consumer, that is, with the number of informants consisting of 3 middlemen, 3 local traders, 3 non-local traders this is intended to make it easier for researchers to obtain data related to research objectives.

Statistical Analysis

The data collected is in the form of primary data and secondary data. The data processing method is carried out by qualitative analysis by creating a Food Supply Chain Network (FSCN) model developed by Vorst (2006). Quantitative analysis through the calculation of marketing margin analysis and farmers' share analysis with a calculator and Microsoft Excel version 2019 by Microsoft Corporation.

Model of FSCN

The research method used is a qualitative descriptive method. Research techniques known as qualitative descriptive methods (as opposed to experiments) are used to examine natural object circumstances. Triangulating (mixed) data gathering methods are used, inductive/qualitative data analysis is used, and the emphasis in qualitative research findings is on meaning rather than generalization **[17]**.

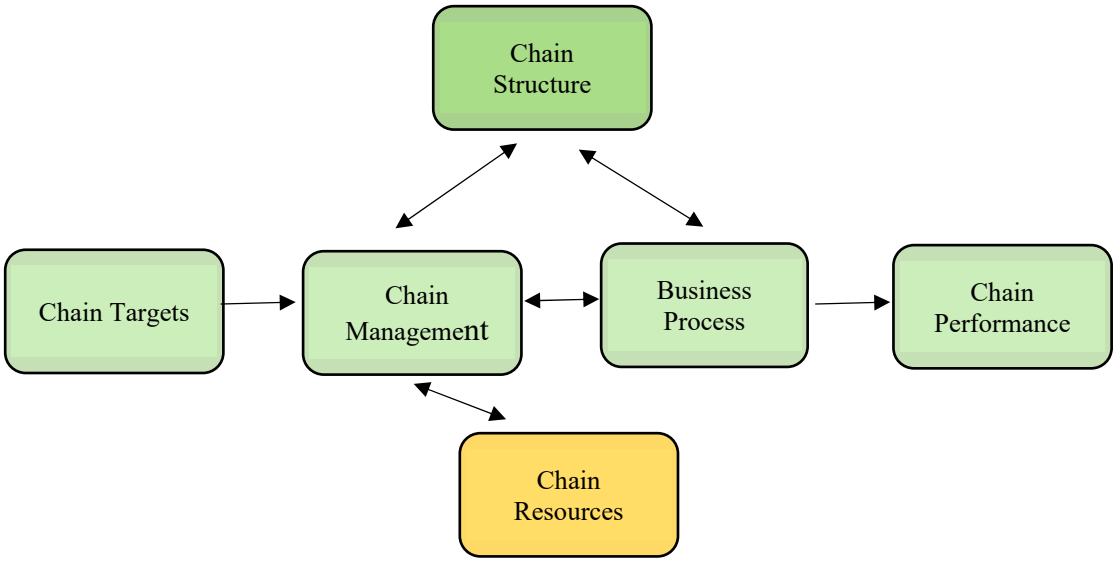

Figure 4 Frame Model of FSCN.

In the FSCN framework (Figure 4), several characteristics that are typical of the supply chain can be identified by distinguishing the following four elements that involve coordination in it and can be used to describe, analyze and/or develop supply chains, namely:

1. The supply chain structure describes the actors involved in the network and each role in the supply chain. The structure also describes the elements in the supply chain that can encourage business processes.
2. Structured supply chain business processes and measurable business activities are designed to produce certain outputs (physical types of products, services, and information) for certain customers or markets.
3. Supply chain management illustrates a form of coordination and management structure in the network that facilitates the decision-making process and the implementation of the process by members of the supply chain by utilizing the resources found in the supply chain to realize the purpose of the supply chain performance.
4. Supply chain resources are used to produce products and give them to customers (called resource transformation). Supply chain resources can be in the form of physical resources, technology, human resources, and capital.

The list of questions addressed to rspondens is present in Table 2.

Table 2 Transcript of the list of respondents' questions.

No.	Respondents	Questions
1.	**Farmers**	How much land for avocado cultivation do you have? What is the average production of avocados once harvest? What is the selling price of the avocado offered? Where are avocados sold or marketed? How to transport avocados to the market? what costs must be incurred in avocado cultivation? Is the income received enough to benefit farmers?
2.	**Collectors Traders/Tengkulak**	What is the purchase price offered to farmers? Are you involved in the capital of avocado farmer farming? What is the cost of transportation incurred? Where are the farmers' produce sold? What is the selling price offered to local or non-local traders?
3.	**Local Traders**	How much does it cost to buy an avocado from a collector? How much does it cost to transport avocados? What is the selling price offered to the buyer?
4.	**Non-Local Traders**	How much does it cost to buy an avocado from a collector? How much does it cost to transport avocados? What is the selling price offered to the buyer?
5.	**Final Consumers**	Where do you usually buy avocados? How much does it cost to buy an avocado in the market?

Marketing Margin Analysis

Marketing margin is the price difference between the price paid by consumers and the price received by farmers, which can be systematically formulated as follows:

$$\boldsymbol{M = Pr - Pf}$$

Where:

M = Makerting Margin; Pr = Prices at the consumer level; Pf = Prices at the Farmers level.

Farmer's Share Analysis

One indicator of marketing efficiency is the Farmer's share which is analyzed to find out how much the farmer receives from the price paid by the final consumer. Farmer's share is mathematically formulated as follows:

$$\boldsymbol{Fs = \frac{Pf}{Pk} x\ 100\%}$$

Where:

Fs = Farmer's Share; Pf = Prices at the Farmers level (Rp/Kg); Pk = Price that consumers pay end (Rp/Kg).

RESULTS AND DISCUSSION

Avocado farm revenue analysis

Production costs are all costs that must be incurred in carrying out avocado farming, which consist of fixed costs and variable costs. Fixed costs on avocado farming in South Warkuk Ranau District are equipment costs and depreciation, including spray tanks, hoes, and machetes. As for variable costs consisting of seeds, fertilizers, pesticides, herbicides, and labor costs **[18]**. Fixed fees for depreciation and variable costs in the avocado farm result in total production costs, as presented in Table 3.

Table 3 The Average Cost of Avocado Farmers' Production in South OKU Regency.

Description	Total Costs (Rp/Ha/Thn)
Fixed Costs and Depreciation	
Spray Tank	Rp 134,285
Hoes	Rp 30,428
Machetes	Rp 37,500
Total Fixed Costs	Rp 202,214
Variable Costs	
Seeds	Rp 7,464.286
Fertilizer	Rp 1,203,142
Pesticides	Rp 159,428
Herbicides	Rp 514,286
Labor	Rp 8,501,071
Total Variable Costs	Rp 17,842,213
Total Production Costs	Rp 18,044,427

The average total production cost in Avocado farming in South Warkuk Ranau District is Rp 18,044,427 ha/year. Where the cost of depreciation of the tool is the cost incurred from the initial cost minus the cost of residues and divided by the period of use (year). The average fixed cost/depreciation of tools consisting of spray tanks, hoes, and machetes is Rp 202,214 ha/year, and the average variable cost consisting of seeds, fertilizer, pesticides, herbicides, and labor is Rp 17,842,213 ha/year. Production is the result obtained by avocado farmers in each harvest season. The average production of avocado farming in West Warkuk Ranau District is 13,436 kg/ha/year. The average Avocado farm reception can be seen in Table 4 below:

Table 4 Average Avocado Farmers in South Warkuk Ranau.

Description	Total (Rp/Ha/Year)
Average production (Kg/Ha/Thn)	13,436
Price (Rp/Kg)	Rp 8,000
Revenue (Rp/Ha/Thn)	Rp107,488,000

Table 5 shows that the average revenue in avocado farming in the South Warkuk Ranau District from an average production of 13,436 kg/ha/yr multiplied by the selling price of Rp8,000 is Rp107,488,000 ha/yr. Next is to see how much the income earned by avocado farmers is through farming income analysis. Income is the difference between the amount of revenue from avocado farming and costs incurred as production costs. Avocado farmers' average income can be seen in Tables 5 and 6 below:

Table 5 The Average Revenue of Avocado Farmers in South Warkuk Ranau

Description	Total (Rp/Ha/year)
Revenue (Rp/Ha/Thn)	Rp 107,488,000
Total Production Costs (Rp/Ha/Thn)	Rp 18,044,427
Income (Rp/Ha/Thn)	Rp 89,443,573

Table 6 Total Income of Avocado Farmers in South Warkuk Ranau

Description	Total (Rp/Ha/Thn)
Average Income (Rp/Ha/Thn)	Rp 89,443,573
Capital Loan (Rp/Thn)	Rp 10,528,571
Profit Sharing 1%	Rp 160,000
Total Income (Rp/Ha/Thn)	Rp 78,755,002

Based on the calculations in Table 5 above, the average revenue reduced the return of capital loan costs and profit sharing of 1% (Rp.10,000/harvest season) based on the cooperation agreement to obtain a total revenue of the farming of the cooperative partnership pattern in the South Warkuk Ranau District of Rp78,755.002 Ha/yr.

Avocado Supply Chain Management

Avocado farmers in South Warkuk Ranau District, South OKU Regency, are still dealing with several problems; based on the explanation of the farmers, it is known that the problem of capital farming, marketing of avocado fruit production, and price fluctuations. Inadequate infrastructure or capital can limit smallholders' ability to meet high production standards **[19]**.

The beginning of the discussion within the FSCN framework is the chain target (chain objectives) by identifying specific supply chain characteristics, integrating quality, and optimizing the chain. Furthermore, chain management emphasizes management between each process of selecting partners, intertwined contracts, the transaction system, the extent of government support, and the collaboration of the supply chain **[20]**.

Furthermore, starting with discussing the chain structure to answer the question of who the members are in FSCN, their roles and the rules. In Figure 5, the following is the flow of the Food Supply Chain Network (FSCN) method in this study:

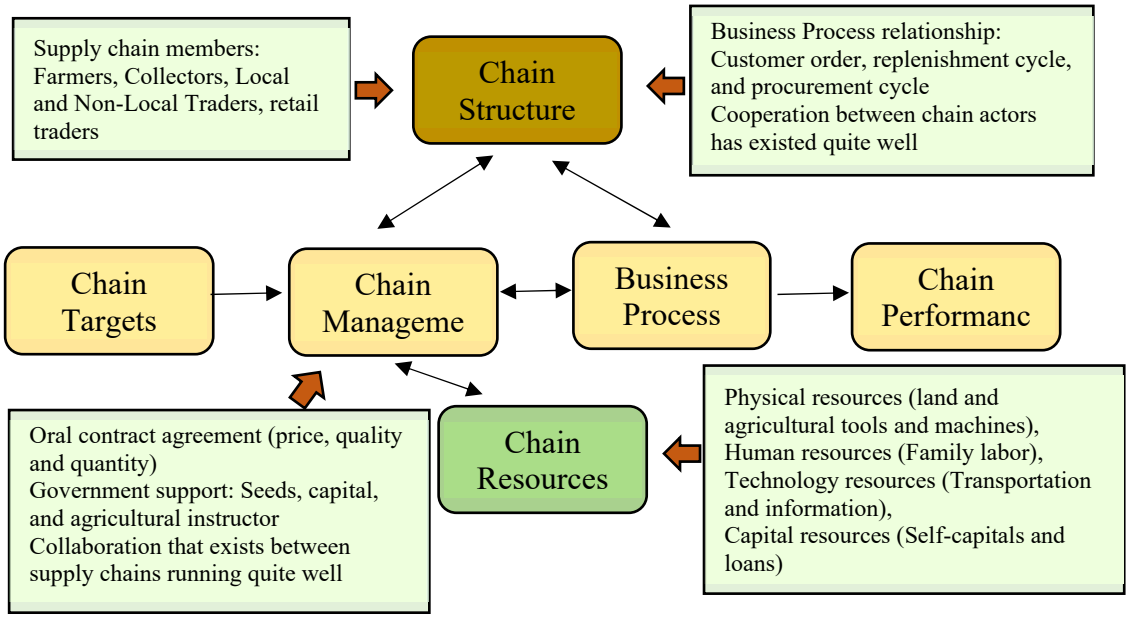

Figure 5 FSCN Method Flow in Avocado Supply Chain.

Supply Chain Targets

Avocado supply chain targets in South Warkuk Ranau District include market targets in the region. Avocado market targets in South Warkuk Ranau District are still dominated to meet the domestic market and products in the form of fresh avocados for consumption. Many farmers sell directly to the collectors rather than selling directly to the local market in Ranau because some collectors have directly taken the harvest on farmers' land so farmers do not need to pay labour costs and costs for transportation and cleaning avocados. In addition, farmers have also partnered with collectors in capital loans for avocado cultivation activities. Avocados are sold to collecting traders who are valued at Rp. 8,000/kg, Rp. 10,000/kg, and Rp. 12,000/kg, depending on the quality of the avocado.

Supply Chain Management

Some things discussed in Avocado Supply Chain Management are the selection of partners, the contractual system between the supply chain members, the transaction system, government support, and the coordination and collaboration between the chain members to determine the decision process of the supply chain management activities.

1. Partner Selection

Avocado farmers in South Warkuk Ranau District will choose partners to spend their harvests to prospective partners based on price offers. Partners who have contracts with farmers during this study are collecting traders. Although it does not determine a certain quality, the collecting traders will set prices by considering the quality of the products that farmers have harvested. In addition, some avocado farmers also partner with cooperatives to advance with a profit-sharing system in each harvest season of 1%, namely Rp.10,000.00 for cooperatives. In this case, farmers who partner with cooperatives like to advance also get capital assistance and must deposit Rp. 50,000.00 of members/farmers who partner as agreed staples. But in reality, more farmers partner with collectors

than cooperatives directly. Smallholders' only flexible option for financing production is to borrow capital input (money) from the 'tengkulak' **[21]**.

2. Contractual Agreement

The contractual agreement details the terms that the supply chain participants have agreed upon, such as the restrictions that must be adhered to by the partner and allows them to operate for the predetermined amount of time. Contracts are designed to share risks, share benefits and create incentive structures to encourage supply chain members to use optimal policies for all chain members **[22]**.

The contractual agreement occurs as an informal contract between members of the Avocado Supply chain from the farmers, collectors, and traders in the form of price agreements and land area sold to the collector. The collector decides avocado prices by adjusting to prices in the market/quantity of commodities to prevent losses on the farmer's side.

3. Transaction System

The transaction system that occurs in all transactions between avocado supply chain members is a cash system and delay. Based on observations in the field, many collectors buy from farmers with delayed payment systems compared to cash systems. This is because the farmers already know and believe in the collecting traders. The deficiency in delayed payment transactions is that there are a lot of delays in the payment process so farmers get lacking profit.

4. Government Support

Policy support to improve Avocado supply chain management by the government, especially in the South Warkuk Ranau District, is currently not much done. The form of support carried out by the South Warkuk Ranau District government is in the form of Avocado Seed Assistance for as many as 200 seedlings in order GSMP (Gerakan Sumsel Mandiri Pangan) program. Capital assistance through KUR in partnership with government-owned regional banks and the support of agricultural extension workers in delivering avocado cultivation materials and post-harvest processing related to technology socialization to extend the fruit savings so as not to rot quickly.

5. Supply Chain Collaboration

The collaboration of supply chains is shown to the chain members involved in the Avocado Supply chain process regarding the disclosure of information between the existing supply chain **[23]**. Communication between avocado farmers and collecting traders in South Warkuk Ranau District has been well established. The collaboration process is related to the avocado harvesting process from the collecting traders to the farmers. The avocado collection process starts from the reconciliation of the land ready for the collecting traders, then the collector traders take the harvest using pick up and the workforce of the collecting traders. Farmers claim that this approach is simpler because it eliminates the need to arrange vehicles, allowing them to simply wait on their fields. Farmers sell their harvests to collecting traders for this reason as well.

Supply Chain Structure

The trustworthiness of each link in the avocado supply chain in South Warkuk Ranau District, South OKU Regency, impacts the chain's structure. Farmers frequently go via supply chain chains to flow their products, hence these chains form. The avocado supply chain relationship structure can be seen in Figure 6 below:

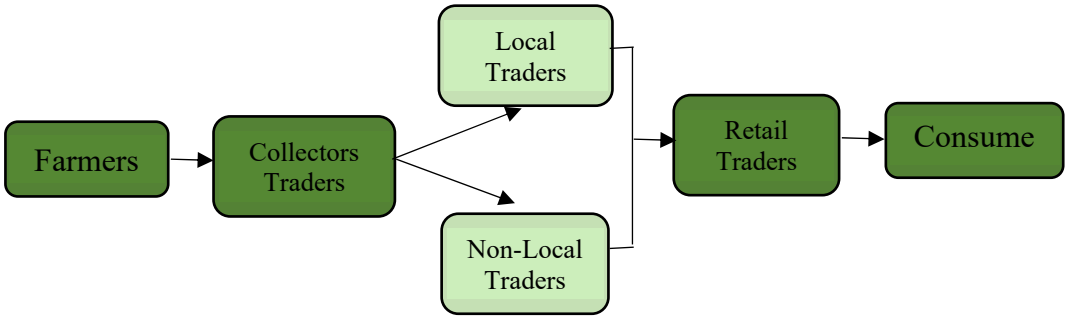

Figure 6 Supply Chain Structure of Avocado Agribusiness.

The supply chain structure involves the supply chain members. Each member performs marketing functions which can be seen in Figure 6. Members of the supply chain are the perpetrators who are incorporated and have a role in the Avocado Supply chain (Table 7).

Table 7 The role of the Supply Chain Members of Avocado Agribusiness.

Level	Members	Process	Activity
Producer	Avocado Farmers	Purchase, cultivation, and sales	Purchase agricultural production facilities and seeds, cultivate avocados, sell avocados to collectors.
Distributor	Collectors Traders	Purchase, storage, and sales	Purchasing avocados from farmers, supplying large traders in the market and to traders outside the region (non-local).
	Local Traders	Purchase, storage, and sales	Purchase avocados from collectors, supply to the local market, and to the retailer.
	Non-local Traders	Purchase, storage, and sales	Purchase avocados from collectors, supply to the non-local market, and to the retailer outside the area.
Retailer	Retailer Traders	Purchase, storage, and sales	Purchase avocados from distributors and sell avocados to consumers.
Consumers	Final Consumer	Storage and consumption	Make avocado purchases from retailers.

1. Avocado Farmers

The average avocado farmer cultivates land with an area of 2.00 hectares. Avocado farmers have an important role because it determines the existing avocado's quantity, quality, and continuity. The number of harvests produced will determine the quantity of carrot availability. The average avocado farmer produces 3 tons per harvest season (every two weeks). There is a need for continuity to maintain the availability of avocados in South Warkuk Ranau District, South OKU Regency.

2. Collection Traders

Collecting traders sell harvests from farmers to local traders, with average sales ranging from 2 to 3 tons per one-time shipping that can be done in the afternoon or evening. Most farmers prefer to sell avocados through collecting traders rather than selling themselves to large traders. The selling price of collecting traders to local traders is between 10,000-13,000 per kg, depending on the quality of the avocado.

3. Local Traders

Local traders involved in Avocado Supply chain activities come from other regions in South OKU Regency. Local traders will sell avocados to non-local traders from outside South OKU to Palembang City, Lubuk Linggua City, and outside South Sumatra Provinces such as Lampung, Jakarta, and East Java. Avocado butter is a type of avocado that is exported outside the region. In addition, local traders will also sell avocado harvests to retailers

4. Retailer Traders

Avocado retailers will receive fresh avocados from local traders and sell them to end consumers in various markets in South OKU, such as Muaradua Market, Saka Selabung Market, and Simpang Sender Inpres Market with prices that also vary from 12,000-15,000 per kg.

Business chain process

The business chain illustrates the entire process along the Avocado Supply chain in South Warkuk Ranau District, South OKU Regency.

1. Business process relationship

The process that occurs in the supply chain business has two aspects: *cycle view* dan *push or pull view* **[24]**. *The cycle view* on a supply chain consists of four process cycles. *Procurement cycle*, *manufacturing cycle*, *order cycle*. The cycle in an avocado supply chain consists of a cycle of procurement, replenishment, and Customer Order. Farmers serve as the primary supplier or producers for the procurement cycle, which is carried out by gathering traders who purchase avocados from them. Local retailers and traders complete the replenishment cycle by raising

the number of orders from the actual number of orders. The ultimate consumer, who has more power to influence the final pricing, completes the customer order cycle. In an economy that is becoming increasingly global, channel power has shifted even more in favor of the end user. Customers more often want price reductions and benefits upgrades for products and services **[25]**.

In this case the final consumer by ordering and purchasing avocados directly to sales and booking locations through online media such as WhatsApp and Instagram, where avocado marketing is also done online on Instagram social media with account names @Alpukat Mentega Ranau. Digital-based promotions include simple information and ordering avocados to attract customers, which can increase the benefits of avocados themselves **[26]**.

2. Distribution Pattern

The effective management of product and information flows is clearly a key aspect of Supply Chain Management **[27]**. The distribution pattern in the avocado supply chain in West Warkuk Ranau District illustrates the pattern of product flow, financial flow, and information flow that occurs between members of the avocado supply chain (Figure 7).

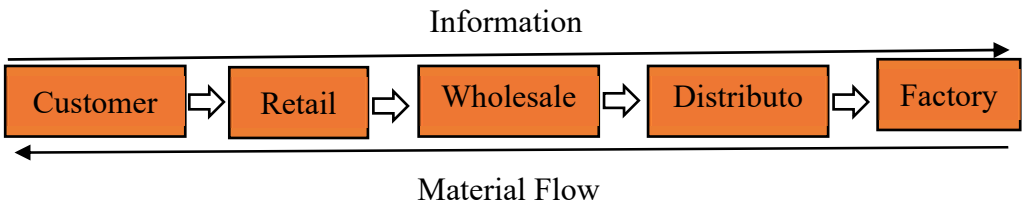

Figure 7 Flow of Goods and Information **[28]**.

1. Product Flow

The product flow channeled in the supply chain is a fresh avocado harvested from farmers' land. The product flow starts with avocado farmers. Avocado farmers do plant until they are ready to harvest at the age of 180 days or at 6-7 months after planting. Some village collecting traders will go to avocado farmers' land to take yields then there is a process of determining the price of the collecting traders with the purchasing system per kg. Farmers, suppliers, and traders, the main actors in the agricultural product supply chain, play an important role in meeting the needs of consumers **[29]**.

2. Financial Flow

The existence of money in a business is like the blood in a person, or it can be said that its existence is absolutely in the needs. The smooth flow of money or finances is very supportive of the achievement of an effective supply chain **[30].** The financial flow in the Avocado Supply Chain in the South Warkuk Ranau District is in the form of payment money on products sold from farmers as the first supply chain actors to the next supply chain actors. Payment money is used as capital to return to cultivate avocado plants, thus forming a financial cycle. The financial flow starts from the final consumer to avocado farmers. For now, the selling price of fresh avocados received by farmers is Rp 10,000-15,000 per Kg.

3. Information Flow

The information flow in the South Warkuk Ranau District's avocado supply chain, including information on commodity prices, quantity, and quality. Unlike the product flow and financial flow, information flows reciprocally from avocado farmers to end consumers and vice versa **[31]**, **[32]**. Information provided by avocado farmers to collecting traders is related to conditions in avocado farming land, such as land area, plant age, and estimated avocado harvest yields. At the same time, the information the gathering traders provides is in the form of a purchase price for the commodity.

Supply Chain Resources

Supply chain resources are needed to support efforts in developing and making activities that take place in the Avocado Supply chain in South OKU Regency consist of four main resources: physical, human, technology, and capital **[33]**.

1. Physical Resources

Physical resources owned by avocado farmers are land whose area is different from the total land area for avocado plants in South Warkuk Ranau District of 20,951.50 ha. Farmers are cultivated by avocado through the cone sari method. Equipment owned by farmers for cultivation to postharvest avocados in the form of hoes, pest spraying devices, flush tools, and sacks.

2. Human Resources

Compared to other resources like capital and technology, human resources make up the majority of an organization since people are in charge of them. For example, using mobile phones to improve communication within the supply chain. For example between farmers and collectors **[34]**. Human resources in the Avocado Supply chain in South Warkuk RanauDistrict involved labor in the family and workers outside the family. Work carried out by workers in the family, namely routine activities such as watering, maintenance using pest spraying, and fertilization. While the harvest of farmers is helped by family labor, other operations like land treatment and avocado land cleaning after harvest use daily workers from outside the family.

3. Technology Resources

Technology resources used by farmers in the avocado cultivation process still use traditional methods such as hoes for land treatment, and the harvesting process is carried out manually using human labor. Refer to research **[35]** weeding by hand is the most common practice in Nepal. Most farmers still manually remove weeds with small hand tools such as spades, various hand hoes (kuto, kodalo, kodali), and sickles. Farmers' use of information technology Farmers' use of information technology is only limited to mobile phones to facilitate communication between supply chain members. For example, between farmers and collectors.

4. Capital Resources

Based on the research results on business capital obtained by farmers come from their own capital and collectors. Own capital is obtained from the revenue of the harvesting period of plantation crops such as coffee and rubber. In addition, some farmers also chose to borrow capital from collectors or middlemen. Farmers with narrow lands who lack a strong capital base (proletarian) are used as opportunities for middlemen and trader farmers as capital owners (bourgeois) to be utilized or exploited by providing loans to farmers even without loan interest and the need for labor from farmers with narrow lands **[36]**. As a result, because they previously felt helped during the agricultural production process, these smallholder farmers sell their crops to middlemen or farmer-traders.

Figure 8 Avocado farmers and avocado fruit marketing.

Supply Chain Performance

Supply chain performance is the performance of activities related to the flow of goods, information, and funds from suppliers to end consumers **[37]**, **[38]**. To find out the performance of the supply chain in avocado agribusiness is carried out through marketing margin analysis and farmer's share analysis.

Marketing Margin

Marketing margin is the price difference producers receive from the final consumer's cost of goods. Marketing margins are used to see receipts received by each component in the marketing channel. Each stage of the marketing

chain takes a percentage of the final weighted average selling price **[39]**, **[40]**. The components in the marketing channel are farmers, collecting traders, local and non-local traders, retailers and consumers. Marketing margins on each supply chain can be seen in the following Table 8:

Table 8 Marketing Margin and farmer's Share of Avocado in South OKU Regency.

Marketing Actors	Marketing Channel			
	Chanel I	Channel II	Channel III	Channel IV
Producer (Farmers)				
Selling Price (Rp/Kg)	8,000	8,000	9,000	10,000
Marketing Costs (Rp/Kg)	825	825	1,025	1,225
Collector Traders				
Buying Price (Rp/Kg)		8,000		
Marketing Margin		2,000		
Marketing Costs (Rp/Kg)		825		
Profits (Rp/Kg)		1,175		
Selling Price (Rp/Kg)		10,000		
Local Traders				
Buying Price (Rp/Kg)		10,000	10,000	
Marketing Margin		1,500	2,000	
Marketing Costs (Rp/Kg)		425	625	
Profits (Rp/Kg)		1,075	1,375	
Selling Price (Rp/Kg)		11,500	12,000	
Non-Local Tarders				
Buying Price (Rp/Kg)		10,000		
Marketing Margin		2,500		
Marketing Costs (Rp/Kg)		1,025		
Profits (Rp/Kg)		1,475		
Selling Price (Rp/Kg)		12,500		
Retailer Traders				
Buying Price (Rp/Kg)	9,000	11,500	11,500	12,000
Marketing Margin	2,500	1,500	1,500	1,500
Marketing Costs (Rp/Kg)	925	425	425	225
Profits (Rp/Kg)	1,575	1,075	1,075	1,275
Selling Price (Rp/Kg)	11,500	13,000	13,000	13,500
Consumer				
Buying Price (Rp/Kg)	12,500	13,000	13,000	14,000
Total Margin	**2,500**	**7,500**	**3,500**	**1,500**
Farmer's Share (%)	**64,00**	**61,53**	**69,23**	**71,42**

The table above shows marketing margins for each component in the marketing channel. The marketing margin will differ in each marketing channel because each marketing actor has a different selling price. The lowest total marketing margin is found in channel IV with a margin value of Rp 1,500 per kg. Channel IV is the shortest supply chain because it only involves two marketing institutions, namely farmers and retailers. According to the research **[41]**, **[42]**, if the marketing margins are expended is equal to the use for which it was created. This indicates that the margins are equal.

Farmer's Share

Farmer's Share is the distribution of prices received by farmers (Farmer Share) which is a price comparison of prices paid by farmers with prices at the consumer or retail level **[43]** can describe one indicator of marketing efficiency and marketing justice **[44]**. *Farmer's share* is an indicator that measures how many parts are received by avocado farmers as a service for contributions made to the final selling price of each marketing channel.

Based on the results of Farmer's Share in Table 8 show that Farmer's Share was obtained for channels I, Channel II, Channel III, and Channel IV respectively, namely 64.00%, 61.53%, 69.23 and 71.42%. Channel IV has the highest percentage of Farmer's Share which is 71.42% means that the part received by farmers is 71.42% of the price paid by the final consumer. *Farmer's share* with a value of ≥40 % can be said as an efficient channel **[45]**. The four avocado marketing channels in South Warkuk Ranau District have a Farmer's Share ≥40% so it is categorized as an efficient channel.

CONCLUSION

Avocado supply chain management in West Warkuk Ranau District, South Oku Regency based on Vorst's Food Supply Chain Network model, includes supply chain targets, supply chain structures, supply chain management, and network chain, supply chain resources, and supply chain business processes.

Avocado supply chain targets in South Warkuk Ranau District include market targets in the region. Avocado market targets in South Warkuk Ranau District are still dominated to meet the domestic market and products in the form of fresh avocados for consumption. Avocado Supply Chain Management includes partner selection, contract systems between supply chain members, transaction systems, government support, and coordination and collaboration. The supply chain structure involves the supply chain members of avocado agribusiness consist of producer (avocado farmer's), distributor (collectors traders, local traders, non-local traders), retailer (retailers traders), consumer (final consumer/final buyer). Supply chain resources to support activities avocado cultivated in South OKU Regency consist of four main resources: physical, human, technology, and capital.

Based on the results of marketing margin of avocado, marketing margin efficiency found in Channel IV because it is the lowest total marketing margin with a margin value of Rp 1,500 per kg. Channel IV is the shortest supply chain because it only involves two marketing institutions, namely farmers and retailers. The shorter a product's marketing chain, the lower the costs, allowing for greater marketing efficiency. Based on the results of Farmer's Share, the four avocado marketing channels in South Warkuk Ranau District have a Farmer's Share ≥40% so it is categorized as an efficient channel. Channel IV has the highest percentage of Farmer's Share, which is 71.42%, meaning that the part received by farmers is 71.42% of the price paid by the final consumer.

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Accelerated technology for bread preparation using activated water

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ABSTRACT

In this study we studied the production of bakery products with an accelerated production cycle using different dispersed flour and ion-ozoned water. The dough was prepared by mechanical loosening of compressed air under pressure (1.5-3 atm). The accelerated technology of bread production combined with wholemeal flour increases the independence of the bakery and reduces the production time of the finished product. The air bubbles in the cavitation process create a finer texture and more airy porous products resulting in higher-quality bread with excellent sensory and textural properties. The accelerated method eliminates yeast from the formulation and expands dietary varieties of yeast-free bread and flour confectionery products. This study used new accelerated technology to quickly intensify the colloidal and biochemical processes that occur during dough preparation. The technology made it possible to eliminate the dough fermentation and proofing process, thereby reducing the duration of the production process of bakery products, increasing labour productivity, and increasing the yield of bread. Qualitative, organoleptic, physicochemical and microbiological indicators and safety indicators evaluated the bakery products. The results showed that the quality of fine and ultrafine disperse flours met the recommended standards for baking yeast-free bakery products. According to laser diffraction data, the average particle size of flour obtained by whole grain milling was 194.9 μm (micron) for fine wheat flour, 609.4 μm for fine wheat flour and 830.0 μm for medium wheat flour. The finest flour fractions (less than 75 μm) provide higher gluten quality, resulting in a better balance of elasticity and extensibility in the dough, according to particle size studies of flours used to create bread. Thus, bakers can give their bread the desired texture. The overall quality of the bread is also affected by the flour's protein content, with the 10-11.5% range considered ideal. The addition of sourdough has improved the taste of baked goods. Bread products made from different dispersed flour and ion-ozoned water had good quality, organoleptic, physicochemical and microbiological indicators, and safety indicators. They could be stored for up to 5 days. As a result of using the accelerated method of dough preparation will improve the structural-mechanical, rheological and technological properties of bread, bakery and flour confectionery products.

Keywords: bread, particle size, dispersity, wheat, ion-ozoned water

INTRODUCTION

Bread is the staple food consumed worldwide, and its production and consumption have increased significantly in recent years. Consequently, there is a constant need to develop new technologies that can increase bread production without compromising its quality. In times of economic recession and trade wars, the issue of food security becomes acute. Access to basic foodstuffs can be disrupted, leading to shortages and rising prices. One way to solve this problem is to develop technology for accelerated bread production with a long shelf life.

Benefits of this technology include cost effectiveness, reduced food waste and increased efficiency. Bread with a longer shelf life can reduce food waste and lower costs for producers and consumers. Accelerated bread technology can also produce bread faster and in larger quantities, satisfying demand in a shorter time **[1]**, **[2]**.

In this research paper, we propose the development and implementation of the technology of accelerated bread production with a long shelf life as a solution to the problems arising during the crisis, economic recession and the instability of raw material supply. The article will discuss the advantages of this technology and provide evidence of its effectiveness in solving food security problems. By highlighting the importance of this technology, we hope to encourage further research and investment in developing solutions to food security problems.

Previous studies have not considered the importance of phytic acid breakdown, which has an important role in the digestion of cereal, seed, and legume origin foods in the human gut. Phytic acid, also known as inositol hexaphosphate (IP6), is a naturally occurring compound in many plant foods, especially seeds, grains and legumes. It is a stored form of phosphorus in plants and plays a crucial role in their growth and development **[3]**, **[4]**.

Phytic acid has been shown to have both positive and negative effects on human health. On the one hand, it is thought to have antioxidant properties and may help reduce the risk of certain cancers, cardiovascular diseases and osteoporosis. On the other hand, it may bind to essential minerals such as iron, zinc and calcium, reducing their bioavailability and absorption in the gut.

Some traditional cooking methods include soaking, fermenting or germinating grains, seeds and legumes to reduce the negative effects of phytic acid. These methods help break down phytic acid and improve the bioavailability of essential minerals.

This naturally occurring compound found in many grains and legumes can reduce the bioavailability of important minerals such as iron, zinc, and calcium. This can lead to mineral deficiencies, especially in populations that rely heavily on plant-based foods **[5]**, **[6]**, **[7]**.

Although accelerated bread production has successfully met the growing demand for bread, the production process may not have considered the impact of phytic acid on the nutritional value of bread. However, recent studies have shown that incorporating sourdough into the bread-making process can significantly reduce phytic acid content and improve mineral bioavailability.

Lactic acid bacteria and yeast in sourdough produce phytase, an enzyme that breaks down phytic acid. This can help increase the availability of minerals in bread and improve the nutritional quality of this staple food **[8]**, **[9]**, **[10]**.

Therefore, it is important to consider the effects of phytic acid when making bread, especially in populations that rely heavily on plant-based foods. Using traditional fermentation methods, such as sourdough, can help reduce the negative effects of phytic acid and improve the nutritional quality of bread. To address the phytic acid problem in bread making, we used the traditional method of soaking the grains and incorporating sourdough into the recipe. Sourdough contains lactic acid bacteria and yeast that produce phytase, an enzyme that breaks down phytic acid and improves the bioavailability of minerals. Using sourdough in the production process of bread, we were able to reduce the negative effects of phytic acid and increase the nutritional value of the bread **[11]**, **[12]**, **[13]**.

One technology that has attracted attention in recent years is the use of ion-ozoned water in the bread-making process. It has been found that activated water effectively disinfects and improves the quality of baked goods. However, it is necessary to investigate the effect of using different dispersed flours in combination with ion-ozone water on the quality and production cycle of baked goods.

Ion-ozone technology and dough sheeter have a number of undeniable advantages over other bread-making methods. Ion-ozone technology uses ozone to break down gluten, resulting in a smoother, more elastic dough that can be handled more efficiently. This results in higher productivity and lower costs, as less time and energy are needed to produce high-quality bread.

In addition, ion-ozone technology and the dough mixer improve bread shelf life by reducing oxidation and ensuring better dough quality. This can reduce food waste and increase profitability for bakeries and food manufacturers.

Compared to other methods of bread production, ion-ozone technology and the dough mixing machine represent a more environmentally friendly and cost-effective solution. Traditional bread production methods often require chemical oxidizers, which can harm the environment and lead to increased costs. In contrast, using ozone in ion-ozone technology is a more environmentally friendly and cost-effective alternative.

Ion-ozone technology and the dough mixer can also reduce the allergenicity of bread, which can be helpful for people with gluten sensitivity or celiac disease. By breaking down the gluten protein, ion-ozone technology helps reduce the amount of gluten in the final product, making it safer for people with gluten sensitivity.

In general, ion-ozone technology and the dough mixer provide a number of advantages over traditional methods of bread production. These advantages include improved dough quality, increased efficiency, longer shelf life, reduced allergenicity and environmental safety **[14]**, **[15]**, **[16]**.

Recent studies have shown that whole wheat dispersed flour can significantly improve the quality of baked goods. Dispersed flour is produced by processing wheat grains into fine particles. Such flour has a higher ability to absorb water and improves baked goods' texture and shelf life. Therefore, the combination of dispersed flour and ozone water can lead to baked products with improved quality and an accelerated production cycle.

This study aimed to develop a new bread product with an accelerated production cycle using various dispersed flours and ion-ozonated water. To achieve this goal, we set the following objectives: (1) to investigate the quality of various dispersed flours and choose the most suitable ones for bread product; (2) to develop a recipe for bread product based on the selected flours and evaluate its physicochemical and sensory characteristics; and (3) to check the effectiveness of ion-ozone water for reducing production time and improving the microbiological safety of bread product.

The study materials consisted of three wheat flour types, obtained from local mills. The physical and chemical properties of the flours were analyzed using standard methods. The most appropriate type of flour dispersion for bread production was selected based on the results obtained. Then, a recipe for a bread product using the selected flour was developed, and the physicochemical and sensory characteristics of the bread were evaluated. To test the effectiveness of ion-ozone water, bread was baked traditionally, and with the addition of ion-ozone water, the microbiological safety of the bread was evaluated using standard methods.

"Activated water" describes water treated by various methods to increase its oxidative potential. Ion-ozone technology is one method that can be used to activate water because it involves passing air or oxygen through the water to create ozone and other reactive species that can help increase the oxidative capacity of water. Therefore, ion-ozone water can be considered a type of activated water.

A feature of activated water is its higher oxidative potential than ordinary water. This can make it more effective for disinfecting and cleaning surfaces and potentially beneficial to health when consumed internally or used in certain therapies.

Ion-ozone technology generates ozone and other reactive species by passing air or oxygen through water. This can be done by passing air or oxygen through water or using an electrical discharge to create ozone. The resulting ion-ozone water can be used for various purposes, such as disinfection or water treatment **[17]**, **[18]**.

Ion-ozone technology can potentially increase the oxidative potential of water, which can help kill bacteria and other microorganisms that may be present in bread, thereby improving its safety and shelf life. Ion-ozone technology can potentially increase the oxidative potential of water, which can help kill bacteria and other microorganisms that may be present in bread, thereby improving its safety and shelf life. Ion-ozone technology can potentially increase the oxidative potential of water, which can help kill bacteria and other microorganisms that may be present in bread, thereby improving its safety and shelf life.

Scientific hypothesis

Activated water may have high physicochemical and biological activity, suggesting that it may have unique properties that could make it useful for various applications in food and other industries (as suggested by result No.9). Another hypothesis suggests that activated water may removes molecules from water due to its ability to bind to certain substances (as suggested by result No.2). There is also the possibility that activated water can be used to purify water through photocatalysis, as shown in result No.7. However, it is important to note that the hypotheses must be tested through careful experimentation and data collection to determine whether or not they are true. Although some initial evidence supports these hypotheses about activated water, more research is likely needed to fully understand its properties and potential applications.

MATERIAL AND METHODOLOGY

Samples

The objects of the study were: ion-ozone water of high and minimum concentration, whole wheat flour from Al-Farabi's first crop of 2020 and other cereals, and Tsesna first-grade wheat flour. All analyses were conducted in an accredited laboratory of the Almaty Technological University.

The objects of the study were:

1. Fine wholemeal wheat bread No. 1 (ion-ozone 3 cavitation 3 kneading 8 speed 600).
1. Bread made of fine wholemeal wheat flour No. 2 (ion-ozone 1.5 cavitation 3 kneading 8-speed 600).
2. Fine wholemeal wheat bread No. 3 (ion-ozone 3 cavitation 2 kneading 8 speed 600).
3. Bread made of fine whole wheat flour No.4 (ion-ozone 1.5 cavitation 2 kneading 8 speed 600).
4. Bread made of fine whole wheat flour No.5 (ion-ozone 3 cavitation 3 kneading 4 speed 600).

5. Bread of fine wholemeal wheat flour No.6 (ion-ozone 1.5 cavitation 3 kneading 4 speed 600).
6. Wholemeal Fine Wheat Bread No.7 (ion-ozone 3 cavitation 2 kneading 4 speed 600).
7. Wholemeal Fine Wheat Bread No.8 (ion-ozone 1.5 cavitation 2 kneading 4 speed 600).
8. Bread made of fine wholemeal wheat flour No.9 (ion-ozone 3 cavitation 3 kneading 8 speed 300).
9. Bread made of fine whole wheat flour No.10 (ion-ozone 1.5 cavitation 3 kneading 8 speed 300).
10. Fine wholemeal wheat bread No.11 (ion-ozone 3 cavitation 2 kneading 8 speed 300).
11. Fine wholemeal wheat bread No.12 (ion-ozone 1.5 cavitation 2 kneading 8 speed 300).
12. Bread of fine wholemeal wheat flour No.13 (ion-ozone 3 cavitation 3 kneading 4 speed 300).
13. Bread made of fine wholemeal wheat flour No.14 (ion-ozone 1.5 cavitation 3 kneading 4 speed 300).
14. Bread made of fine whole wheat flour No.15 (ion-ozone 3 cavitation 2 kneading 4 speed 300).
15. Bread made of fine wholemeal wheat flour No.16 (ion-ozone 1.5 cavitation 2 kneading 4 speed 300).

All analyses were conducted in an accredited Almaty University of Technology laboratory.

Chemicals

All reagents were of analytical purity and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

Instruments

An automatic fat extractor SER 148/3 (Velp Scientifica, Italy), a high-speed dispersant, a vacuum dough mixer, a convection dryer, a grain analyzer (Infratek 1241), a laboratory dough mixer were used.

Laboratory Methods

In work the following indicators of used raw materials and obtained assortment of bakery products were investigated: organoleptic indicators according to GOST 5667-85, and mass fraction of moisture according to GOST 21094-75. Mass fraction of fat according to GOST 5668-68. The mass fraction of protein was determined according to GOST 10846-91. The mass fraction of carbohydrates was determined according to GOST 25832-89. The mass fraction of porosity was determined according to GOST 5669-96. Acidity content was determined according to GOST 5670-96. In addition, microbiological parameters were determined based on the following standards: the number of mesophilic aerobic and facultative anaerobic microorganisms (KMAFANM) - according to GOST 10444.15-94, the number of E. coli bacteria (coliform bacteria) - according to GOST 31747-2012.

The method determined moisture content in flour according to GOST 9404-88. The content of raw gluten was controlled according to GOST 27839-88. The quality of raw gluten was determined by measuring its elastic properties according to GOST 27839-88. Protein mass fraction was determined according to GOST 10846-91, fat content - according to GOST 29033-91, mass fraction of fiber - according to the Wend method. The Glassiness of wheat grains was determined by diaphragmoscope according to GOST 10987-76.

Experimental Design and Procedure

The methodological basis for studying accelerated bakery technology can include a systematic analysis of the process, the ingredients involved, and the various factors affecting the final product. This could include an analysis of the effects of temperature, hydration, fermentation, flour type and other variables on bread quality. The research will likely include a combination of laboratory experiments and data collection, sensory analysis and consumer testing to evaluate the final product regarding flavour, texture and other characteristics. The ultimate goal of the study will be to determine ways to optimize the production process and ingredients to achieve the desired characteristics of the bread.

Laboratory unit for ultrafine grinding and mechanoactivation of vegetable raw materials, consisting of a body with opposite-made loading and unloading pipes, rotor rotating cylinder with grinding grinding grinding balls, installed inside the grinding unit with a gap concerning its upper surface and bottom, acting as a classifier, the loading pipe supplied with a device for regulating the feed of crushed materials differs in that it allows obtaining fine flour with a dispersion range h.

Initial material is placed in the field of centrifugal forces, loaded into the rapidly rotating rotor, and excited by wave vibrations. At the same time, the solid particles come together and are intensively crushed by relative vibrations up to the colloidal state. Grinding media and liquids can be added to intensify the process. The processing mode is controlled by varying the oscillation frequency and the strength of the centrifugal force field. Below is a picture of a dough mixer, accelerated dough, and ion-ozone cavitation unit for accelerated dough preparation (Figure 1).

Figure 1 Accelerated dough kneader, ion-ion cavitation unit for accelerated dough preparation.

The dough was obtained by mechanical loosening under pressure in an experimental ion-ozone cavitation machine for preparing dough with an accelerated cycle developed at ATU. The dough mixing machine works as follows. Recipe dough components are fed through the charging port to the kneading body of a batch mixer which has a kneading body driven by an electric motor through a speed variator. At the end of the loading, the kneading body of the kneading machine is hermetically closed with a lid, and the dough is kneaded for 3-5 minutes at a speed of kneading organ of 5 s^{-1}. Then ion-ozone cavitation air is fed into the kneading chamber at a pressure of 0,20, 0,40 and 0,60 MPa and dough is beaten for 3-10 minutes at a speed of kneading organ rotation of 2-3 and 4-5, 7-8 $seconds^{-1.}$ While beating the prescribed components, the dough mass is saturated with air. The dough prepared this way is a foamy mass with stable physical and chemical characteristics. The process of beating dough from wheat flour of 1 and 2 grades was investigated under the pressure of 0.20, 0.40 and 0.60 MPa and the rotation frequency of kneading organ 2-3 and 4-5, 7-8 s^{-1} during 2-3, 4-5 and 7-10 minutes and without giving compressed air.

The process intensifies because during the preparation of dough, there is no need to use a proving cabinet and other machines used in traditional baking, which reduces the production area and the time spent on the preparation of bread.

As a result, using the proposed invention using an ion-ozone technological line and methods of dough preparation will improve the structural-mechanical, rheological and technological properties of dough and bread, bakery and confectionery products obtained from it.

The dough was prepared using mechanical loosening, which requires special equipment. The equipment used in this study included a planetary mixer, a dough-leavening machine, and a bakery oven.

The recipe for the yeast-free bread dough included flour, water, and salt. Flour was obtained from three different dispersions (medium, fine and fine) and was determined to meet the standards of GOST 26574-2017. The amount of water in the dough was adjusted according to the flour's gluten content as determined by a Chopin CD1 gluten meter.

The dough was prepared as follows: first, the flour and salt were mixed in a planetary mixer. Then water and sourdough were added, and the dough was kneaded for a certain time using the MKP-50 dough leavening agent. The dough was then shaped into loaves and baked in the oven.

In the studies, we studied the dependence of quality indicators of dough from fine whole-meal flour "Al-Farabi" using activated ion-ozoned water and bread prepared from it on the technological regimes of dough processing:

- ion-ozone concentration, $C*10^{-9}$ mg/unit;
- pressure P, atm;
- dough kneading time τ, min;
- dough mixer shaft rotation speed, rpm.

Planning methods for multifactorial experiments were applied to reduce the number of experiments and obtain reliable experimental results. The quality of dough and bread prepared from it in the studies were evaluated according to the following indicators:

y_1 - moisture content of the dough, %
y_2 - alkalinity of the test, deg;
y_3 - the mass of the dough, g;
y_4 - total deformation of the dough, mm;
y_5 - plasticity of the dough, mm;
y_6 - elasticity of the dough, mm;
y_7 - moisture content of bread, %;
y_8 - porosity of bread, %;
y_9 - alkalinity of bread, deg;
y_{10} is the volume of 100 grams of bread, see^3 ;
y_{11} - the mass of bread, g;
y_{12} - total deformation of the bread, mm;
y_{13} - plasticity of bread, mm;
y_{14} - elasticity of bread, mm;
y_{15} - protein, %;
y_{16} - starch, %;
y_{17} - fiber, %;
y_{18} - fats, %;
y_{19} - ash, %;
y_{20} - sugar, %.

To reduce the influence of uncontrollable factors on the results of the experiments, the experiments were randomized using random number tables. Conditions of experiments and obtained results of determining the quality parameters of dough from fine whole-meal flour "Al-Farabi" using ion-ozone water and bread prepared from it. Table 1 shows the conditions of the experiments of fine whole-milk flour "Al-Farabi" using ion-ozoned water and bread prepared from it.

Table 1 Conditions of experiments of fine whole-meal flour "Al-Farabi" using ion-ozone water and bread prepared from it.

No.	Factors			
	C*10^{-9} mg/unit	**P, atm**	**τ, min**	**v, rpm**
1	0.5	3	8	600
2	0.003	3	8	600
3	0.5	2	8	600
4	0.003	2	8	600
5	0.5	3	4	600
6	0.003	3	4	600
7	0.5	2	4	600
8	0.003	2	4	600
9	0.5	3	8	300
10	0.003	3	8	300
11	0.5	2	8	300
12	0.003	2	8	300
13	0.5	3	4	300
14	0.003	3	4	300
15	0.5	2	4	300
16	0.003	2	4	300

The appearance of bread samples carried out in multiple planning experiments are shown in Figure 2.

Figure 2 Samples of bread carried out multiple planning experiments.

Number of samples analyzed: 16 samples were analyzed.
Number of repeated analyses: All tests were performed in triplicate.
Number of experiment replication: Replications were conducted 2 times.
Design of the experiment: Experimental design for an accelerated bakery technology study can include a combination of laboratory experiments and sensory analysis. For example, the study may involve changing temperature, hydration, fermentation time, flour type, or other baking process variables and observing changes in the final product. Sensory analysis may include evaluating bread in terms of taste, texture, and other attributes either through surveys or by trained experts.

Statistical Analysis

The work used the ANOVA program, the PLAN sequential regression analysis program, and the Statistika 10.0 software to analyze the data collected during the study. The analysis included data processing using various mathematical and statistical measures to determine significant sample differences. Data processing and calculations were carried out using the PLAN sequential regression analysis program developed at the Odessa

National Technological University **[19]**, **[20]**. The data was analyzed using MS Excel for Windows version 10 Pro, 2010, and various statistical measures such as mean and standard deviation were used to estimate the values.

RESULTS AND DISCUSSION

Sixteen samples of bread made from fine wheat whole-meal flour were examined. The results are shown in Tables 1 and 2 of Figure 3. As a result of multiple planning experiments 2^n ion-ozone according to four criteria, three samples with the best performance were identified. Samples numbered 2, 5, and 9 outperformed the results obtained with the distinctive regimes regarding dough elasticity and volume of the final product. All samples used activated water of ion-ozone type with maximum and minimum values. Sample number 2 had a minimum concentration of ion-ozone water when kneaded at 1.5 atm, for 8 minutes with a shaft rotation of 600 rpm. The volume of the final product was 2.6 g/cm^3. An admixture of the dough was detecte, as the rheological indices of the dough decreased. Sample number 5 was the maximum concentration of ion-ozone water when kneaded at 3 atm, for 4 minutes with a shaft rotation of 600 rpm. The volume of the final product was 2.6 g/cm^3. With multiple planning experiments, the distinctive feature of this mode is the rapidity of kneading and the best performance on all measured parameters. The experiment with finely dispersed wholemeal flour of the first degree from the grain variety Al-Farabi was carried out at the same regimes. The revealed volume of the final product is 2.3 g/cm^3, which is inferior to indicators from fine-dispersed whole-meal flour of the same variety of grain. Sample number 9 was the maximum concentration of ion-ozone water when kneaded at 3 atm, for 8 minutes at 300 rpm. The volume of the final product was 2.5 g/cm^3 . The indicators of this sample were identical to sample number 5. This is explained by the fact that all the modes were similar except for the kneading time and speed of the shaft. The outcome was similar to 4 minutes at 600 rpm in the early sample. This sample was achieved by extending the kneading time, resulting in several turns to 600 rpm. During the baking process, all samples were found to have two criteria affecting the volume and rise of the dough. A pressure of 3 atm saturated the dough more intensely than 1.5 atm, and ion-ozone-activated water with a maximum concentration more effectively affected the final product's volume than the minimum ion-ozone concentration. The quality indicators of the test below are shown in Table 2.

Table 2 Quality indicators of dough from fine whole-meal flour "Al-Farabi" using ion-ozoned water and bread prepared from it.

	Dough and bread quality indicators																		
No.	**y_1, %**	**y_2, deg.**	**y_3, g**	**y_4, mm**	**y_5, mm**	**y_6, mm**	**y_7, %**	**y_8, %**	**y_9, deg**	**y_{10}, cm^3**	**y_{11}, g**	**y_{12}, mm**	**y_{13}, mm**	**y_{14}, mm**	**y_{15}, %**	**y_{16}, %**	**y_{17}, %**	**y_{18}, %**	**y_{19}, %**	**y_{20}, %**
1	54.3	3.0	500	13.6	12.0	1.6	53.2	60.1	2.0	210.0	448.96	3.5	1.1	2.2	9.16	33.52	6.87	1.41	1.54	3.63
2	52.2	3.4	500	11.0	8.5	2.1	49.6	64.8	2.0	260.0	442.89	4.5	1.7	3.0	9.03	30.55	6.76	1.88	1.59	4.12
3	54.3	2.5	450	13.6	12.0	1.6	53.2	60.1	2.0	233.3	400.39	3.5	1.1	2.2	9.14	28.55	5.95	1.02	1.65	4.31
4	52.2	3.4	450	11.0	8.5	2.1	49.6	64.8	2.0	266.7	395.83	4.5	1.7	3.0	8.75	29.67	6.92	1.25	1.72	5.04
5	53.0	3.4	500	14.8	13.5	1.2	52.5	59.7	1.4	260.0	457.58	4.2	1.9	2.2	9.07	42.72	7.05	0.93	1.75	2.95
6	50.1	3.0	500	17.2	15.0	2.2	52.0	55.3	2.0	200.0	444.95	3.0	0.9	2.1	8.81	33.88	7.19	0.71	1.71	3.12
7	53.0	3.4	450	14.8	13.5	1.2	52.5	59.7	1.4	233.3	348.16	4.2	1.9	2.2	7.96	25.39	6.53	0.92	1.69	4.71
8	50.1	3.0	450	17.2	15.0	2.2	52.0	55.3	2.0	218.9	394.28	3.0	0.9	2.1	7.52	20.54	5.71	1.52	1.22	5.15
9	52.5	3.6	500	16.8	15.5	1.1	52.0	60.0	2.0	250.0	439.82	4.0	1.5	2.5	8.79	30.76	7.28	1.58	0.92	5.30
10	52.8	3.4	500	26.7	25.0	1.7	51.3	56.1	2.0	210.0	447.45	4.5	2.1	2.5	8.20	42.68	8.11	2.08	1.64	3.87
11	52.5	3.6	420	16.8	15.5	1.1	52.0	60.0	2.0	249.8	352.66	4.0	1.5	2.5	8.05	32.18	6.67	0.66	1.62	4.47
12	52.8	3.4	400	26.7	25.0	1.7	51.3	56.1	2.0	237.5	385.09	4.5	2.1	2.5	9.12	46.66	5.98	1.67	1.55	2.85
13	55.2	3.6	500	21.0	18.5	2.2	52.5	59.8	1.2	200.0	445.91	2.5	0.7	1.3	8.76	34.42	5.87	0.91	1.49	3.24
14	52.1	3.4	500	25.8	24.5	1.1	46.9	58.0	1.4	220.0	410.44	3.3	0.9	2.7	9.17	36.18	6.29	0.87	1.47	5.14
15	55.2	3.6	383	21.0	18.5	2.2	52.5	59.8	1.2	234.6	351.13	2.5	0.7	1.3	9.09	33.75	7.37	1.51	0.78	4.72
16	52.1	3.4	450	25.8	24.5	1.1	46.9	58.0	1.4	233.3	376.80	3.3	0.9	2.7	8.34	30.45	6.43	0.89	1.39	4.85

From the data in Table 2 we can see the quality indicators of dough from fine whole-meal flour "Al-Farabi" using ion-ozone water and bread prepared from it according to the applied modes.

Flour contains magnesium, calcium, iron, copper, zinc, cadmium and other minerals. These Swedish researchers compared several bread from industrial bakeries with sourdough bread from a local bakery, intending to see if the bread makes a difference as it passes through the stomach and intestines. By simulating this process in the lab, they found that time and pH levels are important for the breakdown of phytic acid. In an acidic environment, phytic acid breaks down faster. Phytic acid in flour prevents the body from absorbing these nutrients. The long fermentation process breaks down the phytic acid and releases minerals for the body to assimilate. Results show that the body does not assimilate minerals in industrial bakery bread.

In contrast, almost all nutrients from bakery products can be assimilated by the body through a long fermentation process **[21]**, **[22]**. Based on this, a sourdough starter was added to the recipe for the bread to break down phytic acid. Cooking technology was according to the recipe for 1 kg of flour: 20 g sourdough, 950 ml of water, 25g salt, 20 g sugar, improver 2 g, vegetable oil 10 g. Sourdough was dissolved in warm water at 1 kg of flour (950 ml). In the case of fine-dispersed flour, the first and second-degree water take 100ml more per 500 g of flour, because the moisture absorption is higher than that of wholemeal flour finely ground.

The initial weight of the dough pieces was 500 g. The selected bakery products were as follows:

The main technological characteristics for all objects:

- obtaining (dividing, rounding) the product of a mass of 500 g.
- proofing for 20 minutes at 40 °C and 75% relative humidity.
- baking at 220 °C for 30 minutes with steam; the mass of the finished product is 450 g.

The importance of choosing the right techniques for kneading dough and the influence of the processes of creating dough structure on the quality of the finished products have been established by experimental studies. Given the studies, we can propose the following method of kneading the dough: 4 minutes instead of 8 minutes. Significant deformation was observed when making bread from the dough at a speed of 600 rpm, but the bread quickly acquired a normal shape. Prolonged kneading for more than four minutes is ineffective and harmful to the dough structure. Data analysis showed that prolonged kneading destroys the gluten structure of the dough. Bread made after a long kneading time retains its original shape worse.

Based on the results of the studies, regression equations were obtained, which adequately (by Fisher's criterion) describe the effect of technological modes of dough processing C, P, τ, v on the indicators mentioned above of quality of dough and bread prepared from it.

To determine the error variance (reproducibility), 3 parallel experiments were performed in the center of the experiment plan.

Regression coefficients were calculated using matrices in natural dimensionality, and accordingly, the equations themselves were also obtained in natural dimensionality.

The general form of regression equations for the 4 factors is as follows:

$$y_i = b_0 + b_1 C + b_2 \mathrm{P} + b_3 \tau + b_4 \mathrm{v} + b_{12} CP + b_{13} \mathrm{C}\tau + b_{14} Cv + b_{23} \mathrm{P}\tau + b_{24} Pv + b_{34} \tau v \quad (1)$$

Where:

y_i – *i-th* quality indicators of dough and bread prepared from it; C – ion-ozone concentration, $\mathrm{C}*10^{-9}$ mg/d; P – pressure, atm; τ – duration of kneading dough τ, min; v – rotational speed of the dough mixer shaft, rpm.

Summary data on the obtained regression equations in natural variables are given in Table 3. In the same table, the mean square errors of experiments S_e and inadequacy $S_{in.ad.}$, as well as calculated F_c and critical F_{cr} values of the Fisher criterion, testifying that all obtained equations adequately describe the experimental data at confidence probability $p = 0.05$.

More detailed data on the statistical characteristics of the obtained regression equations are given in the listings of their calculations.

The regression equations are mathematical models that allow us to predict the quality indicators of processed dough and bread obtained from it, depending on the values of technological modes of dough processing, i.e. factors C, P, τ, v.

From the data in Table 3 we can see that a brief analysis of the obtained regression equations shows that of the 20 studied indicators of dough and bread quality, only bread porosity (y_8) does not depend on the dough processing modes.

One mode, factor C determines the dough moisture (y_1) and bread moisture (y_7), and factor P determines the dough weight (y_3) and fiber content (y).$_{17}$

The two-mode factors C, v determines the alkalinity of the dough (y_2), C, P determines the mass of the bread (y_{11}) and C, τ determines the elasticity of the bread (y).$_{14}$

The three mode factors *C*, τ, *v determine the* total deformation of the dough (y_4), the plasticity of the dough (y_5) and the elasticity of the dough (y_6). Bread volume (y_{10}) and fat content (y_{18}) depend on factors *C*, *P*, τ. Factors *C*, τ, *v* determine the alkalinity of the bread (y_9), the total deformation of the bread (y_{12}), and the plasticity of the bread (y_{13}). The factors *P*, τ, *v determine* the protein content (*y*).[15]

And only the starch (y_{16}), ash (y_{19}), and sugar (y_{20}) content depend on all 4 factors *C*, *P*, τ, *v*.

To optimize the technological modes of dough processing, the volume of bread was selected as the target function.

$$y_{10} = 222.772 + 71.680\text{-}C*10^{-9} - 12.175\text{-}P + 6.408\text{-}\tau - 10.915\text{-}C*10^{-9} - \tau \rightarrow max \quad (2)$$

Table 3 Regression equations in natural variables and statistical characteristics of dependencies of quality indicators of dough from fine whole-meal flour "Al-Farabi" using ion-ozone water and bread prepared from it on factors C, P, τ, v influencing them.

Regression equations in natural variables	Standard deviation		Fisher's criterion	
	experimental	inadequacies	billing	critical
$y_1 = 51.788 + 3.924\text{-}C*10^{-9}$	0.90	1.11	1.53	19.42
$y_2 = 3.300 + 1.654\text{-}C*10^{-9} - 0.00351\text{-}C*10^{-9} - v$	0.15	0.25	2.68	19.42
$y_3 = 294.875 + 68.375\text{-}P$	21.20	19.25	1.21	3.74
$y_4 = 42.502 - 29.779\text{-}C*10^{-9} - 0.6687\text{-}\tau - 0.04065\text{-}v + 0.04997\text{-}C*10^{-9} - v$	0.91	1.64	3.26	19.40
$y_5 = 41.787 - 33.199\text{-}C*10^{-9} - 0.6562\text{-}\tau - 0.04351 + 0.05869\text{-}C*10^{-9} - v$	0.82	1.54	3.54	19.40
$y_6 = 1.396 + 4.239\text{-}C*10^{-9} - 0.1184\text{-}\tau - 0.3018\text{-}C*10^{-9} - \tau - 0.00651\text{-}C*10^{-9} - v + 0.00040\text{-}\tau\text{-}v$	0.082	0.35	18.13	19.39
$y_7 = 49.934 + 5.231\text{-}C*10^{-9}$	1.13	1.52	1.81	19.42
$y_8 = 59.225$	1.12	2.82	6.34	19.43
$y_9 = -1.534 + 0.255\text{-}\tau + 0.00304\text{-}v + 0.1899\text{-}C*10^{-9} - \tau - 0.00039\text{-}\tau\text{-}v$	0.087	0.088	1.03	19.40
$y_{10} = 222.772 + 71.680\text{-}C*10^{-9} - 12.175\text{-}P + 6.408\text{-}\tau - 10.915\text{-}C*10^{-9} - \tau$	5.00	20.94	17.54	19.40
$y_{11} = 412.256 - 439.710\text{-}C*10^{-9} + 170.540\text{-}C*10^{-9} - P$	19.44	24.45	1.58	19.42
$y_{12} = 0.6792\text{-}\tau + 0.00402\text{-}v\text{-}0.4739\text{-}C*10^{-9} - \tau + 0.00508\text{-}C*10^{-9} - v\text{-}0.00076\text{-}\tau\text{-}v$	0.17	0.38	4.92	19.40
$y_{13} = -2.053 + 1.006\text{-}C*10^{-9} + 0.6265\text{-}\tau + 0.00432\text{-}v\text{-}0.5030\text{-}C*10^{-9} - \tau + 0.00402\text{-}C*10^{-9} - v\text{-}0.00083\text{-}\tau\text{-}v$	0.053	0.20	14.24	19.38
$y_{14} = 1.866 - 1.056\text{-}C*10^{-9} + 0.1187\text{-}\tau$	0.11	0.35	10.00	19.42
$y_{15} = 7.756 + 1.224\text{-}P\text{-}0.00533\text{-}v\text{-}0.1411\text{-}P\text{-}\tau + 0.00088\text{-}\tau\text{-}v$	0.21	0.41	3.77	19.40
$y_{16} = 37.935 + 6.567\text{-}\tau - 0.1213\text{-}v\text{-}5.224\text{-}C*10^{-9} - \tau + 0.06478\text{-}C*10^{-9} - v\text{-}1.886\text{-}P\text{-}\tau + 0.03497\text{-}P\text{-}v$	1.66	2.76	2.76	19.38
$y_{17} = 5.480 + 0.4825\text{-}P$	0.21	0.61	8.59	19.42
$y_{18} = 3.515 + 1.393\text{-}C*10^{-9} - 1.297\text{-}P\text{-}0.4075\text{-}\tau - 0.3131\text{-}C*10^{-9} + 0.2356\text{-}P$	0.060	0.24	16.37	19.39
$y_{19} = -1.592\text{-}C*10^{-9} + 0.5736\text{-}P + 0.2445\text{-}\tau + 0.00304\text{-}C*10^{-9} - v - 0.09054\text{-}P\text{-}\tau$	0.072	0.20	7.60	19.40
$y_{20} = 5.435 - 3.576\text{-}C*10^{-9} - 0.9116\text{-}\tau + 0.01276\text{-}v + 0.5621\text{-}C*10^{-9} - \tau + 0.3045\text{-}P\text{-}0.00534\text{-}P\text{-}v$	0.19	0.65	11.85	19.38

Analysis of the above equation shows that the bread volume is completely unaffected by the speed of rotation of the dough mixer shaft *v*. The pressure *P* linearly changes the bread volume – with increasing *P,* the bread volume decreases.

The equation also shows that the factors ion-ozone concentration C and duration of kneading dough τ have a joint contradictory pair influence (coefficient -10.915 has a minus sign). Therefore, it is difficult to uniquely analytically determine the effect of each factor on the bread volume.

More clearly, the character of the joint mutual influence of the ion-ozone concentration C and the dough kneading time τ on the bread volume (y_{10}) can be determined from the response surface (Figure 3), constructed by the above equation (1).

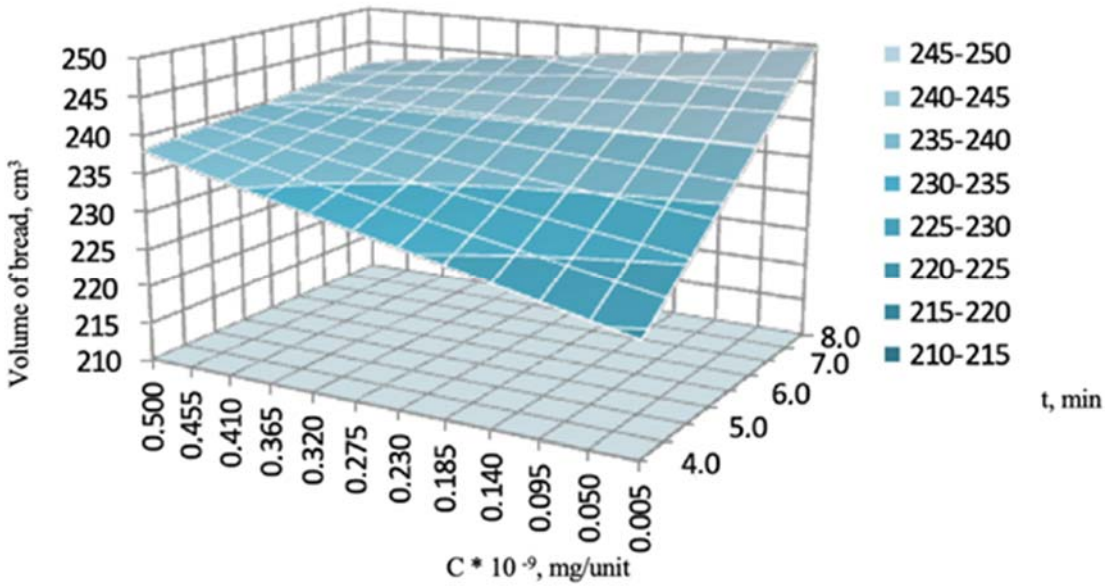
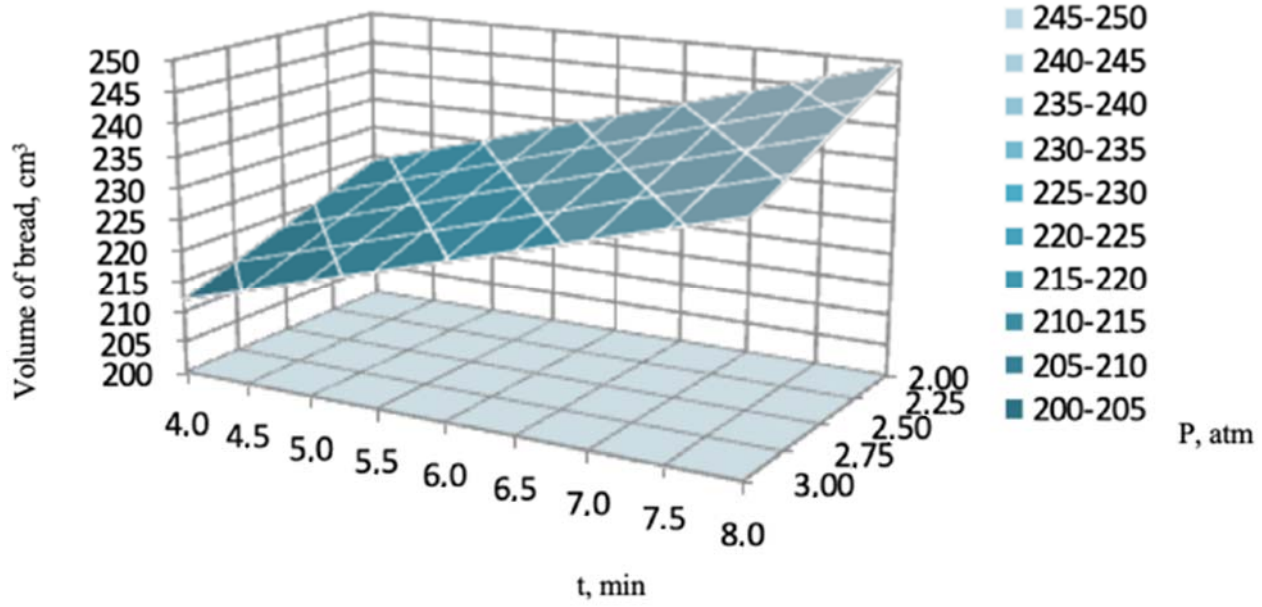


Figure 3 Response surface of nonlinear joint effect of factors C and τ on bread volume (at P = 2 atm).

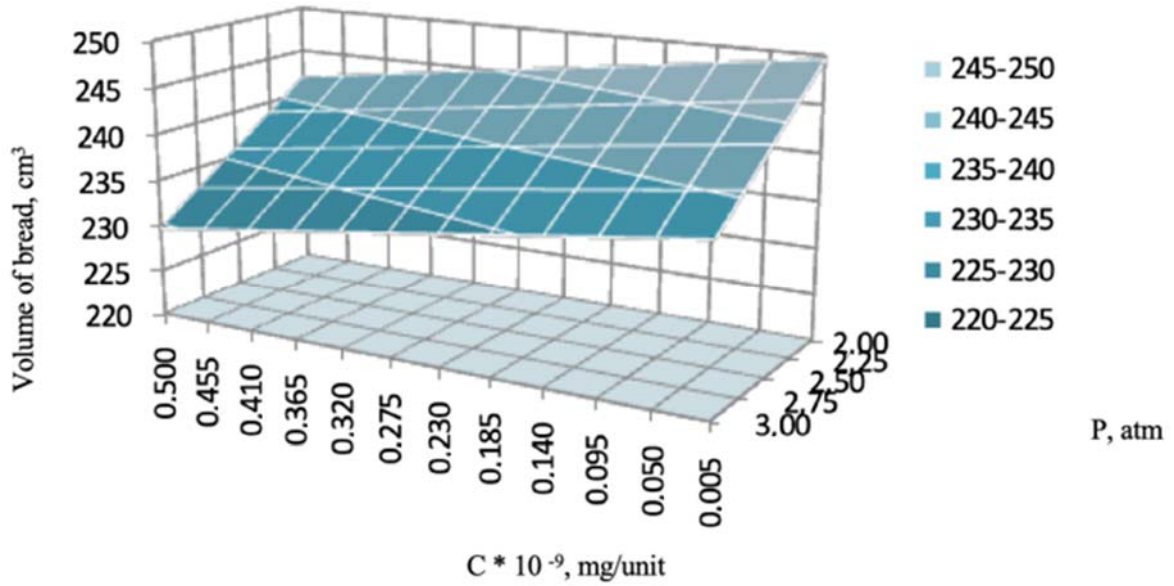
Figure 3 shows that increasing the duration of kneading dough τ unambiguously increases the bread volume. However, at the minimum concentration of ion-ozone (C = 0.003*10^{-9} mg/unit), increasing the duration of kneading dough from 4 to 8 minutes increases the volume of bread from 223.1 to 249.6 cm^3 , i.e. by 11.4%, and at a concentration of C = *0*.5*10^{-9} mg/unit – only from 238.1 to 241.9 cm^3 , i.e. only by 1.6%.

Considering the effect of kneading duration τ on bread volume at different concentrations of ion-ozone C we can see that due to the mutual influence of factors C and τ a different character of changes in bread volume is observed. Thus, at τ = 4 min, decreasing the concentration of ion-ozone C reduces the bread volume from 238.1 to 224.1 cm^3 (by 6.2%), and at τ = 8 min, on the contrary, increases it from 241.9 to 249.6 cm^3 (by 3.2%). Thus, to increase the bread volume at P = 2 atm, the concentration of ion-ozone C should be reduced, and the dough kneading duration τ should be increased.

Figure 4-a and Figure 4-b show the response surfaces characterizing the linear effect of pressure P and dough kneading duration τ on the bread volume.



a)



b)

Figure 4 Response surfaces of linear dependences of bread volume on pressure *P* and dough kneading duration τ: a) linear influence of factors *P* and τ on bread volume (at $C = 0.5\text{-}10^{-9}$ mg/d), b) linear influence of factors *C* and *P* on bread volume (at τ = 8 min).

Figure 4-a shows that increasing the dough kneading time and reducing the pressure *P* leads to a linear increase in bread volume. The largest bread volume is observed at the maximum kneading time τ and the minimum pressure *P*. Figure 4-b shows that decreasing the concentration of ion-ozone C and reducing the pressure *P* leads to a linear increase in bread volume. The maximum bread volume is observed at minimum concentration *C* and pressure *P* values. Optimization of the process of bread production was carried out with the following limitations on the quality indicators of the processed dough, taking into account the necessary quality indicators obtained from the bread:

Table 4 Optimization of the process of bread production.

Min				Max
48.0	≤	y_1 =51.788+3.924-C*10^{-9}	≤	58.0
2.0	≤	y_2 =3.300+1.654-C*10^{-9} -0.00351-C*10^{-9} -*v*	≤	5.0
375	≤	y_3 =294.875+68.*375-P*	≤	530
10.0	≤	y_4 =42.502-29.779-C*10^{-9} -0.6687-τ-0.*04065-v+0*.04997-C*10^{-9} -*v*	≤	29
7.0	≤	y_5 =41.787-33.199-C*10^{-9} -0.6562-τ-0.04351++0.05869-C*10^{-9} -*v*	≤	26
0.9	≤	y_6 =1.396+4.239-C*10^{-9} -0.1184-τ-0.3018-C*10^{-9} -τ-0.00651-C*10^{-9} -*v*+ +0.*00040-τ-v*	≤	2.5
44.0	≤	y_7 =49.934+5.231-C*10^{-9}	≤	55
53.0	≤	y_8 =59.225	≤	66.0
0.95	≤	y_9 =-1.534+0.255-τ+0.*00304-v+0*.1899-C*10^{-9} -τ-0.*00039-τ-v*	≤	2.8
100	≤	y_{10} =222.772+71.680-C*10^{-9} -12.*175-P* + 6.408-τ-10.915-C*10^{-9} -τ	≤	360
325	≤	y_{11} =412.256-439.710-C*10^{-9} +170.540-C*10^{-9} -*P*	≤	500
2.2	≤	y_{12} =0.6792-τ+0.*00402-v-0*.4739-C*10^{-9} -τ+0.00508-C*10^{-9} -v- -0.*00076-τ-v*	≤	5.5
0.14	≤	y_{13} =-2.053+1.006-C*10^{-9} +0.6265-τ+0.*00432-v-0*.5030-C*10^{-9} -τ+ +0.00402-C*10^{-9} -*v-0.00083-τ-v*	≤	2.5
1.0	≤	y_{14} =1.866-1.056-C*10^{-9} +0.1187-τ	≤	4.5
7.0	≤	y_{15} =7.756+1.*224-P-0.00533-v-0*.1411-P-τ + 0.*00088-τ-v*	≤	10.5
20.0	≤	y_{16} =37.935+6.567-τ-0.*1213-v-5*.224-C*10^{-9} -τ +0.06478-C*10^{-9} -v- -1.886-P-τ+0.*03497-P-v*	≤	52
2.7	≤	y_{17} =5.480+0.*4825-P*	≤	10.0
0.30	≤	y_{18} =3.515+1.393-C*10^{-9} -1.*297-P-0*.4075-τ-0.3131-C*10^{-9} +0.*2356-P*	≤	2.6
0.42	≤	y_{19} =-1.592-C*10^{-9} +0.*5736-P+0*.2445-τ+ 0.00304-C*10^{-9} -*v* -0.09054-P-τ	≤	1.82
2.2	≤	y_{20} =5.435-3.576-C*10^{-9} -0.9116-τ+0.*01276-v* +0.5621-C*10^{-9} -τ+ +0.*3045-P-0.00534-P-v*	≤	6.6

Protein content was maximized within the range of changes in the regime factors $t_в$, τ, w, and $t_з$ given in the experiment planning matrix (T a Table 5).

Non-linear programming was done to optimise the processing modes of dough from fine whole-meal flour of "Al-Farabi" variety using activated ion-ozone water. The following optimal technological regimes of dough processing were obtained:

- ion-ozone concentration, $C*10^{-9}$ = 0.003 mg/d;
- pressure P = 2 atm;
- dough kneading time τ = 8 min;
- dough mixer shaft rotation speed, v = 300 rpm.

At these optimal modes of grain processing the target function (volume of 100 g of bread) was 249.6 cm^3. The values of other indicators of the quality of dough and bread at the optimal modes of grain processing are shown in Table 5.

Table 5 Values of quality indicators of dough from fine wholemeal flour "Al-Farabi" using ion-ozoned water and bread prepared from it.

Indicators	min		opt		max
y_1 – moisture content of the dough, %	48.0	≤	51.8	≤	58.0
at_2 – alkalinity of the test, deg;	2.0	≤	3.3	≤	5.0
y_3 – the mass of the dough, g;	375	≤	431.6	≤	530
at_4 – total deformation of the dough, mm;	10.0	≤	24.9	≤	29
at_5 – plasticity of the dough, mm;	7.0	≤	23.4	≤	26
at_6 – elasticity of the dough, mm;	0.9	≤	1.4	≤	2.5
at_7 – moisture content of bread, %;	44.0	≤	50.0	≤	55
at_8 – porosity of bread, %;	53.0	≤	59.2	≤	66.0
at_9 – alkalinity of bread, deg;	0.95	≤	2.0	≤	2.8
at_{10} – is the volume of 100 grams of bread, see^3 ;	100	≤	249.6	≤	360
at_{11} – the mass of bread, g;	325	≤	412.0	≤	500
at_{12} – total deformation of the bread, mm;	2.2	≤	4.8	≤	5.5
at_{13} – plasticity of bread, mm;	0.14	≤	2.3	≤	2.5
at_{14} – elasticity of bread, mm;	1.0	≤	2.8	≤	4.5
at_{15} – protein, %;	7.0	≤	8.5	≤	10.5
at_{16} – starch, %;	20.0	≤	44.8	≤	52
at_{17} – fiber, %;	2.7	≤	6.4	≤	10.0
at_{18} – fats, %;	0.30	≤	1.43	≤	2.6
at_{19} – ash, %;	0.42	≤	1.65	≤	1.82
at_{20} – sugar, %.	2.2	≤	3.64	≤	6.6

Thus, the processing of fine whole-meal flour of the "Al-Farabi" variety using activated ion-ozone water according to the optimal technological modes allowed to provide the maximum amount of bread and maintain within the permissible limits of the studied indicators of quality of dough and bread obtained from it.

Unleavened bread has a better effect on the human body. It promotes fast bowel movements and cleanses of unnecessary toxins and toxins. Many believe that yeast, which are living organisms and need vitamins, proteins and other useful substances, when they enter the body, simply take them away from a person. Another disadvantage and difference of yeast bread is that yeast bacteria cause fermentation and flatulence in the human body **[23]**, **[24]**.

Due to its organoleptic properties, it contributes to the smooth functioning of the intestines, stimulates the active work of the muscles of the gastrointestinal tract. It is its rather high density and rigidity that contribute to better absorption of food and the efficient functioning of the digestive system **[25]**, **[26]**.

The results of the study of qualitative, organoleptic, physical, chemical and microbiological parameters, as well as safety indicators, showed that the yeast-free bakery products made from different dispersed flours and ion-ozoned water meet the requirements and norms of Technical regulation of the Customs Union 021/2011 of the Technical Regulations of the Customs Union "On food safety". The results showed that the use of various dispersed flours in the production of yeast-free bakery products could improve the quality and nutritional value of the final product.

Microbiological analysis showed that the total number of microorganisms in the yeast-free bread prepared with ion-ozoned water was significantly lower than in the samples prepared with tap water. This result indicates that ozone water can be used as an effective disinfectant in the production of bakery products **[27]**, **[28]**. Moreover, the use of different dispersed flours can significantly affect the biological value of baked goods, where the highest biological value was found in bread made with wholemeal flour **[29]**, **[30]**. In addition, the energy value was higher in bread made from wheat flour than other flour types.

When gaseous ozone or ozone ion is dissolved, ozonized water or ion ozonated water is obtained, which is a liquid form of ozone for food use. The solubility of ozone and ion-ozone in water is ten times higher than that of oxygen, in addition, when dissolved in water, ozone decomposes much faster **[31]**, **[32]**. It has been established that the rate of ozone decomposition in water is influenced by its concentration, reaction pH, ultraviolet radiation and dissolved anions. Therefore, with an increase in the pH of the medium, as well as a higher content of organic substances and the presence of an insignificant amount of carbonates, the rate of ozone decomposition increases significantly **[33]**, **[34]**.

Sensory evaluation of yeast-free bread products showed that bread made with various dispersed flours and ion-ozoned water had good texture, taste and flavour. Bread made with wholemeal flour had a nutty flavour and was denser than other bread types, which is consistent with the results of previous studies. The use of ozone and ion-ozoned water in producing yeast-free bakery products also improved the flavour and taste of the final product.

Overall, the results show that the use of various dispersed flours and ion-ozoned water can improve the quality and nutritional value of yeast-free bread products. Adding ozone water to the production process can also reduce the number of microorganisms and improve the palatability of the final product **[35]**, **[36]**. Future research could focus on optimizing ion-ozoned water and various dispersion flours to further improve the quality and nutritional value of yeast-free bread products **[37]**, **[38]**.

Over the past decade, significant progress has been made in improving bread products' quality and chemical composition through various methods **[39]**, **[40]**. One of these methods involves mechanically loosening the dough by whipping it in a specialized whisk for 5 minutes, then knead it in a conventional dough mixer, and then cutting and baking. However, these methods have been used only for long-term dough preparation and have not found practical application in the industry **[41]**, **[42]**.

This technology for functional whipped bread offers potential advantages in terms of shorter production cycles, increased efficiency and improved bakery quality. However, further research is needed to optimize the process parameters and ensure the safety and feasibility of the technology for large-scale production.

Accelerated technology of mechanical loosening of dough finds practical application in preparing bakery and flour confectionery products **[43]**, **[44]**. Dough-loosening technology eliminates yeast from the recipe and prepares dietary varieties acceleratedly without yeast bread and flour confectionery products.

The baking properties of the flour undoubtedly influence the amount of optimum mechanical action on the dough during kneading. Flour dough with strong gluten requires more intensive processing; in contrast, flour with weak gluten has a less mechanical influence on the dough during kneading **[44]**, **[45]**. Therefore, the quality of the flour was investigated at the beginning of the experiment. Accordingly, the dough was prepared with a 55-56% moisture content. Recipes and modes of dough preparation for yeast-free bakery products from fine and fine dispersion flours were developed. Further, the qualitative indicators of manufactured yeast-free bakery products were investigated. Organoleptic, physicochemical and microbiological parameters, and the biological and energy value of yeast-free bakery products, were analyzed.

It was found that the technology of yeast-free dough preparation by mechanical loosening under pressure on the experimental laboratory setup allows reducing the duration of the production process of bakery products from 3 to 6 hours, increases labour productivity by 2-3 times, increases the yield of bread by 14-18%, improves organoleptic characteristics of the finished products.

Based on the technology of accelerated dough preparation, allowing to exclude yeast from the formulation, reducing the duration of the production process, improving the quality of finished products, increasing labour productivity, increase socio-economic indicators of bakeries, a new direction in the production of yeast-free bakery products can be developed.

The semi-finished product is intensively saturated with air oxygen when the dough components are whipped under compressed air pressure. This improves the structural and mechanical properties of the dough, reducing its bulk weight. This is associated with a decrease in the number of SH-groups and the formation of bonds in the structure of protein S-S, which contribute to the strengthening of the protein structure and, consequently, the foam film. Thus, intensive mixing and beating of the dough with air saturation with oxygen in the presence of the enzyme preparation GK-106 accelerate the processes of protein hydrolysis, thus increasing their solubility, increasing the foam formation of semi-finished products, intensifying the formation of substances involved in melanoid formation reactions, reducing the specific power per kneading **[46]**, **[47]**, **[48]**.

Consequently, enzymatic hydrolysis of the main components of flour with mechanical loosening and its intensification can allow obtaining dough and bakery products with optimal structural and mechanical properties and rich flavour and aroma. After kneading before cutting, the dough fermentation stage is reduced by intensifying the colloidal and biochemical processes occurring during dough preparation.

CONCLUSION

The study showed that the new method of bread production, which uses mechanical kneading under pressure and activated water, can significantly reduce kneading time by up to 50%, while improving the physical and sensory properties of the bread. The bread produced by this method had a higher specific volume, softer crumb and more elastic texture than traditional bread-making processes. The economic effect can be achieved by reducing the duration of the production process from 3 to 6 hours, reducing the number of pieces of equipment by eliminating the fermentation and proofing processes (dough mixers, barrels, fermentation tanks, proofing cabinet), etc. The advantages of our technology compared to the known ones are: reduction of production cost due to the elimination of yeast and reduction of a technological process; an increase of bread yield by 14-18%; an increase of labour productivity by more than 2-3 times, and also an increase of enterprise income by two times. In this study, the quality of flour and yeast-free dough was studied using an accelerated method. The dough was prepared by mechanical loosening under compressed air pressure. The study results showed that the quality of flour of the highest, first and second grades correspond to GOST 26574-2017 and is recommended for baking yeast-free bakery products. To improve flavour and aroma, sourdough was added to the dough. Yeast-free bakery products made of fine-dispersed flour with ion-ozoned water met the requirements and norms of Technical regulation of the Customs Union 021/2011 of the Technical Regulations of the Customs Union "On food safety". The biological value in the yeast-free bakery products of second-grade flour was the highest (62.4%), and the energy value was higher in the yeast-free bakery products of the highest-grade flour (877 kJ). Accelerated technology of mechanical loosening of dough is practically used to prepare bakery and pastry products. This technology makes it possible to exclude yeast from the formulation and prepare dietary varieties of yeast-free bread and flour confectionery products by an accelerated method. This study used new accelerated technology to quickly intensify the colloidal and biochemical processes occurring during dough preparation. The technology made it possible to eliminate the dough fermentation and proofing process, thereby reducing the duration of the production process of bakery products, increasing labour productivity, and increasing the yield of bread. A new direction in producing yeast-free bakery products can be developed based on accelerated dough preparation technology.

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Changes in the level of consolidation of the fatty acid profile of *Hermetia illucens* larvae grown on a substrate contaminated with heavy metals

Svyatoslav Loskutov, Yan Puhalsky, Pavel Sorokoumov, Dmitry Ryabukhin, Nikolay Vorobyov

ABSTRACT

We conducted a comparative investigation to examine the alterations in the composition and content of the fatty acid complex in the larvae of the Black Lion fly (*Hermetia illucens*) as they were reared under different concentrations and combinations of heavy metals. The use of the method of mass spectrometric analysis of the obtained biomass showed that linoleic, lauric and oleic fatty acids predominated in the composition of the larvae. The use of the mathematical method of fractal analysis based on the data on the profile distribution of fatty acid components in the insect body according to the experimental variants showed that samples with metal concentrations of 20 mg of cadmium, 800 mg of cobalt and Mix (200 mg of copper, 20 mg cadmium, 200 mg cobalt, 20 mg aluminium and 50 mg lead) per kilogram of dry food. The variation in the values of the indices of the biosystemic consolidation of acids, based on the conversion to their molar masses, ranged from 0.41 to 0.82.

Keywords: *Hermetia illucens*, heavy metal, fatty acid, fractal, bioconsolidation index

INTRODUCTION

One of the topical issues in the development of society at the moment is the world's hunger problem. In 2050 the world population is expected to grow from 7 to 10 billion. However, out of 800 million people who are already malnourished, 650 million are in developing third-world countries. Overall, two billion people suffer from nutritional deficiencies. The problem of producing and using cheap feed additives is also felt in the livestock industry when grazing and keeping livestock. One of the possible solutions here is to introduce non-traditional sources of fodder protein into the diet of humans and animals, which can be larval forms of edible insects.

The most frequently produced species of forage insects is the Black soldier fly (*Hermetia illucens*), from the lionfish family (*Stratiomyia chamaeleon*) **[1]**, **[2]**. Individuals of this species in natural conditions are mainly distributed in tropical and subtropical countries. In Russia, the insect is called the "Black Lion", abroad - "Black Soldier" **[3]**. The first experiments on the cultivation of *Hermetia illucens* began in the 90s of the twentieth century in search of an effective way to utilize organic waste by converting it into biomass rich in proteins and fats. The biomass obtained from their larvae or pupae is a rich source of protein with essential amino acids (in particular, arginine, histidine, leucine and isoleucine, lysine, phenylalanine, tyrosine, valine and others), fatty acids (lauric, myristic, palmitic, stearic, oleic, linoleic acid, etc.), vitamins, macro- and microelements, and other biologically active substances **[4]**, **[5]**, **[6]**. For a long time, these ingredients were part of the food consumed by only most Southeast Asia. However, according to the FAO, in our time, dried insects are no longer an exotic food for Western European countries, including the Russian Federation. They are gradually becoming an ordinary source of high protein content for humans, without allergens and toxins **[6]**, **[7]**.

The use of *Hermetia illucens* larvae has so far gained great popularity in animal husbandry **[8]**, **[9]**. The larvae are also used in fish farming, especially aquaculture **[10]**, **[11]**, **[12]**. It is known that large areas of agricultural land with grassy vegetation are required for the production of animal protein. The feeding process here is very long and laborious. Therefore, dried insect biomass can serve as an excellent complementary feed supplement to existing animal food sources or as a promising alternative to replace their traditional food base completely. According to haematological and biochemical studies, this product did not cause serious deviations in the indicators of enzymatic activity in the blood and liver and violations of the main indicators of protein, lipid, and carbohydrate metabolism in animals **[5]**. Among the European industrial companies producing large-scale feed protein derived from Black Lion fly larvae, the following companies can be distinguished: Hermetia Baruth GmbH (Germany), Agri Protein Technologies (South Africa), Enterra Feed Corporation (Canada), Protix (Netherlands), Bühler Insect Technology Solutions (Switzerland) **[13]**. In the Russian Federation, this direction's representatives are the Biogenesis company. Because *Hermetia illucens* larvae are polyphagous, companies feed them on substrates derived from various waste sources.

It should be noted that in the European Union there are general principles of food and feed safety - the "General Food Law" and the "Hygiene Package", by which the use of manure and food waste for fattening is prohibited **[14]**. In Russia, however, the ecological assessment of plant and animal substrates used for feeding larvae may often differ from the generally recognized level (standard) of European quality. A particular problem here is the possible contamination of substrates with heavy metals. Laboratory experiments conducted with insect larvae reared using standardized chicken feed supplemented with various concentrations of cadmium (2-50 mg/kg), lead (5-125 mg/kg), and zinc (100-2000 mg/kg) showed that the bioaccumulation factor (the ratio of the amount of metal in the body compared to the amount of metal in the substrate) varied considerably. It was revealed that in insect prepupae, the value of the factor for cadmium was 2.32-2.94, for lead - 0.25-0.74. For zinc, the value of the factor decreased with an increase in its concentration in the substrate and ranged from 0.97 to 0.39 **[15]**. Based on the results of the work, it was also concluded that none of the three heavy metals significantly affected the life cycle determinants (pupal weight, development time, sex ratio) of larvae.

It is known that, compared with other insects, Black Lion larvae contain a large amount of fat, especially in the form of saturated fatty acids with an average carbon chain length **[5]**, **[16]**, **[17]**, **[18]**, **[25]**, **[26]**. However, more studies on the effect of their synthesis on the mechanism of regulation and adaptation of the insect to metal-induced stress is needed. This work aimed to study the composition and level of organization of fatty acids in the larvae of *Hermetia illucens*, grown under conditions of contamination of the nutrient substrate with heavy metals.

Scientific Hypothesis

Insects can be a new protein source in feed and food production upon risk assessment. But today, the presence of the availability of accurate data raises the suspicion of the danger of the risk associated with the presence of toxic elements in stable biomasses of insects. The aim of our work was to investigate the alleged detection of metals in the larvae of one of the most popular objects shortly for proposal as a source of protein - the Black soldier fly (*Hermetia illucens*). We are seeing an increase in the structure of acidic fat organization for permanent inclusion in the trophic food chain.

MATERIAL AND METHODOLOGY

Insect

The eggs of the black soldier fly were selected from the brood colony of insects kept in the insectarium of the laboratory for the structural processing of biomass of the All-Russian Research Institute of Food Additives (St. Petersburg, Russia).

Samples

Before the current experiment, newborn larvae were grown in a light chamber (insectarium), 60×50×50 cm in size. The front wall of the chamber was a glass window, and the rest were made of chipboard. The temperature in the working area for breeding flies was maintained at 30 ±2 °C, relative humidity was 65 ±5% Testo 174H (Testo SE & Co. KGaA, Germany) (Figure 1).

Figure 1 Insectarium: development of the authors.

Small ventilation holes were made on the cover of the insectarium. On the ceiling of the incubator, two fluorescent lamps with a power of 30 watts each were installed, connected to the network. The colour temperature of both lamps was 6500 K. The length of the day was 12 hours.

Feed

Initially, the larvae were fed wheat bran moistened with deionized water (water content 75%), then switched to standardized chicken feed (PC-2 compound feed, Gatchinsky Feed Mill JSC, Leningrad region, Russia).

Chemicals

To contaminate the substrate with heavy metals, the chicken feed was thoroughly mixed with the appropriate volume of the salt solution of the toxicant until the desired concentration was reached. Copper salts ($CuSO_4$) were used in the experiment – 200 and 800 mg/kg of dry food, designated as Cu 200 and Cu 800; cadmium ($CdSO_4$) – 20 and 80 mg/kg of dry food, designated as Cd 20 and Cd 80; cobalt ($CoCl_2$) – 200 and 800 mg/kg of dry food, designated as Co 200 and Co 800; aluminium ($AlCl_3x6H_20$) – 20 and 80 mg/kg of dry food, designated as Al 20 and Al 80 and lead (PbOAc) – 50 and 200 mg/kg of dry food, designated as Pb 50 and Pb 200 (Sigma-Aldrich Pty Ltd., Darmstadt, Germany). Next, two special nutrient substrates were prepared to contain mixtures of metals with a minimum (Cu 200, Cd 20, Co 200, Al 20, Pb 50) – mix 200, and maximum (Cu 800, Cd 80, Co 800, Al 80, Pb 200) – mix 800 concentrations of each element. The selected metal concentrations for research are in the range of metal concentrations found in contaminated manure or commercial organic fertilizers **[19]**, **[20]**, **[27]**. In addition, the same volume of deionized water without adding heavy metals was mixed with chicken feed as a control (40, 60 and 80 mg/kg).

Instruments

The content of fatty acids in insect biomass was determined using gas chromatography with mass spectrometric detection, a Varian 450-GC gas chromatograph with CP-Wax 58 FFAP CB, 50 m x 0.32 mm x 0.50 µm column coupled with a Varian 240-MS mass spectrometric detector (Varian, USA).

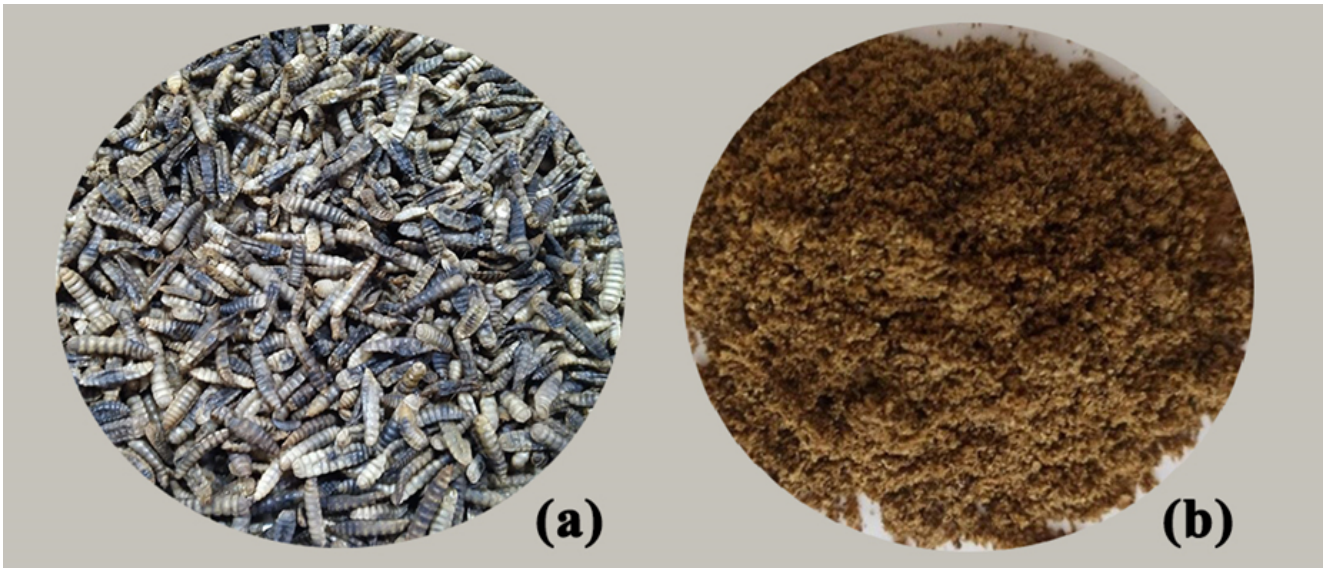

Figure 2 Dried larva of the Black soldier fly *Hermetia illucens*: (a) – whole; (b) – ground.

Analysis conditions

Carrier gas flow (Helium) rate 1 ml/min, injector temperature 250 °C, split 1:15, the start of chromatogram registration from 9 minutes. The temperature program is presented in Table 1.

Table 1 Temperature analysis program.

Temperature, °C	Heating rate, °C/min	Time at a given temperature, min	Total time, min
50	-	4	4
190	6	15	42.33
250	4	10	67.33

Description of the Experiment

Sample preparation: Each experiment was carried out in triplicate. For each repetition, 150 larvae were collected. Insects were placed in plastic containers (12×10×8 cm) located inside the insectarium and fed daily with wheat bran with or without the addition of metals. Feeding was stopped after 8 days. At the end of the experiment, the larvae and their faeces were separated by manual sieving using 3 mm sieves and then ground in a laboratory mill IKA A11 basic (IKA-Werke GmbH & Co. KG, Germany) for further analysis (Figure 2).

The resulting ground samples were evaporated to dryness in JEIO TECH vacuum oven OV-12 with cold trap bath CTB-10 coupled with Woosung Vacuum Co vacuum pump MVP 6 600 µl of a 15% sulfuric acid solution in methanol was added to the evaporated samples, and 600 µl of chloroform was added. Eppendorf was carefully sealed with Parafilm and placed in a heater for 1 hour at 65 °C. After the sample was cooled, 200 µl of deionized water was added and thoroughly mixed. The organic layer was analysed and injected directly into the chromatograph at 1 µl using a CPAL autosampler and a 10 µl Hamilton chromatographic syringe.

Statistical Analysis

The experimental data were subjected to statistical analysis using the R program (version 4.1.0, https://cran.r-project.org/bin/windows/base/) for Windows **[21]**, **[22]**. Statistical processing of the results of determining the profile of fatty acids in the samples was carried out using ANOVA analysis of variance and Fisher's test (F). Differences were considered significant, and the presence of a relationship between the indicators was recognized at a probability level not exceeding 0.05. Fractal portraits and indices of biosystematic determination (IF) of fatty acids were calculated based on the mathematical algorithm incorporated in the original computer program **[23]**.

RESULTS AND DISCUSSION

Biochemical tests showed that the composition of the larvae was dominated by linoleic (C18:2n-6), oleic (C18:1n-9) and lauric (C12:0) fatty acids p <0.01 (Table 2). The latter is known for its antimicrobial activity against pathogenic Gram-positive bacteria **[18]**, **[24]**. For example, it has been shown that lauric acid can modulate gut health in humans **[28]** and mice **[29]**. Lauric Acid is an inhibitor of *Clostridium* - a gram-positive spore-forming anaerobic pathogen of the gastrointestinal tract **[30]**. Lauric acid also showed the best result in terms of inhibition of multiplication of the Junin arenavirus (JUNV) **[31]**.

Table 2 Average values of the content of fatty acids in the sample, %.

Variant	1	2	3	4	5	6
g/mol	172.26	200.3	228.37	226.36	242.4	256.43
Control	1.50 ±0.07	22.89 ±0.22	9.61 ±0.31	0.34 ±0.01	BDL	12.71 ±0.52
Al 20	1.75 ±0.09	24.76 ±0.91	7.93 ±0.29	0.38 ±0.02	0.44 ±0.01	9.12 ±0.36
Al 80	1.57 ±0.04	22.98 ±0.42	7.92 ±0.13	0.50 ±0.01	0.31 ±0.01	10.13 ±0.13
Cd 20	1.93 ±0.04	24.88 ±0.81	7.71 ±0.02	0.40 ±0.01	0.33 ±0.01	10.21 ±0.09
Cd 80	1.50 ±0.10	21.92 ±2.22	7.99 ±0.77	0.25 ±0.00	0.32 ±0.01	12.67 ±1.04
Co 200	2.29 ±0.05	23.62 ±0.38	7.82 ±0.11	0.22 ±0.01	0.32 ±0.01	9.40 ±1.56
Co 800	1.61 ±0.03	26.82 ±0.53	9.53 ±0.22	0.26 ±0.02	0.52 ±0.08	10.80 ±0.18
Cu 200	1.89 ±0.07	27.71 ±0.69	8.59 ±0.08	0.62 ±0.01	0.47 ±0.01	9.20 ±0.17
Cu 800	1.79 ±0.08	27.05 ±0.77	8.38 ±0.15	0.46 ±0.05	0.28 ±0.01	9.37 ±0.10
Pb 200	1.50 ±0.07	23.99 ±0.52	7.70 ±0.20	0.40 ±0.06	0.22 ±0.01	9.68 ±0.22
Pb 50	2.66 ±0.07	27.98 ±0.47	7.69 ±0.31	0.35 ±0.02	0.50 ±0.13	9.44 ±0.24
Mix 200	1.11 ±0.01	17.89 ±0.01	6.41 ±0.01	0.31 ±0.01	0.40 ±0.01	10.71 ±0.01
Mix 800	1.28 ±0.01	19.00 ±0.01	6.74 ±0.01	0.31 ±0.01	0.20 ±0.01	11.06 ±0.01
Variant	**7**	**8**	**9**	**10**	**11**	**12**
g/mol	254.41	270.45	284.48	282.46	280.45	278.44
Control	3.73 ±0.19	BDL	6.18 ±0.11	17.54 ±0.24	23.43 ±0.16	2.29 ±0.18
Al 20	3.23 ±0.09	0.45 ±0.01	6.6 ±0.27	14.59 ±1.88	27.57 ±0.68	3.17 ±0.10
Al 80	3.97 ±0.04	BDL	5.94 ±0.07	16.46 ±0.11	27.30 ±0.34	2.70 ±0.21
Cd 20	4.32 ±0.12	BDL	5.61 ±0.65	17.09 ±0.37	24.99 ±0.77	2.46 ±0.04
Cd 80	4.01 ±0.21	0.63 ±0.01	6.14 ±0.84	17.15 ±1.26	25.47 ±1.14	2.49 ±0.35
Co 200	3.72 ±0.12	0.36 ±0.03	7.32 ±0.29	16.23 ±0.52	25.96 ±0.79	2.78 ±0.14
Co 800	3.43 ±0.19	0.50 ±0.02	5.81 ±0.20	15.20 ±0.54	23.04 ±0.43	2.36 ±0.08
Cu 200	4.59 ±0.27	0.34 ±0.07	4.37 ±0.13	14.52 ±0.12	24.48 ±1.31	2.93 ±0.05
Cu 800	4.63 ±0.17	0.48 ±0.02	4.80 ±0.27	15.93 ±0.16	24.26 ±0.39	2.40 ±0.33
Pb 200	4.09 ±0.22	0.58 ±0.01	6.88 ±0.31	17.10 ±0.46	25.53 ±0.45	2.70 ±0.08
Pb 50	3.39 ±0.29	0.50 ±0.01	3.93 ±1.10	14.03 ±0.27	26.66 ±1.05	2.90 ±0.06
Mix 200	3.23 ±0.01	0.50 ±0.01	7.00 ±0.01	16.77 ±0.01	32.18 ±0.01	3.49 ±0.01
Mix 800	3.45 ±0.01	0.45 ±0.01	6.49 ±0.01	15.98 ±0.01	31.33 ±0.01	3.37 ±0.01

Note: Addendum: BDL* – below the detection limit. 1 – Capric acid; 2 – Lauric acid; 3 – Myristic acid; 4 – Myristoleic acid; 5 – Pentadecanoic acid; 6 – Palmitic acid; 7 – Palmitoleic acid; 8 – Heptadecanoic acid; 9 – Stearic acid; 10 – Oleic acid; 11 – Linoleic acid; 12 – Gamma-linolenic acid.

The initial values of fatty acids from percentages were converted into molar masses to approximate the obtained data and construct fractal portraits.

The visual difference between the data in the quantitative composition of the acid profiles for different experimentexperiment variants is shown on the heat map (Figure 3).

Further, based on these data, two-dimensional coordinate planes were constructed, on which point represents each acid represents each acid represents each acid represents each acid with the x-coordinate equal to the fractional part of $\log_2$ (ei/emax), and the y-coordinate equal to $\log_2$ (ei/emax), where ei, emax is an acid with a nominal, established serial number (i) and with a maximum intensity of synthesis. The entire field of the fractal portrait is divided into rectangular sectors by horizontal and vertical lines. The sectors highlighted in color contain dotted images of fatty acids inside. Further, on the basis of a decreasing power series of fatty acid indicators **[32]** for the selected sectors, IF calculations were made under the influence of metal-induced stress **[33]**, **[34]**, **[35]**. To calculate them, it suffices to count the total number of selected sectors in fractal portraits (N0) and the number of selected sectors (NF) located in the columns and diagonals of the fractal portrait grid and uniting groups consisting of three or more sectors. As a result, all IF calculations are reduced to the formula (1):

$$IF = NF/N0 \quad (1)$$

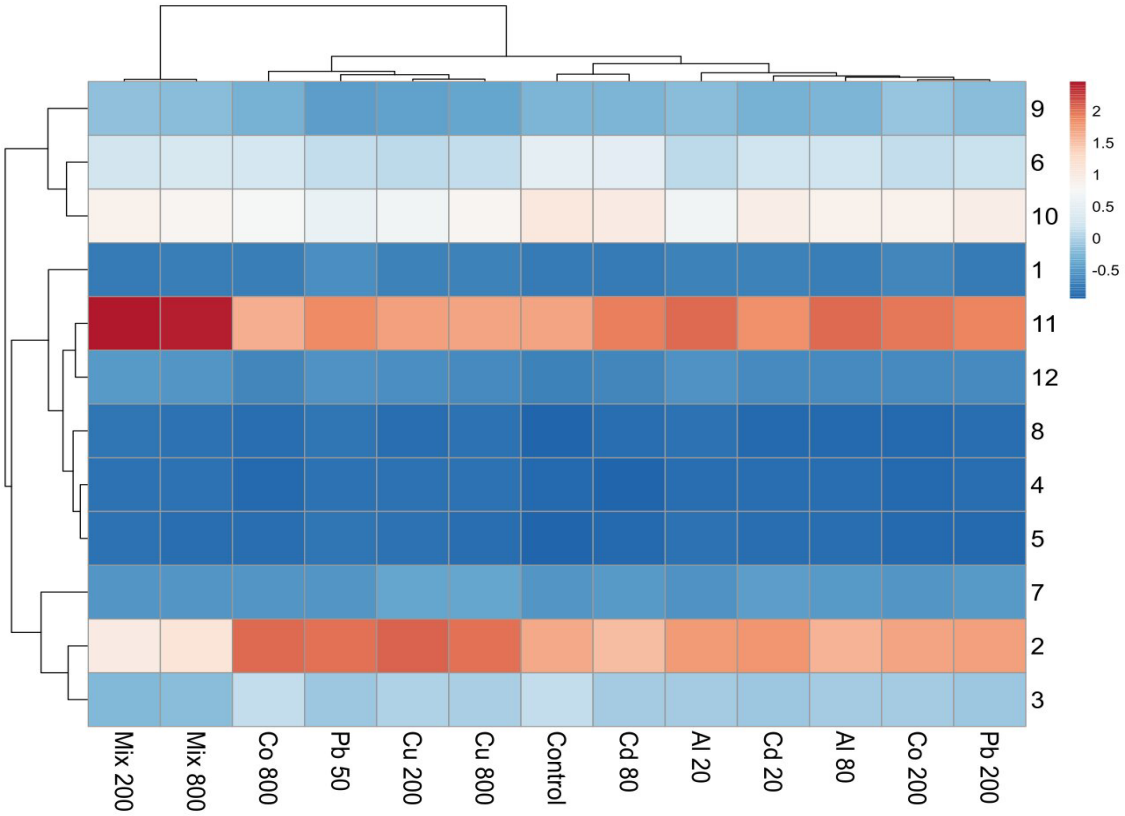

Figure 3 Heat map of changes in the composition of fatty acids in the larvae of the black soldier fly *Hermetia illucens* under metal-induced stress. Red indicates a higher concentration; blue indicates a lower concentration of each component. 1 – Capric acid; 2 – Lauric acid; 3 – Myristic acid; 4 – Myristoleic acid; 5 – Pentadecanoic acid; 6 – Palmitic acid; 7 – Palmitoleic acid; 8 – Heptadecanoic acid; 9 – Stearic acid; 10 – Oleic acid; 11 – Linoleic acid; 12 – Gamma-linolenic acid.

Also, the Simpson and Shannon indices were calculated according to the data obtained **[36]**, **[37]**, **[38]**, most commonly used by ecologists to measure the diversity of communities **[39]**. The results of all calculations are shown in Table 3.

Table 3 Average indices of various indices of systemic organization of fatty acids in insect larvae of *Hermetia illucens*.

No. p/n	Option	IF	Index Simpson	Index Shannon
1	Control	0.51 ±0.05	0.08 ±0.01	0.85 ±0.01
2	Al 20	0.56 ±0.06	0.11 ±0.01	0.80 ±0.01
3	Al 80	0.67 ±0.02	0.10 ±0.01	0.81 ±0.01
4	Cd 20	0.71 ±0.01	0.10 ±0.01	0.80 ±0.01
5	Cd 80	0.53 ±0.12	0.09 ±0.01	0.82 ±0.01
6	Co 200	0.55 ±0.03	0.10 ±0.01	0.80 ±0.01
7	Co 800	0.71 ±0.07	0.11 ±0.01	0.78 ±0.01
8	Cu 200	0.68 ±0.04	0.12 ±0.01	0.78 ±0.02
9	Cu 800	0.66 ±0.10	0.12 ±0.01	0.77 ±0.01
10	Pb 200	0.68 ±0.05	0.10 ±0.01	0.81 ±0.01
11	Pb 50	0.65 ±0.02	0.12 ±0.01	0.77 ±0.01
12	Mix 200	0.72 ±0.01	0.11 ±0.01	0.78 ±0.01
13	Mix 800	0.53 ±0.01	0.11 ±0.01	0.78 ±0.01

As we can see, the Cd 20, Co 800 and Mix 200 samples had the greatest impact on the change in the biochemical fatty acid composition of the larvae. The overall variation in IF values ranged from 0.41 to 0.82. Their fractal portraits and chromatograms are shown in Figure 4.

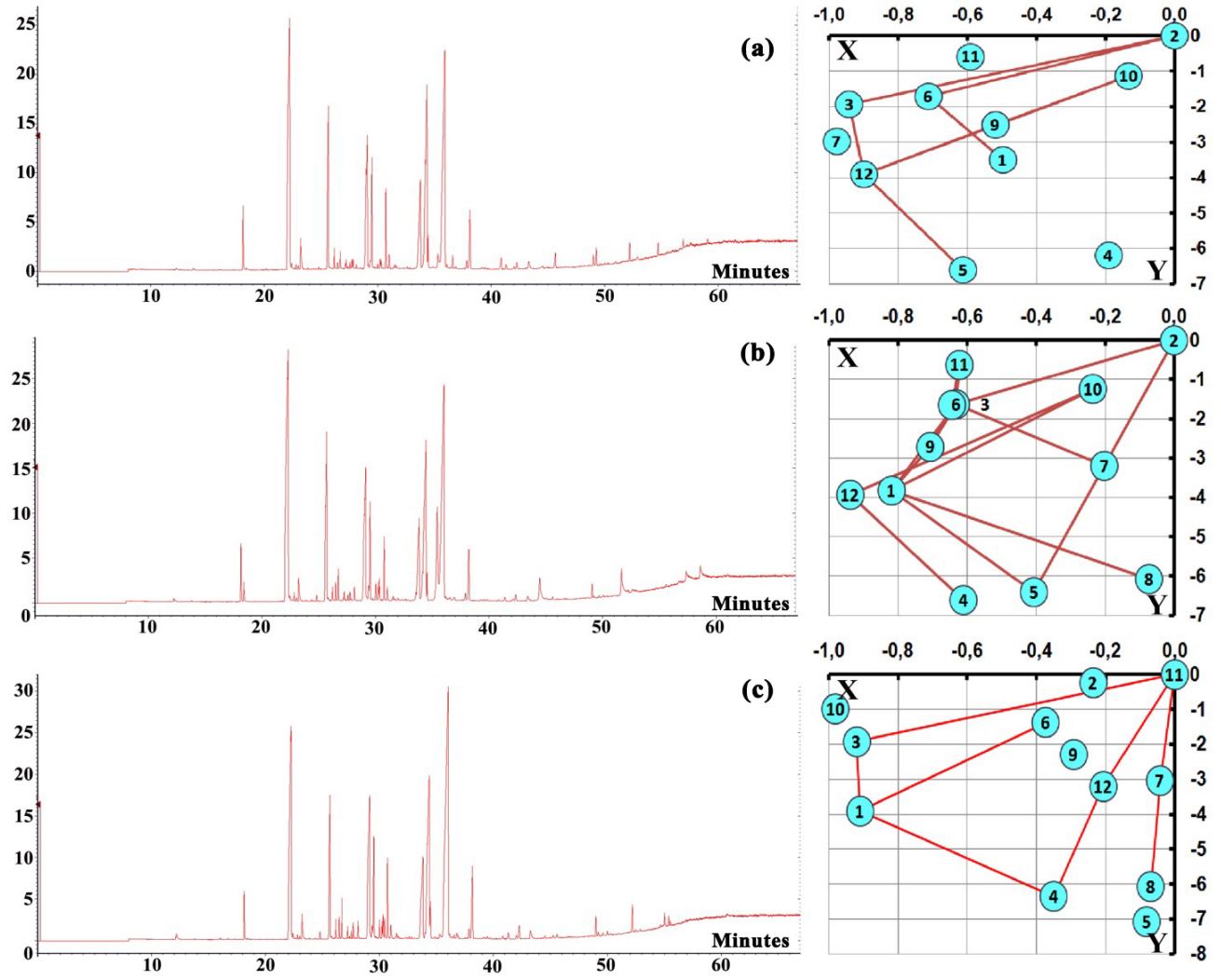

Figure 4 Chromatograms and fractal portraits of the biocomposition of fatty acids in the body of *Hermetia illucens* insects: a – sample Cd 20; b – sample Co 800 and c – sample Mix 800.

So far, only few data have been published on contaminants identified in commercially available insects and insect-based products for human consumption or animal feed. It seems clear that the substrate has an effect on the fatty acid composition, which are the source of energy in insects **[40]**, **[41]**. A study by a fully accredited laboratory in the UK studying the chemical safety of four different species of fly larvae, including *Hermetia illucens*, as a protein source for animal feed showed that only cadmium was above the maximum EC limit in animal feed of 0.5 mg/kg (three of nine samples analyzed) **[42]**. This finding confirms the cadmium accumulation shown in feeding studies under controlled lab conditions **[43]**, **[44]**. In addition to cadmium, the influence of chromium, lead and arsenic has also been studied **[45]**, **[46]**, **[47]**. Pimpla turionellae showed a striking decrease in lipid content, when exposed to Cd **[48]**. Also was demonstrated that whole body lipid concentration of day-3 4th instar larvae Lymantria dispar were significantly reduced in Cd-contaminated **[49]**.

Our results showed that the composition of the larvae was dominated by linoleic, lauric and oleic fatty acids. Insect hemolymph composition is known to change with developmental stage and within one stage **[50]**. Hence, the interpretation of lipid and fatty acid composition in the insects may be difference. In spite of that, this study lends further support to the observation that *Hermetia illucens* a is sensitive to Cd and these results suggest that the whole body lipid concentration are affected directly by Cd concentrarion in nutrient substrate.

CONCLUSION

The use of the mathematical method of fractal analysis based on the data on the profile distribution of fatty acid components in the insect body according to the variants of the experiment showed that samples with metal concentrations of 20 mg of Cd, 800 mg of Co and Mix (200 mg of Cu , 20 mg Cd, 200 mg Co, 20 mg Al and 50 mg Pb) per kilogram of dry food. Since the Mix 200 mixture also contains cadmium ions, and the mean error in the Co 800 variant exceeds those for the other two IF values selected based on the calculation results, it can be concluded that this pollutant, to a greater extent, compared to all other heavy metals, takes part in the launch of typical reactions of fatty acid homeostasis involved in the implementation of the internal program of adaptive lability in this insect species.

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Conflict of Interest:

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

Ethical Statement:

The study titled "Changes in the level of consolidation of the fatty acid profile of Hermetia illucens larvae grown on a substrate contaminated with heavy metals" conducted by the All-Russian Research Institute for Agricultural Microbiology in Pushkin, St. Petersburg, Russia, upholds the principles of ethical research. The welfare of Hermetia illucens larvae was given utmost consideration throughout the study. All necessary measures were taken to ensure their well-being and minimize any potential harm or discomfort caused during the experiment.

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Preparation and examination of the quality of gingerbread made with composite flour and sugar beet

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ABSTRACT

The production of confectionery products is one of the most in-demand industries. Due to this, various assortments of confectionery products and production technologies have expanded. Following modern requirements, the product's appearance, taste, aroma, and nutritional value should be appropriate. Accordingly, to create a new range of gingerbread products, chickpea and bean flour, and sugar beet powder as a sugar substitute were introduced into the recipe: 10%, 15%, and 20% of the wheat flour in the original recipe were replaced by chickpea flour and bean flour; also, 30 and 60 g of the 125 g of sugar in the recipe was replaced by beet powder. A fully factorial experimental design was created to perform the work. According to this plan, control and 8 research samples of gingerbread were cooked and prepared. Organoleptic and physicochemical analysis of these finished products was performed. Sample No. 6, the sample with 5% chickpea flour, 10% bean flour, and 30 g of sugar beet powder, had the best organoleptic indicators. It is evenly light golden in colour, smooth in shape, undamaged, and well cooked. The taste and smell are sweet and are not inferior to the control sample in all parameters. Moisture content, water absorption properties, ash, acidity, fat, vitamins, toxic elements, and microbiological indicators were determined from the physicochemical parameters. Analysing the research results, gingerbread product No. 6 was the optimal regimen, because it contained a high amount of mineral elements and vitamins, and no toxic elements or microbiological indicators were found. In addition, the density and water absorption were relatively close to the control sample.

Keywords: gingerbread, sugar beet, chickpeas, beans, organoleptic and physicochemical parameters

INTRODUCTION

Nowadays, mass nutrition is gradually turning towards industrialization. Modern companies with advanced technological tools and various factories and workshops are being created. They use advanced technologies, introduce scientific organization of work and production, and use new types of service.

The confectionery industry is an important branch of the food industry. Confectionery products are characterized by a high caloric content and quick absorption by the body. These properties are characteristic of confectionery products due to the use of sugar, caramel syrup, oils, milk and milk products, egg products, cocoa beans, fruits, and nuts, as well as flours from various grains **[1]**, **[2]**, **[3]**.

In general, confectionery production is divided into two main groups: sugar and flour confectionery production.

Confectionery products made of flour are of great importance in the people's diet. Their basis is flour, which contains many carbohydrates from starch and vegetable proteins.

Confectionery made from flour generally includes products made with flour, sugar, milk, butter, eggs, and yeast. These products contain proteins, fats, minerals, carbohydrates, and vitamins. These will not be the same in all products; it will vary depending on the type of flour used and the recipe used.

Confectionery made from flour makes up 40% of the total confectionery production **[4]**, **[5]**, **[6]**.

Their chemical composition influences the nutritional value of confectionery products made from flour. This determines the group of substances that form the main and additional raw materials used in their production. But not all substances that enter the body with food remain stable; not all of them are completely absorbed. Some substances change, some become less digestible. Therefore, energy, biological, physiological, and organoleptic values are considered in assessing overall nutritional value. On average, flour confectionery products contain 5% to 29% water, 3% to 10.6% protein, 3% to 74% carbohydrates, and 40% fat.

Nutritional value describes the completeness of the necessary parameters of the product and its taste benefits due to the various nutrients it contains. The higher the nutritional value, the more the product meets the body's physiological needs for these substances and ensures its normal functioning.

Confectionery products are characterized by high nutritional value because they are the main source of carbohydrates and fats in the human diet **[7]**, **[8]**, **[9]**, **[10]**.

The assortment of this group of products is constantly expanding and being updated; new types of products appear, which means that the industry has developed somewhat.

This work aims to use composite flour from chickpeas, beans, and sugar beet powder to increase the nutritional value of gingerbread products.

Adding chickpea flour makes it possible to expand the range of confectionery products made from flour and increase the nutritional value of the products. It contains 31.0% protein, 7.0% fat, and 5.2% fibre. Chickpea protein is close to animal proteins in its biological value, as it contains all essential amino acids. In addition to biologically valuable proteins, chickpeas contain elements such as potassium, phosphorus, manganese, selenium, and zinc **[11]**, **[12]**, **[13]**, **[14]**.

Beans contain a lot of vitamins of group B, especially vitamin B6, which affects the function of the immune and nervous systems and improves the skin. Beans are rich in starch, carbohydrates, and proteins. In addition, there are vitamins C, B1, B2, B6, PP, macro- and microelements (especially copper, zinc, and potassium), various acids, and carotene **[15]**, **[16]**, **[17]**.

Sugar beet powder was also used in the work. It is very rich in useful vitamins and minerals. Its energy value per 100 g is 40-45 kcal and it contains 1.5 g of protein, 0.1 g of fat, 8.8 g of carbohydrates, 2 g of fibre, 2.5 g of dietary fibre, and 1 g of ash. In beet growing, the dry matter contains sucrose. In addition, sugar beet contains vitamins A, C, E, PP, B1, B2, B3, B6, and B9.

Scientific hypothesis

Improving the quality and useful properties of gingerbread will depend on the raw materials used, and the mode and technology of preparation. The content of composite flour and dried sugar beets significantly impact the content of vitamins, minerals, and other beneficial properties of flour confectionery.

MATERIAL AND METHODOLOGY

Samples

The study used chickpea, bean flour, and dried sugar beets to make gingerbread from premium flour. Chickpea and bean flour were obtained from raw materials collected in the summer of 2022 from peasant farms of the Zhambyl region (Kazakhstan); sugar beet was obtained from the Koksu Sugar Plant LLP (Koksu village, Almaty region).

Chemicals

All reagents were of analytical grade and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

Instruments

A Chizhov ELEKS-7M Tagler instrument (Sibagropribor, Russia), KVANT-Z-ETA atomic absorption spectrometer (Kortek, Russia), and Agilent 1100 HPLC (Agilent Technologies, USA) were used.

Laboratory Methods

The following indicators of raw materials and the resulting product were studied in the work:

- wettability according to GOST 15810-2014:, The standard applies to gingerbread products: gingerbread. The method is based on a change in the mass of a gingerbread product when immersed in water at a temperature of 20 °C for a certain time. Wetness is characterized by the ratio of the mass of gingerbread products after wetting to the mass of dry gingerbread products and is expressed as a percentage;

- humidity according to GOST 5900-2014: the standard applies to confectionery and semi-finished products and establishes methods for determining the mass fraction of moisture and solids. The essence of the method lies in drying the analyzed sample of the product at a certain temperature and calculating the weight loss in relation to the mass of the analyzed sample before drying. The method is intended for the following products: flour confectionery, muffins, semi-finished products for cakes and pastries, oriental sweets, rolls, halva, chocolate and chocolate icing, praline, marzipan, fondant, milk sweets, toffee, whipped products, products containing alcohol. The method is applicable in the measurement range of mass fractions of moisture from 0.5% to 50.0%.

- organoleptic indicators according to GOST 15810-2014. According to organoleptic indicators, gingerbread products must comply with Table 1.

Table 1 Organoleptic characteristics of gingerbread products.

Name of indicator	Characteristic
Taste and smell	Products with a pronounced sweet taste and aroma characteristic of this name of a gingerbread product, corresponding to the added flavoring additives, without foreign taste and smell.
Structure	Products with a soft, bonded structure that do not crumble when broken.
Color	From white-cream to dark brown with shades of varying intensity. The color of the crumb is uniform throughout the entire volume of the product. The surface may be darker than the crumb, the lower surface is darker than the upper. A darker color of the protruding reliefs of the imprint of a drawing or inscription is allowed. The general color tone of individual products must be the same in each packaging unit.
Split type	Baked products, with a uniform well-developed porosity, without voids, hardening and traces of non-mixing. In gingerbread with filling, the filling must be inside the product; the filling must not leak onto the product's surface. A slight compaction is allowed in places bordering on the filling. Gingerbread with filling consists of layers of semi-finished gingerbread interconnected by filling. The filling should not protrude beyond the edges of the gingerbread product.
Surface	Dry, without large cracks, swellings, or depressions, not burnt, without sagging. The presence of small cracks is allowed no more than 5% of the surface area. The imprint of a drawing or inscription must be clear, not blurry. Finishing of the top surface is allowed.
Form	Correct, varied, not blurry, without dents, with a convex upper surface (except gingerbread products that imprint a pattern or inscription on the surface). The bottom surface is flat. Cavities not more than 5 mm in diameter are allowed in the amount of not more than 10% of the lower surface area. The cut of the gingerbread should be even, without crumpled edges. The filling should not protrude beyond the edges of the gingerbread product.

- fat content according to GOST 5668-68: The method is based on extracting fat from a pre-hydrolyzed product sample with a solvent and determining the amount of fat by weighing after removing the solvent from a certain volume of the resulting solution.

- ash content according to GOST 5901-2014: the standard applies to confectionery and semi-finished confectionery products (after this referred to as the product) and establishes methods for determining the mass fraction of ash (total and insoluble in hydrochloric acid solution). The essence of the method lies in charring, ashing the analyzed sample of the product at a temperature of 500-600 °C and the subsequent determination of the mass fraction of total ash.

- alkalinity according to GOST 5898-87: the standard applies to confectionery and semi-finished products and establishes methods for determining titratable alkalinity. For degrees of titratable alkalinity, the number of cubic centimeters of a hydrochloric acid (sulfuric acid) solution with a concentration of 1 mol/dm^3 is taken to neutralize the alkaline substances contained in 100 g of the product.

- iron was determined according to GOSTGOST 26928-86: The standard applies to food products and establishes a colorimetric method for determining iron. The method is based on measuring the color intensity of a solution of a complex compound of ferrous iron with red orthophenanthroline.

- the phosphorus content was determined by GOST 30615-99: the standard applies to raw materials and food products and establishes a method for determining phosphorus. The method consists in dry mineralization of the sample, dissolution of ash, carrying out a color reaction with a molybdenum-vanadium reagent and measuring the

intensity of the yellow color of the solution l = (440 ±5) nm using a photoelectrocolorimeter or spectrophotometer. The presence of macro- and microelements does not interfere with the determination.

- the content of vitamin A was determined by GOST R 54635-2011: the standard applies to functional foods and establishes a method for determining the mass fraction of vitamin A in the form of retinol, retinol acetate, retinol palmitate using high-performance liquid chromatography (hereinafter – HPLC). The measurement range of the mass fraction of vitamin A is from 0.5 to 10.0 ppm;

- the vitamin E content was determined by GOST EN 12822-2014: the standard establishes a method for determining vitamin E in food products by high-performance liquid chromatography (HPLC). The determination of the content of vitamin E is carried out by α-, β-, γ- and δ-tocopherols. The activity of vitamin E can be calculated from the content of tocopherols by applying the appropriate coefficients;

- the content of vitamins B1 and B2 was determined according to Method M 04-41-2005 (Certificate of attestation of the measurement procedure No. 224.04.17.035/2006). This document establishes a methodology for measuring the mass fraction of water-soluble vitamins B1, B2, B3, Bs, C, B6, B5 in the form of nicotinamide and nicotinic acid and, depending on the composition of the analyzed sample and the requirements for measurement accuracy, the latter can be analyzed by two methods of capillary electrophoresis – zone (CEZ) and micellar electrokinetic chromatography (MECH).

Other standard conventional chemicals and organoleptic methods were used to study raw materials and finished products.

Description of the Experiment

The study was conducted following the state standard requirements used in producing gingerbread products. A recipe for the preparation of gingerbread products was created. According to this recipe, 8 samples were obtained by replacing 10%, 15%, and 20% of the wheat flour with chickpea and bean flour, and sugar beet powder was added as a sugar substitute (Tables 2-4).

Table 2 Product formulation of a control sample of gingerbread.

Raw material	Amount of raw material used for the production of 500 g
Flour, g	208
Egg, whole	1
Milk, ml	125
Sugar, g	125
Vegetable oil, ml	25

Table 3 Experimental design parameters.

	x_1- chickpea flour, %	x_2- bean flour, %	x_3- sugar beet, g
Max	10	10	60
Medium	7.5	7.5	45
Min	5	5	30

Table 4 Experimental design draft.

Sample	x_1- chickpea flour, %	x_2- bean flour, %	x_3- sugar beet, g
1	10	10	60
2	5	10	60
3	10	5	60
4	5	5	60
5	10	10	30
6	5	10	30
7	10	5	30
8	5	5	30

Number of samples analysed: 9 samples were analysed.
Number of repeated analyses: All tests were performed in triplicate.
Number of experiment replication: Replications were conducted twice.
Design of the experiment: Samples from the finished product were determined using generally accepted analytical methods. During the research, methods were used to describe the organoleptic characteristics of the studied objects, their chemical composition, and nutritional and energy value.

Statistical Analysis

The data obtained during the experiments were processed using the one-way analysis of variance ANOVA to analyze the data and determine if there were significant differences between samples. The data collected during the study were subjected to independent testing. 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.

RESULTS AND DISCUSSION

The quality of flour confectionery products is evaluated according to the following indicators: appearance (color, shape, finish, surface condition), fracture appearance and structure, taste and smell. When evaluating the appearance by examining the products, the correctness of the shape, the presence of deformed products, fractures, tears, bubbles, cracks, shells, burnt products are noted **[18]**, **[19]**.

Evaluating the product in terms of "kind of fracture and structure", they pay attention to the baked goods, the uniformity of the pores, the presence of voids, non-mixing, hardening. Assessing the taste and smell of products, the presence of unpleasant or unusual odors and tastes, as well as a crunch on the teeth due to the presence of mineral impurities, is established **[20]**, **[21]**.

To give preventive, functional properties to gingerbread, it is a promising direction to introduce flour from leguminous crops into the gingerbread recipe, which will enrich products with biologically active substances **[22]**, **[23]**, **[24]**.

Along with the control sample, 8 samples were analysed for organoleptic indicators, physicochemical properties, and microbiological indicators (Tables 5-10 and Figures 1-9).

Table 5 Organoleptic indicators according to research results.

Sample	Taste and smell	Structure	Colour	Split type	Surface	Form
Control sample	According to the ingredients added to the composition, it has a sweet taste and smell, without foreign odours and flavours.	According to the soft standard, which does not scatter when divided.	The surface layer is bright yellow, and a soft cream.	The product is porous, soft; ingredients are completely mixed.	Dry, without cracks, not burnt.	The given form is preserved.
1	The taste and smell of beans are clearly detectable.	The structure is correct; it does not scatter.	Light yellow, soft yellow.	Porous, soft, standard.	Dry, without cracks.	The format is preserved.
2	The taste and smell of beans are clearly detectable.	Stronger than other models.	Light yellow, not burnt.	Porosity is lower.	Dry, without cracks.	The format is preserved.
3	There is a strange smell.	Has a non-scattering structure, conforming to the standard.	Light yellow, soft cream.	The product is porous, soft and, suitable.	Dry, without cracks.	The format is preserved.
4	The taste of beet sugar can be clearly felt. Slightly acidic.	The structure is non-sprinkling and soft.	Light yellow, soft cream.	Porous, compliant.	Dry, without cracks.	The format is preserved.
5	It has a unique smell and taste.	The structure is solid.	Light yellow, soft cream.	Porosity is low.	Dry, without cracks.	The format is preserved.
6	According to the state standard, it has its own characteristic smell and taste.	Non-scattering, soft, conforming to requirements.	The surface layer is bright yellow, and a soft cream.	The product is porous, soft, and the ingredients are completely mixed.	Dry, without cracks, not burnt.	The format is preserved.
7	It has a taste and smell according to its ingredients.	It has an unbreakable structure.	Light yellow, suitable.	Porous, soft.	Dry, with cracks.	The format is preserved.
8	It has a unique smell and taste.	It has an unbreakable structure.	Light yellow.	Porous, soft.	Dry, with cracks.	The format is preserved.

Figure 1 Gingerbread product prepared according to the control sample.

The results of the control sample were under the requirement.

Figure 2 Gingerbread sample No. 1 (10% chickpea flour, 10% bean flour, 60 g of sugar beet).

The smell and taste of beans were evident in sample No. 1, made with 10% chickpea flour, 10% bean flour, and 60 g of sugar beet.

Figure 3 Gingerbread sample No. 2 (5% chickpea flour, 10% bean flour, 60 g of sugar beet).

The taste and smell of beans were also evident in sample No. 2, made with 5% chickpea flour, 10% bean flour, and 60 g of sugar beet.

Figure 4 Gingerbread sample No. 3 (10% chickpea flour, 5% bean flour, 60 g of sugar beet).

In sample No. 3, made with 10% chickpea flour, 5% bean flour, and 60 g of sugar beet, a foreign smell was detected, apart from the added ingredients.

Figure 5 Gingerbread sample No. 4 (5% chickpea flour, 5% bean flour, 60 g of sugar beet).

In sample No. 4, made with 5% chickpea flour, 5% bean flour, and 60 g of sugar beet, a clear taste and smell of sugar beet was observed, and it turned out to be slightly acidic.

Figure 6 Gingerbread sample No. 5 (10% chickpea flour, 10% bean flour, 30 g of sugar beet).

In sample No. 5, made with 10% chickpea flour, 10% bean flour, and 30 g of sugar beet, no porosity was observed when dividing the product compared to other samples.

Figure 7 Gingerbread sample No. 6 (5% chickpea flour, 10% bean flour, 30 g of sugar beet).

Sample No. 6, the sample with 5% chickpea flour, 10% bean flour, and 30 g of sugar beet, had the best organoleptic indicators. The sample was uniformly light in colour, smooth in shape, undamaged, and well-cooked. The taste and smell were sweet. No indicators were inferior to those of the control sample.

Figure 8 Gingerbread sample No. 7 (10% chickpea flour, 5% bean flour, 30 g of sugar beet).

Sample No. 7, made with 10% chickpea flour, 5% bean flour, and 30 g of sugar beet, had crusts and cracks.

Figure 9 Gingerbread sample No. 8 (5% chickpea flour, 5% bean flour, 30 g of sugar beet).

In sample No. 8, made with 5% chickpea flour, 5% bean flour, and 30 g of sugar beet, the shape was preserved, and the surface was dry.

According to the organoleptic indicators of the gingerbread products, it was decided that product No. 6 is the optimal mode.

For flour from legumes, a characteristic disadvantage is the smell and taste of legumes, which constrains the norms of its introduction when enriching food systems, since at a specific dosage, the organoleptic characteristics of products obtained with the addition of flour from legumes decrease **[25]**, **[26]**. The introduction of chickpea and bean flour into the gingerbread recipe is justified by its nutritional properties and high biological value, it is an easily digestible product that is balanced in terms of the composition of proteins, carbohydrates and fats and is also rich in fiber. It is known from the scientific literature that chickpea and bean flour is a valuable biological product that contains vitamins (β-carotene, A, B1, B2, PP) and mineral elements (calcium, magnesium, sodium, potassium, phosphorus, iron) **[27]**, **[28]**.

Flour confectionery differs from sugar confectionery in that their recipe includes flour. These products have a high-calorie content and digestibility, have a pleasant taste and attractive appearance. The high nutritional value of flour confectionery products is due to the significant content of carbohydrates, fats, proteins. Due to the low humidity, most products are valuable food product with a long shelf life. All flour confectionery products are characterized by high nutritional and energy value **[29]**, **[30]**. The low humidity of these products allows them to be stored for a long time **[31]**, **[32]**.

Gingerbread products were analysed according to physicochemical and microbiological indicators; the results are shown in Tables 6-10.

Table 6 Physico-chemical indicators of gingerbread products.

Sample	Indicators								
	Humidity, %	**Fat, %**	**Protein, %**	**Carbohydrate, %**	**Ash, %**	**Gluten, %**	**Density, g/cm^3**	**Alkalinity, degree**	**Water absorbency, %**
Control	11.18	9.99	6.43	60.45	0.89	2.77	0.54	1.83	222
1	8.91	14.22	9.09	57.74	0.95	4.42	0.54	2.22	194
2	9.55	12.93	9.03	55.78	0.75	5.51	0.64	0.80	169
3	11.71	15.62	8.44	66.43	1.26	4.46	0.65	1.59	166
4	7.52	11.45	8.20	55.42	0.79	3.31	0.57	1.60	165
5	8.17	13.55	8.49	53.27	1.19	3.88	0.58	2.08	210
6	9.12	13.02	8.95	58.46	0.76	3.96	0.48	1.07	209
7	9.36	12.53	9.84	60.03	1.05	4.12	0.56	1.72	190
8	8.27	12.15	8.08	52.79	1.12	3.38	0.48	2.0	182

As shown in Table 6, the moisture content of the control sample was 11.18%. Samples No. 1, No. 2, No. 3, No. 4, No. 5, No. 6, No. 7, and No. 8 with chickpea and bean flour and sugar beet powder showed a decrease in moisture content compared to the control sample. The content of carbohydrates ranged from 52.79 to 66.43%. The sweet components that make up the chemical composition of gingerbread lead to the release of happiness hormones into the blood, from which, after a sweet snack, the mood rises sharply **[33]**, **[34]**.

The benefit of flour confectionery products lies in the high content of carbohydrates in the composition of products, which give the human body the necessary energy boost for normal life **[35]**, **[36]**. Due to the special consumer properties of confectionery products, storing certain flour products for quite a long time is possible. In addition, due to their easy digestibility, some sweets and confectionery products are used in sports nutrition **[37]**, **[38]**.

Since the amount of protein in chickpea flour and bean flour is higher than in wheat flour, gingerbread products made with these additives also have a higher protein content.

Sample No. 6 was found to be standard according to physical and chemical indicators.

The study determined the potassium, calcium, iron, and phosphorus content of the control gingerbread products and those made with a mixture of chickpea and bean flour and sugar beet flour.

According to the research results in Table 7, the amount of potassium in samples No. 1, No. 2, No. 4, No. 5, No. 6, No. 7, and No. 8 with the addition of chickpea and bean flour and sugar beet powder was 2% higher than that in the control sample. The potassium content in sample No. 3 increased by 3% compared to the control.

The amount of calcium in the control sample was 20.17 mg, but it increased in the samples with chickpea and bean flour and sugar beet powder. Only the amount of calcium in sample No. 4 was lower than that in the control sample, i.e. 18.59 mg.

The iron content was 1.03 mg in the control sample, increasing only slightly in the samples with chickpea and bean flour and sugar beet powder.

The phosphorus in the control sample was 80.59 mg, increasing several times with chickpea, bean flour, and sugar beet powder.

Table 7 Mineral elements in gingerbread products.

Sample	Indicators, mg/100 g			
	Potassium	Calcium	Iron	Phosphorus
Control	122.24	20.17	1.03	80.59
1	284.79	43.27	2.12	170.11
2	280.11	35.64	1.88	176.07
3	346.52	31.22	1.81	149.16
4	202.35	18.59	1.73	138.24
5	262.59	39.41	1.95	149.09
6	235.09	35.51	2.36	152.94
7	232.59	25.02	1.98	167.81
8	247.77	35.66	1.72	141.19

Table 8 Vitamins contained in gingerbread products.

Sample	Indicators, mg/100 g				
	A	E	B_1	B_2	PP
Control	0.009	2.21	0.145	0.047	2.633
1	0.008	2.37	0.218	0.084	3.581
2	0.007	2.44	0.254	0.052	3.243
3	0.013	2.21	0.235	0.079	3.611
4	0.009	2.39	0.174	0.063	3.128
5	0.012	2.47	0.222	0.078	2.951
6	0.015	2.38	0.281	0.094	3.942
7	0.010	2.33	0.288	0.091	3.884
8	0.009	2.27	0.255	0.082	3.219

As we can see from Table 8, samples with chickpea and bean flour and sugar beet powder contained more vitamins than the control sample.

Such vitamins increase immunity, maintain normal metabolism, stimulate brain activity and strengthen the entire nervous system. Even after baking, some vitamins remain in the products and enter the body. The action of mineral elements is closely intertwined with the action of vitamins, strengthening blood vessels, stimulating blood formation and supporting the musculoskeletal system **[39]**, **[40]**.

Considering the chemical composition, high biological value, and composition of vitamins, we can conclude that the use of chickpea, bean flour and sugar beet is promising in producing gingerbread to give them a functional orientation.

No less important are such hygienic requirements for the quality of confectionery products, as indicators of their safety. The packaging must contain data on testing for toxic elements, radionuclides, pesticides and microbiological indicators (food infections, molds, yeasts). The microbiological quality of confectionery products determines the degree of their safety for humans and the exclusion of the risk of poisoning and disease after consumption. In addition to safety for humans, microbiological indicators determine the degree of freshness and shelf life and the correct storage of confectionery **[41]**, **[42]**.

Table 9 shows the content of toxic elements, Table 10 shows the microbiological parameters of gingerbread.

From the data in Table 9 it can be seen that cadmium was not detected in all samples. Lead was also found in samples No. 1, No. 3 and No. 7, in a small amount and does not exceed the norm, complies with the standards of the Technical Regulations of the Customs Union TR CU 021/2011 - On food safety products (as amended on July 14, 2021 No lead was found in the rest of the samples.

Table 9 Toxic elements in gingerbread products.

Sample	Indicators, mg/kg	
	Cadmium	Lead
Control	Not found	Not found
1	Not found	0.0006
2	Not found	Not found
3	Not found	0.0009
4	Not found	Not found
5	Not found	Not found
6	Not found	Not found
7	Not found	0.0002
8	Not found	Not found

Table 10 Microbiological indicators in gingerbread products.

Sample	Indicators, CFU/g			
	QMAFAnM	*E. coli* in 1.0 g of product	Yeast	Mould
Control	8×10^3	Not found	Not found	Not found
1	18×10^3	Not found	4	6
2	2×10^3	Not found	10	4
3	12×10^3	Not found	8	13
4	4×10^3	Not found	Not found	Not found
5	Not found	Not found	Not found	Not found
6	Not found	Not found	Not found	Not found
7	Not found	Not found	2	2
8	5×10^3	Not found	3	Not found

From the data of Table 10, mesophilic aerobic and facultative anaerobic microorganisms were found in the control sample, also in samples No. 1, No. 2, No. 3, No. 4 and No. 8. Yeast was found in samples No. 1, No. 2, No. 3, No. 7 and No. 8, moulds were also found in samples No. 1, No. 2, No. 3 and No. 7. All these data do not exceed the established norm of the Technical Regulations of the Customs Union TR TS 021/2011 – On food safety (as amended on July 14, 2021). The Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM), Escherichia coli (E. coli), yeasts and molds were not detected in the remaining samples.

Analysing the data in Tables 6-10, we determined gingerbread product No. 6 to be the optimal regimen because it contained many mineral elements and vitamins, and no toxic elements or microbiological indicators were found. In addition, the density and water absorption were relatively close to the control sample's.

As a result of the research, gingerbread product No. 6 had an optimal mode. Thus, the developed gingerbread product is highly nutritional and can be considered a functionally oriented food.

According to organoleptic indicators, sample No. 6, made with 5% chickpea flour, 10% bean flour, and 30 g of sugar beet powder, had the best indicators. It is evenly light golden in colour, smooth in shape, undamaged, and well cooked. The taste and smell are sweet and are not inferior to the control sample in all parameters. Moisture content, water absorption properties, ash, acidity, fat, vitamins, toxic elements, and microbiological indicators were determined from the physicochemical parameters.

Analysing the research results, gingerbread product No. 6 was the optimal regimen, because it contained a high amount of mineral elements and vitamins, and no toxic elements or microbiological indicators were found. In addition, the density and water absorption were relatively close to the control sample's.

CONCLUSION

In order to expand the assortment of high-nutrition flour confectionery products, it is recommended to market sample No. 6, i.e. the recipe with 5% chickpea flour, 10% bean flour, and 30 g of sugar beet powder, from among the research samples made in this study. This is because it meets standard requirements and has more protein and higher nutritional value than ordinary gingerbread products. As a functional food product, it could be popular among young children and the elderly and for dietary purposes. Purchasing imported equipment has allowed enterprises to expand the types of products produced and improve product quality. The optimal amount of chickpea and bean flour to replace wheat and sugar beet to replace sugar was established experimentally by increasing the percentage of replacement and evaluating the organoleptic characteristics of the finished product. Samples with 5 and 10% replacement of wheat flour for chickpea and bean flour, as well as 30 and 60 grams of sugar replacement for sugar beet were investigated. As a result of the research, it was found that an increase in the percentage of replacing wheat flour with chickpea bean flour has a positive effect on the organoleptic and rheological characteristics of the dough, and also increases the nutritional and biological value of the gingerbread. Based on the results obtained, it can be concluded that the change in prescription ingredients makes it possible to obtain new functional gingerbread with high organoleptic characteristics, with reduced calorie content, with increased nutritional value due to the introduction of high-protein chickpea and bean flour, enrichment with dietary fiber, vitamins, macro- and trace elements of sugar beet, which will undoubtedly be in demand among buyers.

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The influence of chitosan on the raspberry quality during the storage process

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ABSTRACT

Raspberry is a perishable berry raw material with a high capacity for mechanical and microbiological damage, and therefore, after harvesting, it is necessary to use appropriate technologies to preserve its quality and extend the storage time. This work aimed to study the influence of different concentrations of chitosan solutions on the quality and duration of storage of raspberries under refrigerating conditions. Raspberries were picked at the consumer maturity stage in perforated plastic containers with a capacity of 500 grams. The berries were processed by spraying with 0.5%, 1.0%, and 2.0% chitosan solution, then removing residual moisture. The storage was carried out for twelve days in a refrigerating chamber at a temperature of 2 °C and relative humidity of 95%. The research was performed according to the physical, chemical, and organoleptic indicators, determining the changes in the mass fraction of ascorbic acid, the mass fraction of sugars during storage, taste, aroma, colour, etc. consistency and appearance at the end of storage. It was found that the loss of ascorbic acid in the processed berries was 1.5-3.9 times less than in the reference sample. A similar situation was observed with the mass fraction of sugars, which prevailed 0.9-2.5 times in the processed samples. According to the organoleptic indicators, the samples with a solution concentration of 1.0% and 2.0% were recognized as the best. According to the results of experimental investigations, it was established that pre-processing of berries with chitosan solutions is a promising method to slow down unwanted metabolic processes that take place after harvesting.

Keywords: chitosan, storage time, raspberries, pre-processing, biopolymer

INTRODUCTION

Raspberry is a highly nutritious berry crop with a short storage time, high water content and thin cover tissues that mechanical and microbiological factors may easily damage; this berry has a high nutritional value and a wide range of applications in the food industry **[1]**. Moreover, it can be used to make:

- jams, confitures and other preserves, which have a pleasant taste and aroma, as well as a high pectin content, which allows obtaining a stable gelatinous product;
- fruit juices, nectars and lemonades with a high content of vitamins and antioxidants;
- as an additive to ice cream and yoghurt;
- confectionery and dried fruits.

Raspberries continue their metabolic activity after harvesting, gradually losing quality, decreased resistance to phytopathogenic damage, and increased percentage loss. Therefore, edible coatings made of biopolymers and their combinations extend the storage time of perishable berry products **[2]**.

Chitosan is a biopolymer obtained from chitin, the main component of the skeleton of crustaceans and insects, which can form a thin layer around fresh products, which acts as a protective agent, extending the storage time, and also serves as an inhibitor of metabolic processes **[3]**. Chitosan has several advantages for processing berries, among which are the following:

‣ berry storage, chitosan can be used as a preserving agent for berry storage as it is a natural antibacterial agent that helps to prevent the development of spoilage microorganisms **[4]**;

‣ the storage time extension, chitosan can increase the storage time of berries by creating a protective layer that helps to retain moisture and to prevent oxidation **[5]**;

‣ the improvement of the quality of berries, chitosan can improve the quality of berries by reducing moisture loss and increasing their viability and resistance to mechanical damage **[6]**;

‣ the environmental component, chitosan is a natural product that decomposes after use, so it is an environmentally compatible means for raspberry processing **[7]**.

Diseases, improper transport, and storage techniques cost the world economy about $220 billion annually, reducing crop productivity and quality and leading to higher food prices and global food insecurity. Reducing losses and scraps of fresh fruit and vegetables can help reduce pressure on food production systems, especially in the context of limited natural resources and climate change.

Edible coatings have become integral for protecting fruits and vegetables from phytopathogenic damage. A wide range of studies demonstrated the antimicrobial activity of edible coatings against *Botrytis cinerea*, *Colletotrichum* spp., *Penicillium* spp. and *Alternaria* spp. **[8]**. Films formed on the surface of fruits can change the atmosphere's composition, creating a barrier for gas exchange, such as oxygen, carbon dioxide and ethylene, which are involved in the process of respiration **[9]**.

There are many edible coatings; among them, chitosan is the safest. Chitosan (β-(1,4)-2-amino-2-deoxy-D-glucose) is a natural biopolymer obtained by deacetylation of chitin, which is the second with ranking most important polysaccharide in nature after cellulose, and which enters into the composition of the exoskeleton structure of marine invertebrates, insects, as well as fungi, algae and yeast **[10]**. Chitosan is one of the most widely used edible coatings due to its biocompatibility, biodegradability, and bioactivity. When applied to fruits, vegetables and berries, chitosan creates a semi-permeable film that protects against the development of fungus diseases and slows down metabolic processes **[11]**. This edible coating is widely used for post-harvest fresh fruits and vegetables preservation. The scientific literature on edible coatings using chitosan has increased in recent years. This can be explained by the importance of chitosan for plant protection as a natural fungicide. In 2014, chitosan hydrochloride was approved as one of the first main plant protection substances by the European Union (EU Regulation 2014/563), and the second chitosan formulation was approved in 2022 (EU Regulation 2022/456) **[12]**.

Therefore, the use of raspberry in the food industry is a promising and relevant direction of research because its use will allow to expand of the assortment, increase the quality and nutritional value of products made on its basis, attract new consumers, and export can be a promising line for the development of the food industry of Ukraine.

Scientific Hypothesis

For further improvement of storing and processing raspberry technology, predicting the storage time depending on various environmental influences may be considered a promising direction. Therefore, the research aimed to develop the most effective method of storing raspberries to improve the prediction of the content of dry soluble substances, sugars and titrating acids in raspberries. By conducting experimental investigations, the expediency of predicting the content of the main components of the chemical composition in raspberries will be determined according to average values, and the factors that have the greatest influence on the accumulation of the studied indicators will be identified.

MATERIAL AND METHODOLOGY

Samples

Raspberries of Patricia (Figure 1 a), Polka (Figure 1 b) and Chervona Koroleva (Figure 1 c) varieties were picked in the fields of the academic and research department of the Uman National University of Horticulture at the consumer maturity stage according to DSTU 7179:2010 **[13]**.

a

b

c

Figure 1 Studied varieties of raspberries: a – berries of the variety of Patricia, b – berries of the variety of Polka, c – berries of the variety of Chervona Koroleva.

Chemicals

Acetone, C_3H_6O (TD Energobudinvest, Ukraine).
Sodium hydroxide, NaOH (Khimlaborreaktiv TOV, Ukraine).
Ascorbic acid, vitamin C (Khimlaborreaktiv TOV, Ukraine).
Metaphosphoric acid, HPO_3 (Khimlaborreaktiv TOV, Ukraine).
Pyrocatechin, $C_6H_4(OH)_2$ (Khimlaborreaktiv TOV, Ukraine).
Chloroform, $CHCl_3$ (CHEMICO GROUP, Great Britain).
Methanol, CH_3OH (CAS, the Netherlands).
Hexane, C_6H_{14} (Hammerite, the Netherlands).

Animals, Plants and Biological Materials

For experimental investigations, the following varieties were used: Patricia raspberry variety (produced by the academic and research department of the Uman National University of Horticulture, Cherkasy Oblast, Ukraine); Polka raspberry variety (produced by the academic and research department of the Uman National University of Horticulture, Cherkasy Oblast, Ukraine) and Chervona Koroleva raspberry variety (produced by the academic and research department of the Uman National University of Horticulture, Cherkasy Oblast, Ukraine).

Instruments

Drying oven SNOL 67/350 (ThermoEngineering TOV, Ukraine), a titrator (Labor-Technik TOV, Ukraine).
Analytical electronic balance KERN ABS 120-4 (Khimtex, the State Enterprise, Ukraine).
Refractometer IRF-454B2M (KOMZ VAT).
pH meter ULAB MP 511 (ULAB, China).
Gas chromatograph Kristallux-4000M (Meta-Chrom, the Research and Production Company).
Refrigerator GGM Gastro (GGM Gastro, Germany).
Refractometer (IRF-454 B2M, manufactured by Inter-Syntez TOV, Ukraine).
Laboratory thermometer (TLS-200, manufactured by Inter-Syntez TOV, Ukraine).
Photocolorimeter (KFK-3, manufactured by Inter-Syntez TOV, Ukraine).
Flame spectrophotometer (Saturn-4, manufactured by Inter-Syntez TOV, Ukraine).

Laboratory Methods

The selection and preparation of samples for analysis of fresh strawberries were carried out according to DSTU (the State Standards of Ukraine) 7205:2009, and the products of its processing – according to DSTU 7244:2009.

The quality assessment of fresh raspberries and preserves was carried out following the following regulatory documents:

– fresh berries - according to DSTU 7205:2009 **[14]**;
– compotes - according to DSTU 8102:2015 **[15]**;
– jams - according to DSTU 4900:2007 **[16]**;

In the test samples, the following parameters were determined:

– an average weight of strawberries by weighing;
– a volume of strawberries by the amount of displaced water when immersed in a measuring cylinder;
– a hardness of berries by calculating the ratio of the mass of berries to their volume;
– a density by piercing a fruit in the equatorial zone with a FT 02 penetrometer;
– a respiration intensity by the amount of released carbon dioxide **[17]**;
– a weight loss of berries by the method of fixed samples weighing **[18]**;
– a shine - visually on a 5-point scale, where 1 – a dull surface of berries,
no shine, and 5 - a shiny glossy surface;
– a content of dry soluble substances according to DSTU 8402:2015 **[19]**;
– a content of sugars according to DSTU 4954:2008 **[20]**;
– organic acids by titrating with alkali according to DSTU 4957:2008 **[21]**;
– pH – by the potentiometric method according to DSTU 6045:2008 **[22]**;
– an acetaldehyde content – by the bichromatic and iodometric method **[23]**;
– an ethyl alcohol content – by the iodometric method **[24]**;
– an ascorbic acid content – by the iodometric method **[25]**;
– a content of nitrates – by the ionometric method according to DSTU 4948:2008 **[26]**;

All investigations were performed in triplicate.

The results of the analysis led to the initial mass according to the formula (1):

$$X = \frac{A \times (100 - b)}{100} \quad (1)$$

Where:
X – the content of substances taking into account a mass loss, %; A – the content of substances at the end of storage, %; b – the mass loss during the storage period, %.

Microbiological studies were carried out by microscopy using a MICROmede XS – 2610 microscope with a magnification of 50 times, taking samples from the surface of berries with an inoculating wire loop. Photomicrographs were taken using a photo camera.

Description of the Experiment

Sample preparation: Bushes, typical for a certain variety, even-aged, with medium intensity of fruiting, were selected for the research. Raspberries of each variety were picked when the pulp of the berries was still dense enough, but the taste and color were appropriate for this variety. The harvesting date was determined by the following characteristics of the quality of fresh berries: the appearance and the size of berries about the largest longitudinal diameter. The selected berries corresponded to the indicators of the first commercial variety: the shape and colour of the variety, berries with stalks, mechanical damage, vermin damage and fungal diseases. Fruits were picked from different bushes.

Experiment 1: Raspberries were processed with chitosan solutions of six concentrations (0.05%; 0.1%; 0.2%; 0.3%; 0.4%; 0.5%) in two ways: spraying and immersion for 1 min. The processed berries were left to dry completely.

Experiment 2: Raspberries were processed with chitosan solutions of six concentrations: 0.05%; 0.1%; 0.2%; 0.3%; 0.4%; 0.5% and left to dry completely.

Experiment 3*:* Raspberries were processed with a 0.5% chitosan solution and stored for seven days in a refrigerator with free access to air.

Number of analyzed samples: To determine the content of dry soluble substances, sugars and titrating acids, a sample of 100 berries of each pomological variety was taken from 6 bushes that had entered into a full fruiting period.

Number of repeated analyses: all instrument measurements and readings were performed 3 times.

Number of experiment replications: The number of repeats of each experiment to determine one value was also 3 times.

Design of the experiment: Experiment 1: Dry processed berries and the control were weighed and placed in perforated plastic (PET) containers with a capacity of 500 g, and stored at a temperature of 20 °C on racks in a ventilated location. Berries without processing were considered as the control.

Experiment 2: Dry processed berries and the control were weighed and packaged in perforated plastic (PET) containers with a capacity of 500 g and in plastic bags with a thickness of 30 microns. The storage was carried out in two ways: with free access to air and in a modified gas environment at a temperature of 0 ±2 °C with an atmosphere relative humidity of 90-95%. Berries without processing were considered as the control.

Experiment 3: After storing, the preserves were made from berries according to the manufacturing specifications: "Raspberry puree", "Raspberry jam", "Raspberry confiture", "Raspberry compote", and "Natural raspberry juice". The "Raspberry in own juice" preserves were produced according to the technology developed by our team. The preserves made from fresh unprocessed raw materials were considered as the control.

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 study was conducted every second day of storage to assess the influence of pre-processing of raspberries with chitosan solutions. We have determined weight loss, a mass fraction of dry soluble substances (a mass fraction of DSS), a respiration intensity, a mass fraction of organic acids, a mass fraction of sugars, an ascorbic acid content, pH level; a density, and a shine degree.

During the storage period ends, the content of ethyl alcohol and acetaldehyde and the yield of commercial products were determined, and the organoleptic evaluation was carried out.

A series of similar scientific investigations, in which the influence of pre-processing with various solutions on various types of fruit and berry raw materials, is described in the following scientific papers:

– the influence of pre-processing of strawberry berries **[27]**, **[28]**;
– the influence of pre-processing of currant berries **[29]**, **[30]**;
– the influence of pre-processing of blackberry berries **[31]**, **[32]**;
– the influence of pre-processing of apple fruits with special solutions **[33]**, **[34]**;
– the influence of pre-processing of apricot fruits with solutions based on sulfites **[35]**, **[36]**;
– the influence of pre-processing of cape gooseberry fruits with special solutions **[37]**, **[38]**.

However, the use of sulfite can cause various types of allergic reactions, so its use is limited.

Raspberries are characterized by a high moisture content, which is lost through thin cover tissues due to quick physiological changes. Berry mass losses during storage are caused by a rather high respiration intensity, a decrease in the content of nutritional substances, and a development of phytopathogenic damage **[39]**.

Pre-processing of raspberry with chitosan solutions made it possible to reduce the weight loss of berries during storage (Figure 2).

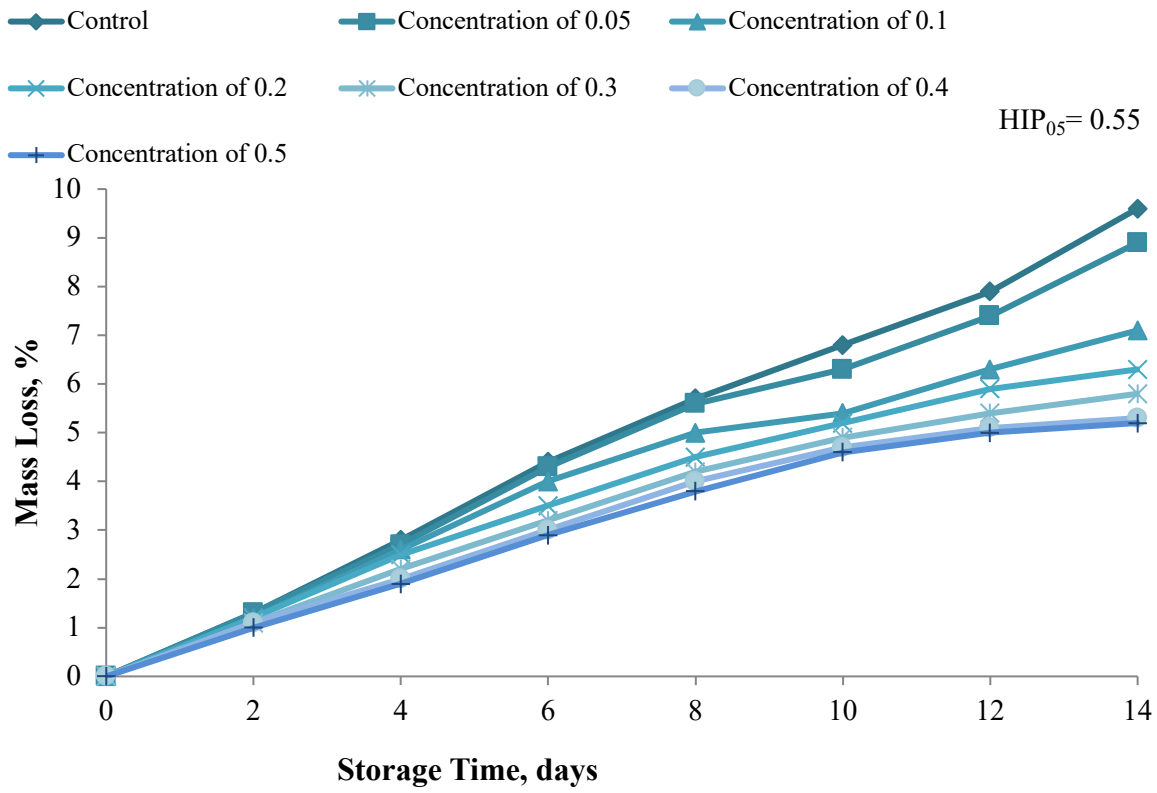

Figure 2 The changes in the natural weight losses of raspberries during refrigerated storage in a modified gas environment (2018-2020).

The mass loss of raspberries increased with each day of storage, and on the second day, it ranged from 0.98 to 1.5% and from 1.0 to 1.3%, depending on the storage method. On the eighth day of the storage, the indicators ranged from 4.3 to 6.3% and from 3.8 to 5.7%. The lowest losses during the entire storage period were detected in the sample with a chitosan processing concentration of 0.5%. During the storage period ends, the mass losses reached 9.6% and 10.9% in control, 6.2-10.4% and 5.2-8.9% in the preprocessed samples.

The analysis of the dynamics of raspberry's weight losses during the two-week storage period demonstrated that the processing with a chitosan solution contributes to weight loss reduction.

Carbohydrates, nitrogenous substances, acids, pectin, vitamins, enzymes, mineral salts and tanning substances represent dry soluble substances. In raspberries, the main part of DSS is carbohydrates, mainly represented by sugars and acids. The change in the mass fraction of dry soluble substances during storage occurs due to the conduction of biochemical processes in raspberries.

The pre-processing of raspberries with chitosan revealed a positive effect on preserving dry soluble substances (Figure 3).

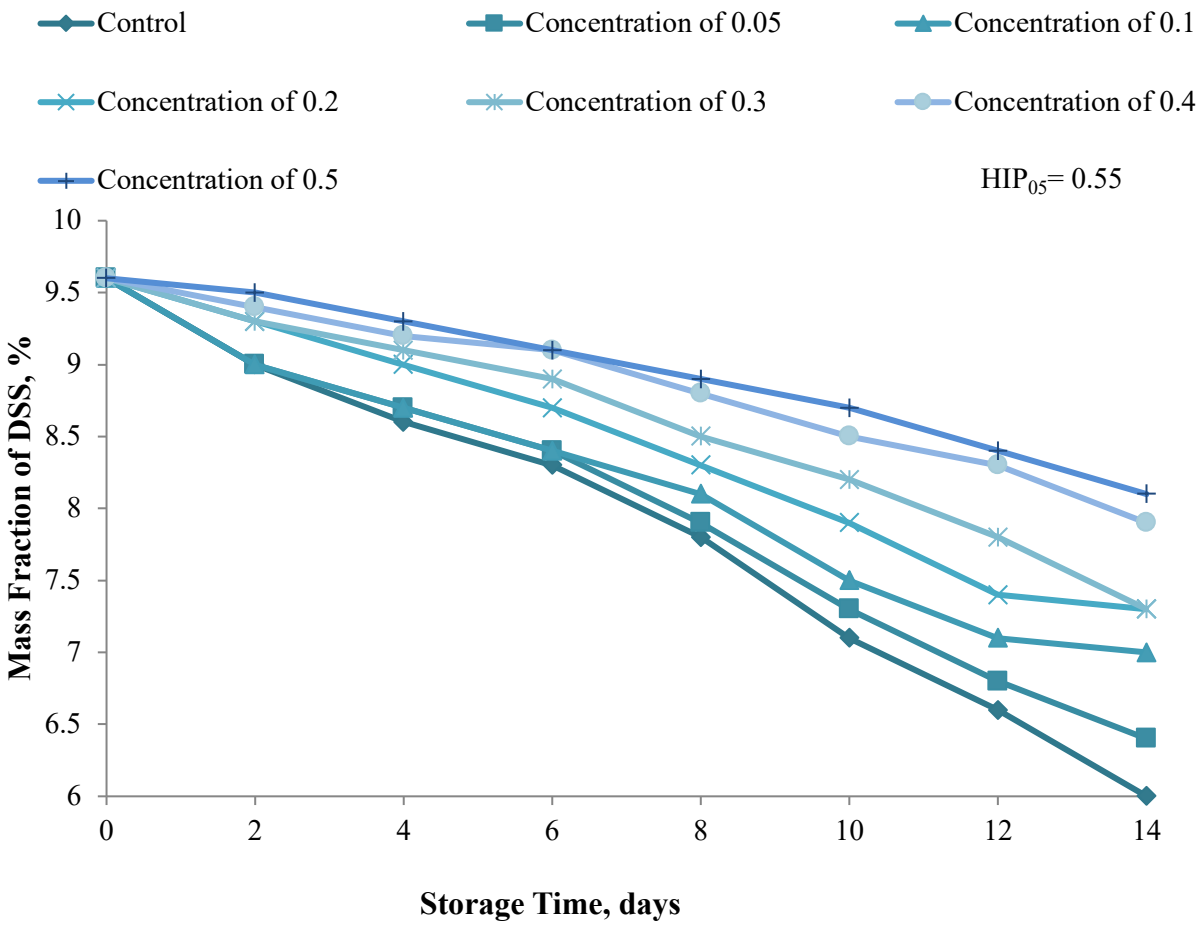

Figure 3 The change in the mass fraction of dry soluble substances of raspberries during refrigerated storage in a modified gas environment (2018-2020).

The investigations have shown insignificant variations in the mass fraction of dry soluble substances among different methods of refrigerated storage of berries. However, the lowest loss of the mass fraction of dry soluble substances during refrigerated storage with free access to air for 14 days was observed when raspberries were processed with chitosan at a concentration of 0.5%, and the highest loss was in control and at a concentration of 0.05%. A similar dependence of the change in the mass fraction of dry soluble substances was revealed during refrigerated storage in a modified gas environment.

It was proved that the mass fraction of dry soluble substances decreased more slowly in the samples processed with chitosan. On the second day, in the control, the accelerated loss rates of the mass fraction of dry soluble substances were observed, negatively affecting raspberries' preservation.

On the sixth day of the storage of berries with free access to air, an equal value (8.0%) was detected in the sample with a processing concentration of 0.05% and in the sample without any processing. In the future, the difference in indicators between these two samples for both refrigerated storage methods was insignificant (0.2-0.4%), which may indicate the inexpediency of using this concentration. It was found that the lowest changes in the mass fraction of DSS were in samples with a processing concentration of 0.4% and 0.5%.

The respiration intensity is the main indicator of metabolic processes in berries. This is the dominant indicator, the slowing down of which allows for extending the storage time of fruits **[40]**. Pre-processing raspberries before their placement in storage helps reduce their respiratory activity (Figure 4).

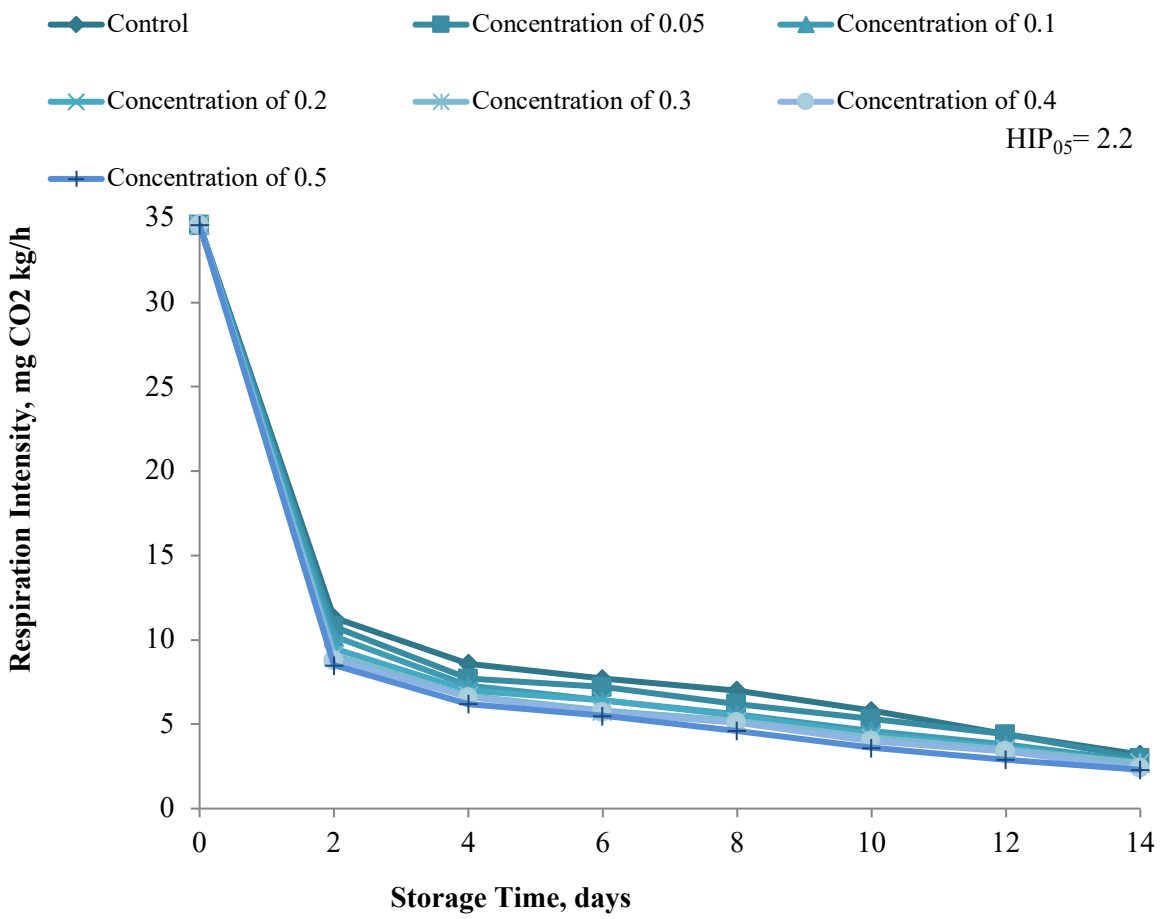

Figure 4 The changes in the respiration intensity of raspberries during refrigerated storage in a modified gas environment (2018-2020).

The physical effect of chitosan is that a thin transparent film forms on the surface of raspberries, which slows down a gas exchange.

The respiratory exchange of raspberries actively continues after their separation from a parent plant, negatively affecting the storage quality and duration.

The average respiration intensity of fresh raspberries was 34.6 mg CO_2.kg/h. On the second day of the storage, the indicator decreased sharply regardless of the processing concentration and the storage method and ranged from 8.9 to 10.5 and from 8.5 to 11.3 mg CO_2.kg/h. This was facilitated by a significant decrease in temperature to 0 ±2 °C. During further storage, the indicators have continued to decrease gradually. In the sample processed with a 0.5% chitosan solution, the respiration intensity was the lowest and on the eighth day was 5.3 CO_2.kg/h when stored with free access for air and 4.6 CO_2.kg/h in a modified gas environment, that by 2.4 and 1.4 less than the control.

Organic acids in raspberries are represented by citric, malic, quinine, salicylic, phosphoric, succinic, shikimic and glycolic acids **[41]**.

During the storage of raspberries, there is a tendency to lose organic acids, which are most involved in respiration. Pre-processing of raspberries with chitosan reduced the respiration intensity, thereby slowing the loss of organic acids by 0.15-0.19% of the counter (Figure 5).

On the second day, the highest percentage of organic acids (0.88%) was found in the sample with a processing concentration of 0.5% for both storage methods.

On the fourth day of the storage, a significant decrease in the content of organic acids was observed in raspberries that were stored with free access to air. However, the samples in a modified gas environment noted a stabilization of the dynamics of losses. On the eighth day of the storage of berries in a refrigerator with free access to air, the lowest content of organic acids (0.50) was detected in the sample with a chitosan processing concentration of 0.1%.

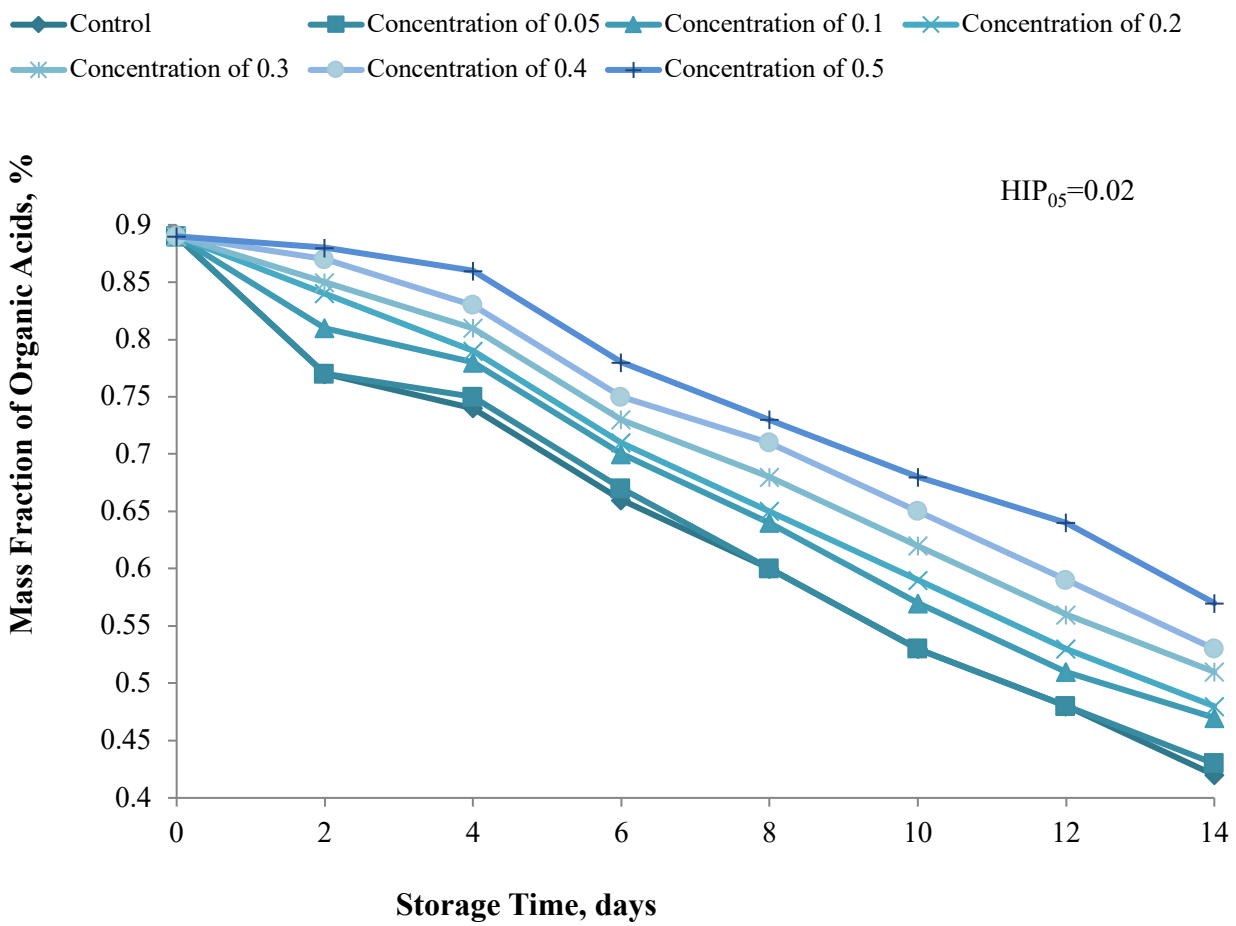

Figure 5 The change in the mass fraction of organic acids of raspberries during refrigerated storage in a modified gas environment (2018-2020).

At the beginning and until the end of the storage period, the best result was observed in the sample with the highest processing concentration, proving its application's effectiveness.

The processing of berries with chitosan solutions significantly reduces the decomposition rate of organic acids.

It is commonly known that sugars in raspberries are represented by glucose, fructose and sucrose. In combination with organic acids, sugars participate in oxidation processes, so their loss is partly caused by the respiration intensity **[42]**.

Our research established that pre-processing of raspberries with a chitosan solution significantly affects the changes in the content of sugars that occur during the storage period. This is explained by the fact that chitosan slows down the respiratory processes in berries, which causes significant losses of sugars.

It is found that the average sugar content in freshly harvested raspberries was 6.0%. During the entire storage period, significant losses of sugars were detected in the control samples.

The authors of scientific papers **[43]**, **[44]** found that the average sugar content in freshly harvested raspberries was 6.0-10%, but raspberry varieties and places where these researches have been conducted, were not indicated. Thus, such statements cause doubts about the obtained research results.

On the second day of the storage, a sharp decrease in the mass fraction of sugars was observed in the control samples (5.2%) and (4.9%) and in samples with a chitosan processing concentration of 0.05% (5.0%) and (5.2%). When raspberries were stored in a modified gas environment (MGE) from the beginning to the eighth day, the value for the control and the sample with the minimum processing concentration was equal to one.

By the end of the storage period, the sugar content in berries gradually decreased and, on the fourteenth day, ranged from 2.7 to 4.3% in samples with free access to air and from 2.5 to 3.6% in a modified gas environment.

The intensity of the use of sugars in the physiological and biochemical processes that occurred during the storage of processed raspberries was significantly lower compared to the control due to the slowing down of respiratory activity.

The content of ascorbic acid usually determines the vitamin value of raspberries. The content of vitamin C depends mainly on the variety and soil and climatic conditions.

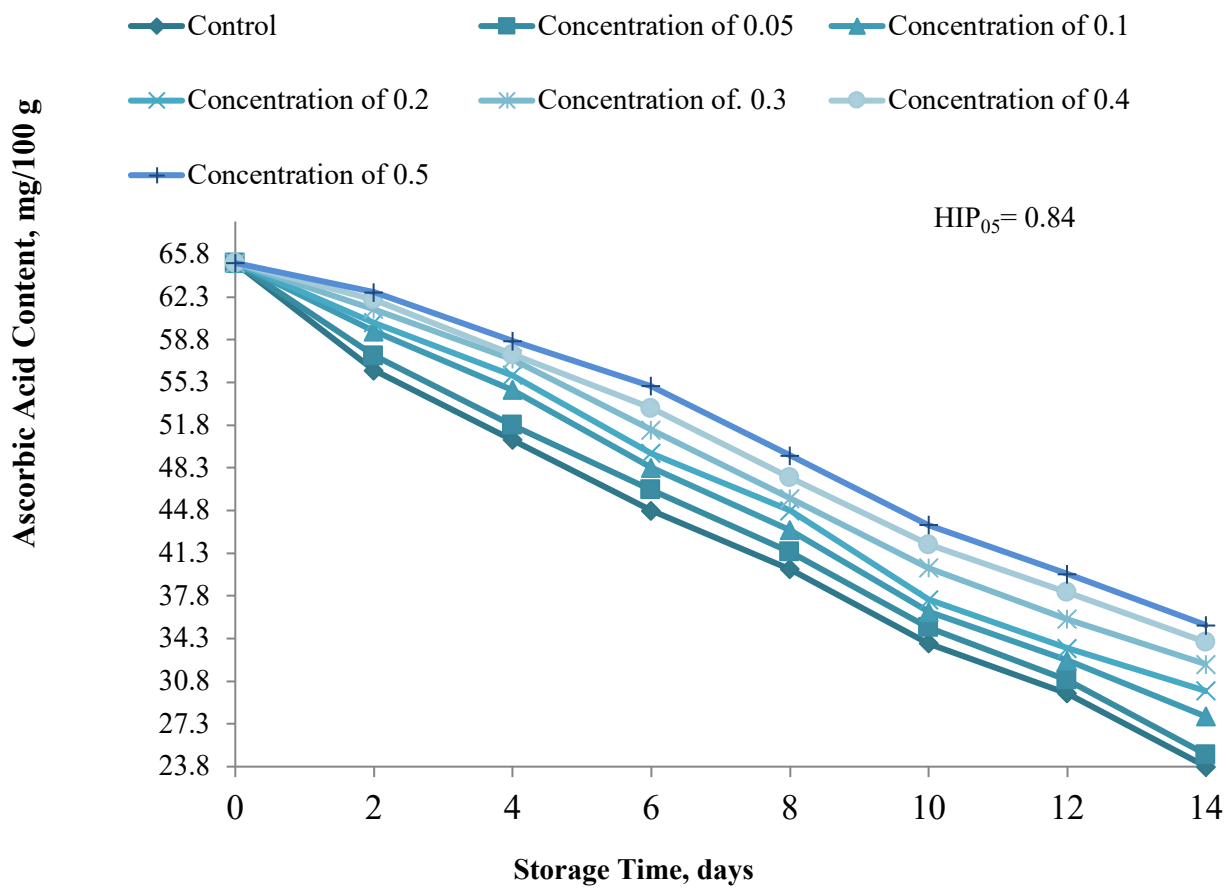

Figure 6 The change in ascorbic acid content in raspberries during refrigerated storage with free access to air (2018-2020).

Ascorbic acid is an unstable compound that is easily oxidized during storage **[45]**. Many factors affect its decomposition; the main of which are light, temperature and pre-processing.

Over the years of investigations, it has been established that the average ascorbic acid content in the Dukat variety raspberries is 65.1 mg/100 g. Depending on the refrigerated storage period, it decreased as well as with free access to air (Figure 6).

It was investigated that the ascorbic acid content in raspberries decreased rapidly, and on the fourth day, it ranged from 50.6 to 58.7 mg/100 g and from 49.5 to 60.2 mg/100 g, depending on the storage method. During the storage period ends, the highest ascorbic acid content was detected in samples with a processing concentration of 0.5% (35.4 mg/100 g) and (37.8 mg/100 g), which is 11.6 and 11.4 more than the control.

The active acidity of berries is an important characteristic of them, as it impacts the microflora's vital activity. The acid taste of fruit and vegetable products is provided by hydrogen ions, which are formed as a result of the electrolytic dissociation of acids and acid salts. The activity of hydrogen ions is characterized by the pH indicator **[46]**.

It was established that the average pH level in fresh raspberries is 3.2. During storage, the active acidity decreases depending on the time and method of storage (Figure 7). Depending on the storage method, the indicator increased by 0.2-0.4 and 0.1-0.3 on the second day. On the eighth day of the storage, the lowest pH level was observed in the sample with a chitosan processing concentration of 0.5% (3.8) and (3.6), 0.5 and 0.4 less than the control.

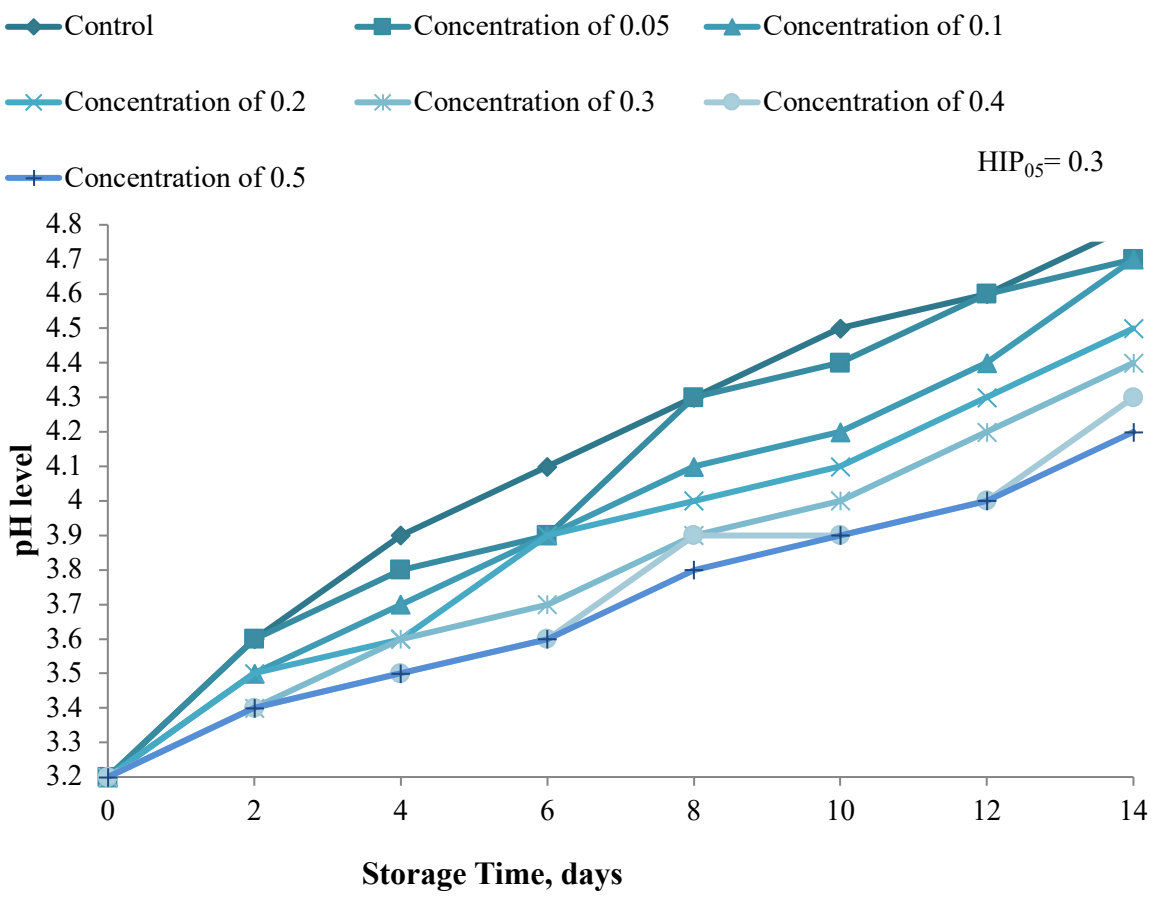

Figure 7 The change in the pH level of raspberries during refrigerated storage with free access to air (2018-2020).

During the storage period ends, the acidity of raspberries ranged from 4.2 to 4.8 in samples stored with free access to air and from 4.0 to 4.5 in a modified gas environment.

The analysis of research results proves that chitosan can slow down the pH level increase.

The tissue density indicates the consumer ripeness (degree of maturity) of fruits. It depends on the variety, fruit size and weather conditions during cultivation. The high density contributes to better storing and transporting of fruit and berry raw materials **[47]**. During storage, the tissue density decreased significantly, and in the middle of the storage period (the 6th day) it ranged from 0.24 to 0.28 kg/cm^2 in processed berries that were under conditions of free access to air, and from 0.25 to 0.28 kg/cm^2 in a modified gas environment.

During ripening, the berry tissue gradually softens, and in the case of frequent rains, it becomes thin and sensitive to mechanical damage.

It was studied that the average density of fresh raspberries after harvesting was 0.30 kg/cm^2.

In berries without any processing, the indicator was 0.21 and 0.24 kg/cm^2. At the raspberry storage period end, the density of processed berries was in the range of 0.14-0.20 kg/cm^2 and 0.17-0.24 kg/cm^2, depending on the storage method, which is 0.01-0.07 and 0.02-0.09 kg/cm^2 more than in control. The analysis of the research results showed that pre-processing of raspberries with chitosan solutions significantly affects tissue density preservation. As the concentration of the solution increased, the indicator decreased more slowly.

Ethyl alcohol is a strong solvent, and due to that, all biochemical processes are accelerated. Its formation in raspberries during storage occurs due to insufficient oxygen when the berries start anaerobic respiration **[48]**.

The highest content of ethyl alcohol was observed in samples stored in a modified gas environment, 0.68-0.85% in processed berries, and 0.88% in the control (Figure 8).

In the samples that were stored with free access to air, the content of ethyl alcohol did not exceed 0.05-0.18%.

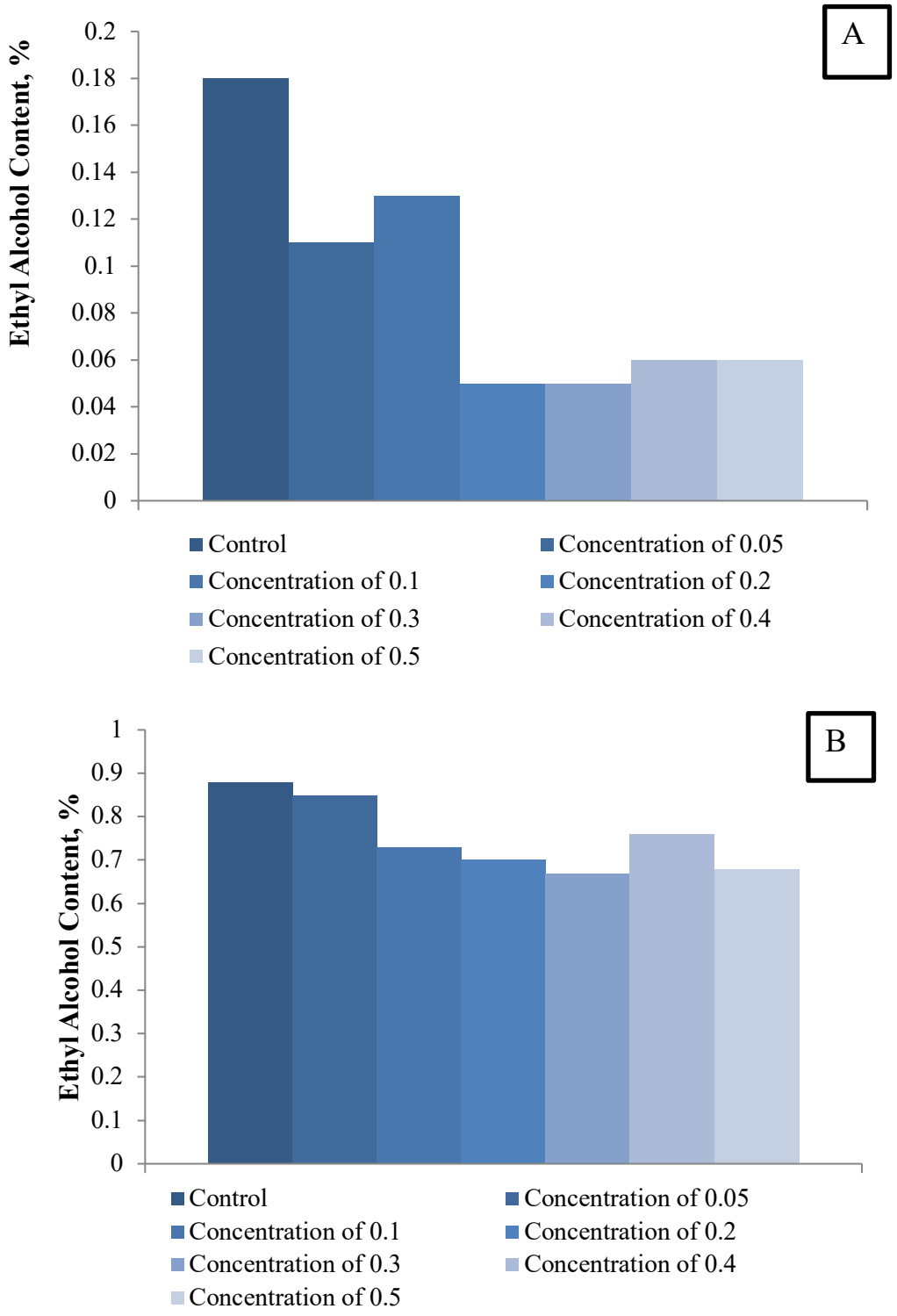

Figure 8 The ethyl alcohol content in raspberries after storage (2018-2020): A – with free access to air; B – in a modified gas environment.

It was established that after fourteen days of storage, the accumulation of acetaldehyde was in raspberries (Figure 9). This indicates the creation of anaerobic conditions in the storage environment.

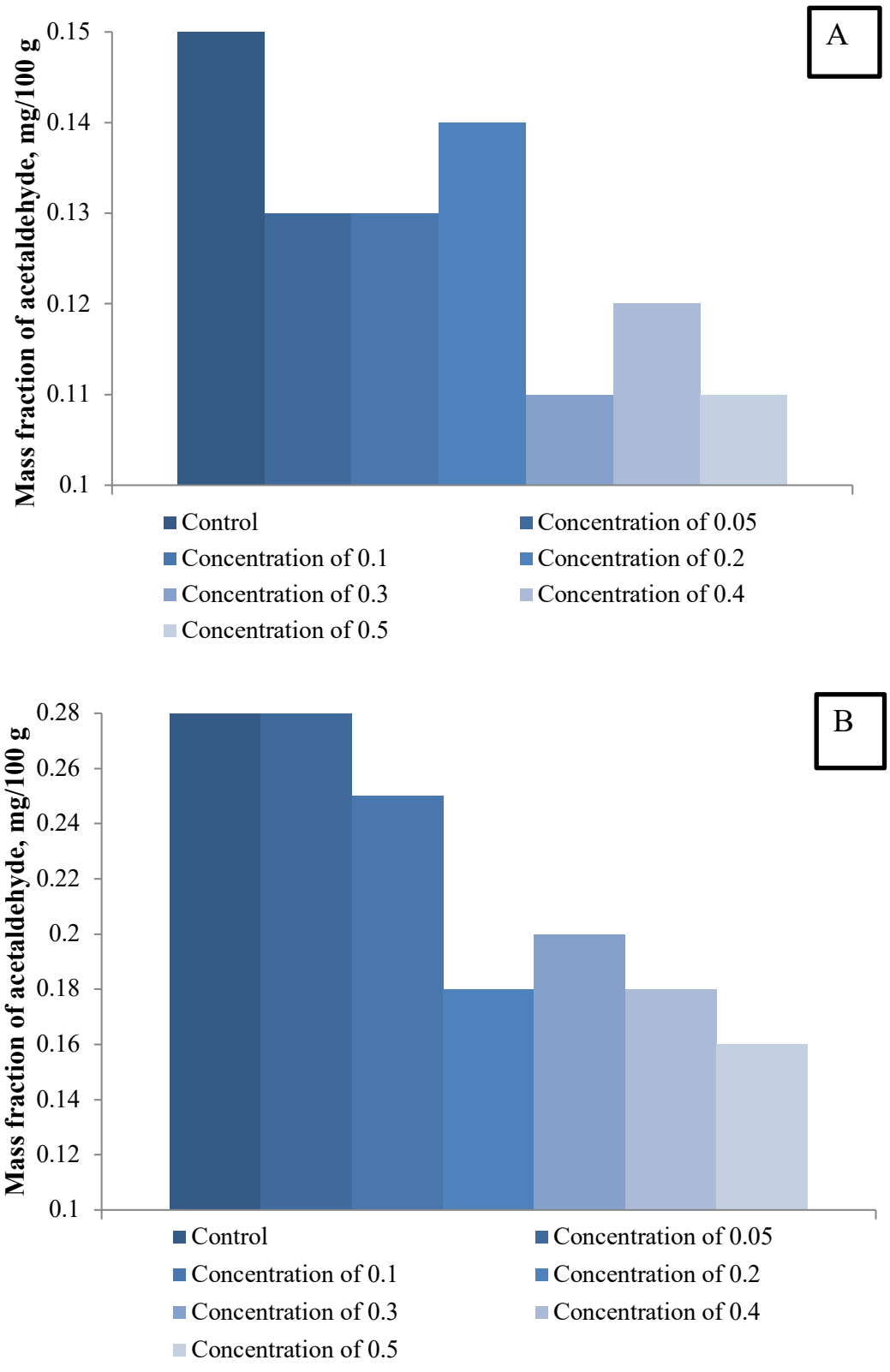

Figure 9 The acetaldehyde content in raspberries after storage (2018-2020): A – with free access to air; B – in a modified gas environment.

It was found that the mass fraction of acetaldehyde was higher in samples stored in a modified gas environment. The indicator ranged from 0.11 to 0.15 mg/100 g when berries were stored with free access to air, and from 0.16 to 0.28 in MGE.

It was established that pre-processing of raspberries with chitosan solutions improved organoleptic properties (Figure 10), better-preserving colour, tissue density and taste.

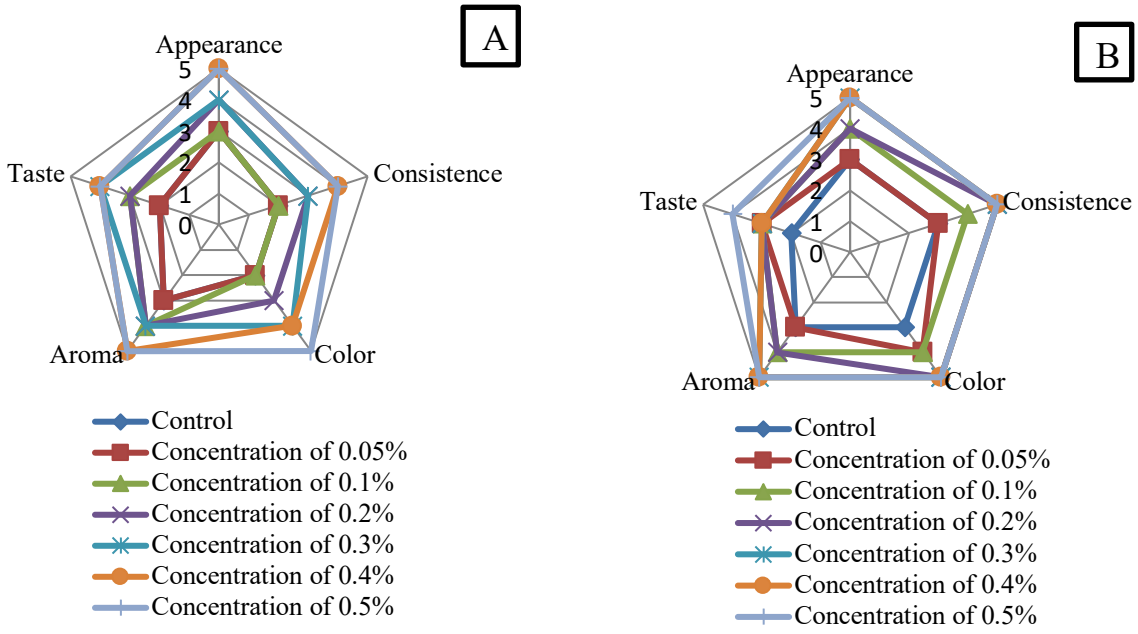

Figure 10 The organoleptic assessment of raspberries after storage (2018-2020): A – with free access to air; B – in a modified gas environment.

An organoleptic assessment is one of the most important indicators of product quality.

The potential buyer, first of all, pays attention to the appearance of the product, its color, aroma and consistency. The maintenance of the natural attraction of berries is a complex process, because during storage, a change in color, loss of elasticity and aroma is inevitable.

The accelerated rates of quality loss of raspberries were detected in the control sample. The sample with a 0.5% chitosan processing concentration was recognized as the most relevant for maintaining the organoleptic properties of raspberries.

Shine is a characteristic feature of the freshness of berry products. Its loss leads to the deterioration of the commercial qualities of the product, resulting in a decrease in the selling price.

Scientific papers **[52]** and **[53]** describe a wide range of organoleptic examinations of different types of berries immediately after harvesting, and also indicate the storage periods without the use of special solutions, but any studies of physical and chemical features were not conducted; it is unclear how the qualitative composition of raw materials changed during storage.

During the storage of raspberries, quick rates of shine loss were detected, particularly in the control sample (Table 1).

Table 1 The changes in the raspberry shine degree before storage and during refrigerated storage (2018-2020).

Processing Concentration, %	Shine Degree, points					
	With free access to air			In a modified GE		
	Before storage	In the middle of the storage period (the 7th day)	At the storage period end (the 14th day)	Before storage	In the middle of the storage period (the 7th day)	At the storage period end (the 14th day)
Control	5	2	1	5	3	1
0.05	5	4	2	5	4	2
0.1	5	5	2	5	5	2
0.2	5	5	3	5	5	4
0.3	5	5	4	5	5	4
0.4	5	5	5	5	5	5
0.5	5	5	5	5	5	5
HIP_{05}	0.2					

It was established that thanks to the film-forming properties of chitosan, the berries had a shiny glossy surface, significantly improving the raspberry's appearance. The obtained research results make it possible to recommend pre-processing raspberry with 0.4% and 0.5% concentration chitosan solutions.

Rapid losses of quality accompany the storage of berries in the conditions of trade transactions. This occurs due to increased physiological activity, particularly the high respiration intensity, which leads to the loss of nutritional substances **[49]**.

During the storage of berries at a temperature of 20-22 °C, the quick deterioration of raspberry quality was observed. The criterion for the end of storage was the appearance of phytopathogenic damage on the surface of the berries. The berries were assessed according to the physical parameters. The investigations were conducted every day. It was found that the weight loss depended on both the storage period and the berry processing concentration. Scientific papers **[50]** and **[51]** describe the process of storing raspberries at temperatures above 22 °C, and the authors do not state the deterioration of quality indicators. Thus, the question arises about what preparation was used for the berries pre-processing; this information is not specified.

Thus, on the second day of non-refrigerated storage of berries, the mass loss ranged from 6.2 to 8.2% and from 6.4 to 8.4%, depending on the processing concentration, and from 8.3% to 8.6% in control. During the following days of the storage, the indicator increased and at the end, was 15.4-19.2% in the berries processed by the spraying method and 21.0% in control, and 15.1-19.0% in the samples processed by the immersion method and 20.9% in control.

The density of the berry tissues also decreased with the loss of mass. Thus, on the second day of the storage, the indicator decreased by 0.06-0.11 kg/cm^2 for both processing methods (Figure 11 and Figure 12).

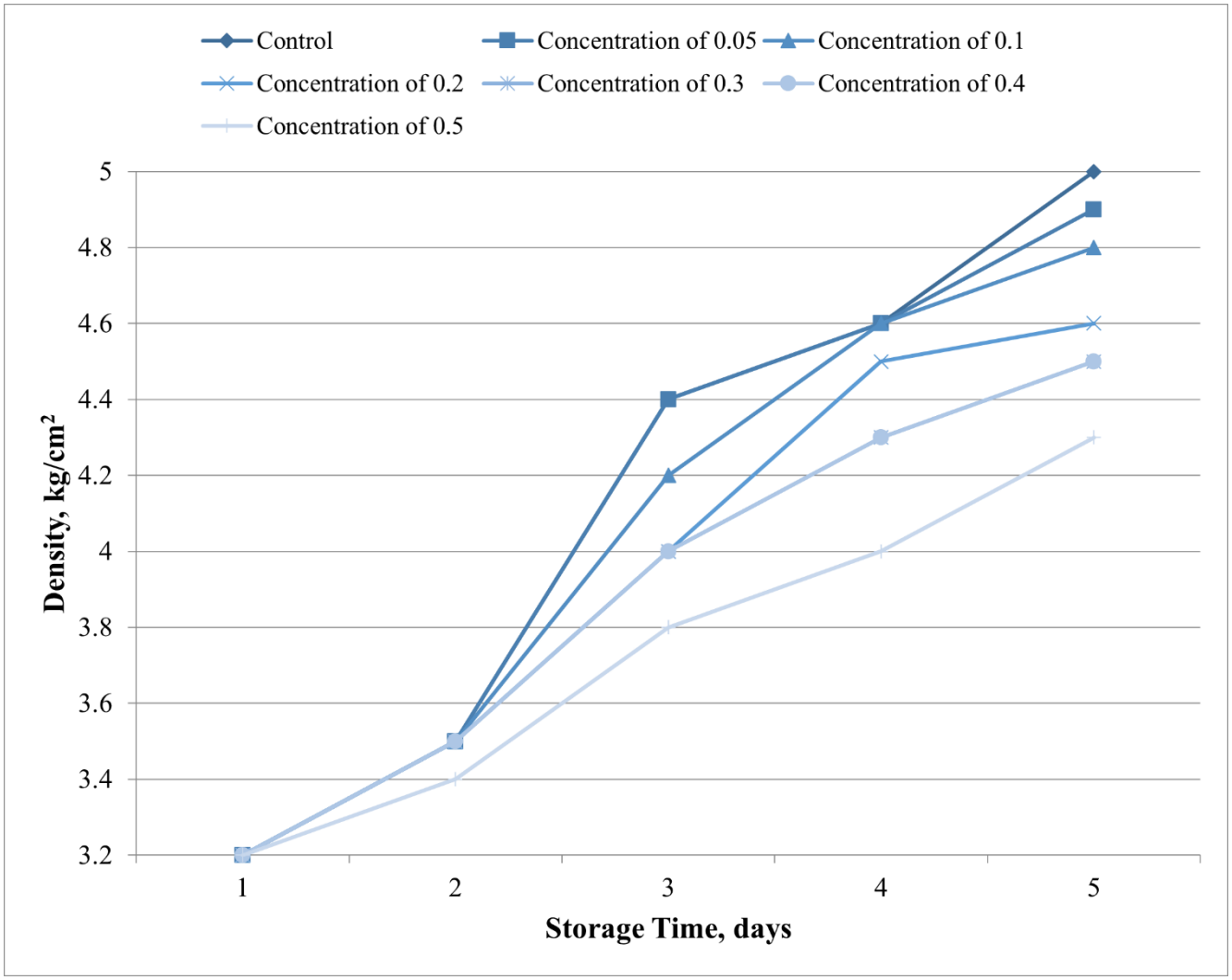

Figure 11 The change in the density of raspberries during non-refrigerated storage using the spraying processing method (2018-2020).

On the third day of storage, the density of berry tissues ranged from 0.15 to 0.20 kg/cm^2. By the end of storage, the indicator decreased rapidly, and on the fifth day, it was 0.12 kg/cm^2 in samples with processing concentrations of 0.4 and 0.5% and 0.10 kg/cm^2 in the remaining samples.

The pH level of raspberries changed very quickly during storage in the conditions of trade transactions. The active acidity has decreased, which led to the deterioration of the quality of berries and the development of fungal diseases.

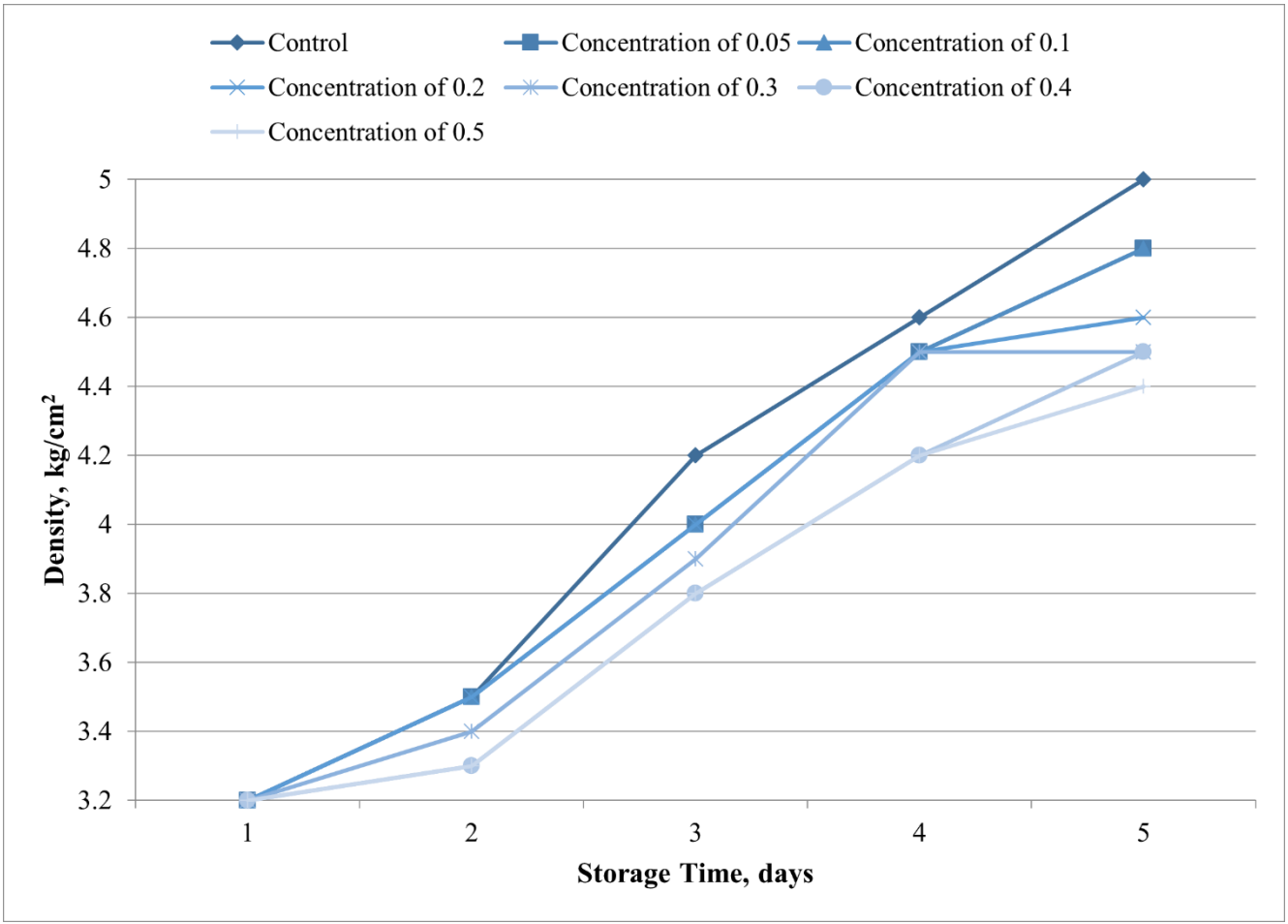

Figure 12 The change in the density of raspberries during non-refrigerated storage with the immersion processing method (2018-2020).

On the second day of the storage, the indicator ranged from 3.2 to 3.5 for both processing methods. On the third day of storage, the pH level decreased to 3.6-4.4 for the spraying method and 3.8-4.2 for the immersion method. At the end of the storage period of the berries, the pH level ranged from 4.3 to 4.9 and from 4.4 to 4.8 in the processed samples and 5.0 in the control.

CONCLUSION

It was established that the chitosan coating positively affects the maintenance of raspberries' quality indicators. It was detected that the processed berries had a loss of ascorbic acid of 1.5-3.9 times less than the control sample. A similar situation was observed with the mass fraction of sugars, which prevailed 0.9-2.5 times in the processed samples. According to the organoleptic indicators, raspberries without processing had an unattractive appearance, a softened consistency and a less pronounced aroma.

It was established that the mass loss of strawberries has increased daily regardless of the storage method. The strawberries' weight loss has a high inverse correlation dependence on the processing concentration. The mass loss has decreased with the increase of the chitosan percentage in the solution. The strawberries stored at a temperature of 20-22 °C for more than five days, both processed and unprocessed, are not advisable due to a significant loss of mass (15.4-21.0%). The ascorbic acid content accumulated during the vegetation quickly decreased on the second day of storage in all studied samples. The strawberries processed with chitosan solutions had lower vitamin C losses than the control. The

high inverse correlation dependence between the change in the ascorbic acid content and the chitosan solution concentration was established.

It was found that the change in the pH level of strawberries depends on the chitosan processing concentration, regardless of the storage and processing methods.

The tissue density decreased during the entire storage period and reached the value of 10 kg/cm^2. It was investigated that strawberries processed with a chitosan solution of different concentrations had a higher density, contributing to long-term preservation.

The content of ethyl alcohol at the end of storage depended on the storage method. The highest values were detected when strawberries were stored in a modified gas environment. It was found that the strawberries pre-processed with a chitosan solution were suitable for preservation after seven days of storage. According to the physical, chemical, and organoleptic indicators, the preserves made from pre-processed berries fully complied with the requirements.

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Thermal performance assessment of an indirect solar dryer: A case study of Bananas

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ABSTRACT

This study presents a design for an absorber used in a solar air collector for an indirect solar dryer. The absorber comprises two aluminium plates corrugated and joined together to form parallel cylinders, enabling airflow within the collector. This research aims to experimentally examine the drying process of two types of bananas, one from Morocco and the other from abroad, using the designed solar air collector. Additionally, the study aims to investigate the peculiarities of the drying process and the performance of the solar dryer employed. The experiments were conducted by subjecting the bananas to the designed solar air collector, and the evolution of drying was monitored. The initial mass of the bananas used was 631.6 g for the Moroccan banana and 713.6 g for the Export banana. After the drying process, the mass of the Moroccan banana reduced to 77.5 g, while the Export banana reduced to 137.3 g, indicating significant moisture removal. The percentage of the amount of water extracted (Q) from the bananas was found to be 87.7% for the Moroccan banana and 80.8% for the Export banana. These results demonstrate the effectiveness of the corrugated aluminium plate absorber in facilitating the drying process in the solar air collector. The significant reduction in the mass of the bananas and the high percentage of water extraction highlight the efficiency of the solar dryer in removing moisture from the agricultural produce. The findings of this study contribute to the understanding of the drying process of bananas and offer valuable insights for the design and optimization of solar drying systems for agricultural applications.

Keywords: indirect solar dryer, solar collector, banana, global irradiance

INTRODUCTION

The increase in prices and the shortage of fuels have prompted extensive studies and research on the use of solar energy as an alternative energy source, particularly in developing countries **[1]**, **[2]**, **[3]**. Solar drying, a widely adopted solar energy system, has been recognized as an effective method for food preservation. Drying fruits and vegetables is an energy-intensive process in the food processing industry and serves as a valuable means to reduce post-harvest losses. Solar drying of crops, fruits, and vegetables has been practiced worldwide for centuries, utilizing the sun's energy in the open air. It has been historically employed for drying grains, meats, and other agricultural products for consumption **[4]**, **[5]**, **[6]**.

The traditional method of drying fruits and vegetables in the sun, without technical aids, continues to be the predominant practice for much of the world's supply. However, large-scale production limits the use of normal outdoor sun drying. This traditional approach suffers from various issues. Among them are the lack of proper control over the drying process, uncertainties caused by weather conditions, high labor costs, the need for extensive space, and the risk of infestation by insects and other foreign bodies. As a result, solutions involving solar energy have been developed, including the use of collection devices or solar dryers **[7]**, **[8]**.

The utilization of a well-designed solar dryer can help mitigate the disadvantages associated with open sun drying and lead to enhanced quality of the final dried product. Numerous scientists have conducted studies on the modeling of solar drying for agricultural products. Additionally, simulation studies have been carried out on solar dryers, both direct and indirect, and the behavior of various vegetables and fruits, focusing on their drying kinetics

[9]. By harnessing free, renewable, and non-polluting energy from the sun, the adoption of solar dryers in developing countries has the potential to reduce crop losses and significantly enhance the quality of the dried product compared to traditional drying methods.

In recent years, significant efforts have been made to develop solar drying systems, particularly for the preservation of agricultural products. The design of solar drying systems needs to be tailored to meet specific drying requirements and accommodate different types of crops. In a study focusing on apricots, researchers presented a mathematical model for a thin-layer solar drying process. They utilized a cabinet solar dryer with a forced convection system operating in mixed and indirect modes **[10]**. The findings revealed variations in the drying rate of apricot samples based on the solar drying technique employed and the drying air's temperature and airflow velocity. Notably, apricots exhibited faster drying rates when subjected to mixed-mode solar drying compared to indirect solar drying.

Pruengam et al. **[11]** developed a solar collector dryer that can be positioned on both sides of a drying chamber. Their study involved drying bananas in the dryer for 5 days, reducing the original moisture content from 68.5% to 17.4%. In comparison, the open sun drying method reduced the moisture content to 27.3%. The solar collector dryer reduced moisture content for bananas by a factor of 1.3-1.5 when compared to sun drying.

In another study, Essalhi **[12]** presented an innovative absorber design for a solar air collector used in an indirect solar dryer. The absorber consisted of two corrugated aluminium plates. The data obtained from the study revealed that after 24 hours of drying pears, the sample's mass decreased from 997.3 g to 135.1 g. The average thermal efficiency of the drying chamber was observed to be 11.1%.

In this paper, our experimental study aims to investigate the drying characteristics of two different banana types **[13]**, **[14]**. By examining the drying evolution of these different banana types , we aim to identify any drying anomalies and potential limitations of the dryer system specific to this agricultural product. Through this research, we aim to gain insights into the optimal drying conditions and potential improvements that can be made to enhance the drying process for these two different banana types. The findings from this study will contribute to a better understanding of banana drying and provide valuable information, including global solar irradiation **[15]**, for developing more efficient and effective drying techniques in the future.

Scientific Hypothesis

By implementing a well-designed indirect air solar dryer and following the recommended temperature range provided by the manufacturer, we have expected that the drying process for agricultural products, specifically sweetened bananas, will be more efficient and effective. Furthermore, by incorporating pre-treatments to eliminate obstacles that hinder drying kinetics and incorporating a heat storage mechanism to retain generated heat during nighttime, we expect to observe improved drying outcomes regarding optimal nutritional and aesthetic qualities within the designated drying time.

MATERIAL AND METHODOLOGY

Samples

We have selected bananas as our chosen "wet product" for the study. Bananas hold significant importance as a food source for various reasons. They are rich in dietary fiber, which promotes digestive health and helps reduce the risk of certain diseases. Additionally, bananas are packed with essential vitamins and minerals such as vitamin C, vitamin B6, and potassium, crucial for maintaining overall bodily functions. Their carbohydrates provide quick energy, making them popular among athletes and active individuals. Moreover, bananas are readily available throughout the year, making them an ideal fruit for drying **[16]**, **[17]**. As depicted in Table 1, they possess substantial nutritional value. With the increasing demand for dried banana products **[18]**, exploring their drying characteristics becomes crucial.

Chemicals

The experiment involves treating the samples with a highly acidic solution of lemon juice, with a pH level ranging from 2 to 3 on the pH scale. This solution exhibits a significantly higher acidity level than water, with lemon juice being nearly 100,000 times more acidic than water. It is important to note that only the exported banana variety underwent the preliminary treatment with lemon juice before the subsequent analysis. A comparative analysis was conducted between the treated exported bananas and the unprocessed Moroccan banana variety.

Animals, Plants and Biological Materials

This study used two types of banana (exported and Moroccan) as they are considered a plant-based food.

Table 1 Nutritional value per 100 g of banana.

Banana nutritional value per 100 g of raw banana			
Water	Total Ash	Fibres	Energetic value
74.9 g	0.8 g	2.6 g	89 kcal
Proteins	Lipids	Carbohydrates	Simple sugars
1.1 g	0.3 g	22.8 g	12.2 g
Trace elements			
Potassium	Magnesium	Phosphor	Calcium
358 mg	27 mg	22 mg	5 mg
Sodium	Copper	Iron	Zinc
1 mg	78 µg	26 µg	25 µg
Vitamins			
Vitamin C	Vitamin B1	Vitamin B2	Vitamin B3
8.7 mg	31 µg	73 µg	665 µg
Vitamin B5	Vitamin B6	Vitamin B9	Vitamin B1
334 µg	376 µg	0 µg	0µg
Vitamin A	Vitamin K		
64 µg	0.5 µg		
Fatty acids			
Saturated	Monounsaturated	Polyunsaturated	Cholesterol
112 mg	32 mg	73 mg	0 mg

Note: Data processed from **[19]**.

Instruments

The solar dryer: This solar air dryer (Figure 1, Table 2) was constructed step by step at the Laboratory of Solar Energies and the Environment at the University of Mohammed V. The project is supported by The Institute for Solar Energy and New Energies Research (IRESEN) (https://iresen.org/amelioration-du-fonctionnement-des-sechoirs-solaires/). All equipment is placed on the roof of the annex where orange trees are located in Rabat, Morocco (34° 1' 15.1752" N and 6° 50' 29.9400" W), facing south at an angle of 34 degrees to maximize solar radiation absorption for our drying experiment **[20]**. The primary objective of this study was to develop and construct an air-type solar collector with an improved cost/performance ratio, specifically designed for solar drying of agricultural products.

Figure 1 The indirect air solar dryer used (34° 1' 15.1752" N and 6° 50' 29.9400" W).

The solar collector: The solar collector used in this study follows a simple and conventional design. It comprises an insulating "cork" on the lower face, a black aluminium absorber with cylindrical cavities, and a standard glass cover on the upper face. The collector functions by allowing fresh air to enter through an opening at the bottom and flow through the 7 cavities of the absorber. This design has been analyzed in a study by **[12]** to assess its impact on the performance of the solar collector. The review concludes that optimizing these parameters can significantly enhance the thermal efficiency of the collector.

Table 2 Solar collector dimensions.

Collector length (cm)	Collector width (cm)	Collector thickness (cm)	Opening diameter (cm)	Cavity diameter (cm)
114	99.5	16	15	9

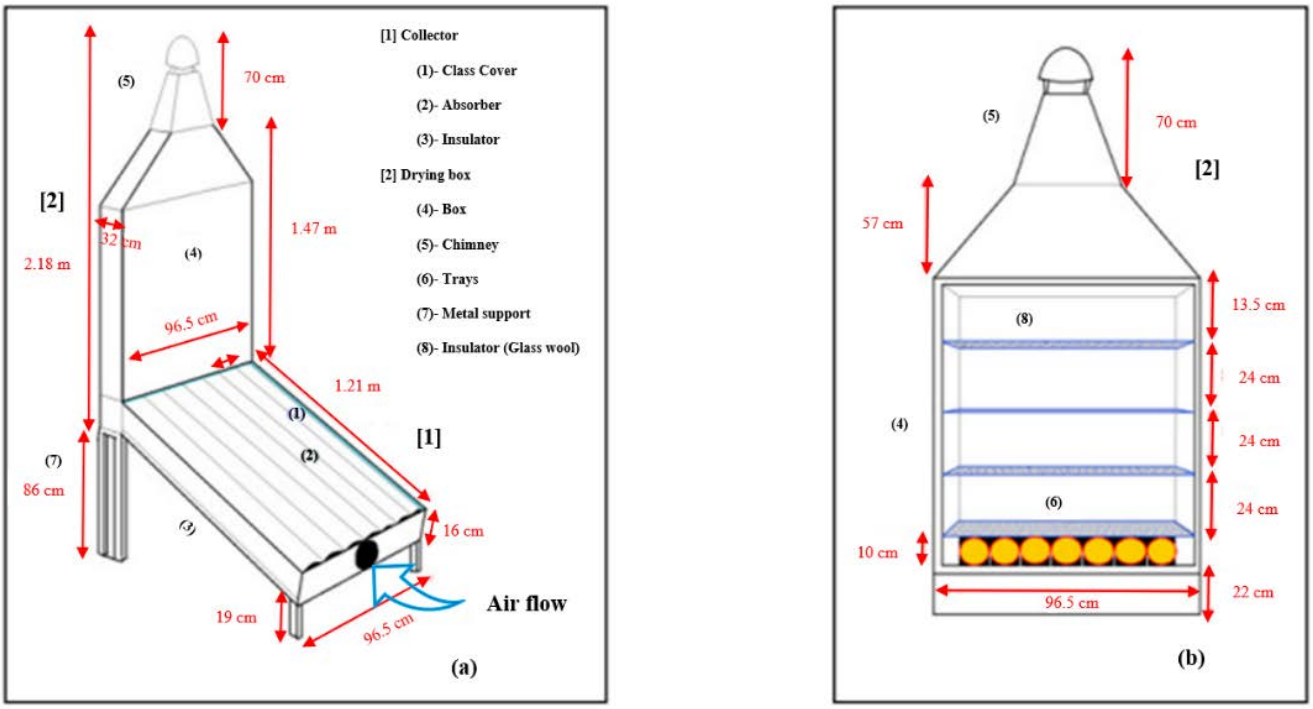

Figure 2 (a) Schematic diagram of the drying system, (b) drying chamber **[12]**.

Drying chamber: The chamber is a parallelepiped-shaped enclosure supported by an iron frame constructed from a durable wood material called "Tréspa." This specialized wood is designed to provide both structural stability and insulation properties. Inside the chamber are four aluminium trays with perforations that allow the drying air to circulate and transfer heat to the bananas. The chamber is equipped with a double-leaf door to facilitate the loading and unloading of the bananas. A chimney is installed at the top of the chamber to release the moist air. For the distribution of the product, only the first three shelves were utilized (Table 3, Figure 2).
The drying box utilized in our study has a capacity of up to 10 kg of bananas, determined by the airflow rate. It is crucial to maintain an optimal airflow in a solar dryer to effectively remove moisture from the product without causing any damage to the fruit. The solar collector's dimensions also constrain the drying chamber's size. In our analysis, we consider the inclusion of the 4th tray within the drying box, as illustrated in (Figure 3).

Table 3 Drying chamber dimensions.

Box length (cm)	Box width (cm)	Box thickness (cm)	Dimension of the 1st tray			Dimension of the 2nd and 3rd tray			Chimney length (cm)
			Length (cm)	Width (cm)	Thickness (cm)	Length (cm)	Width (cm)	Thickness (cm)	
114	96.5	32.5	93.5	9.5	0.2	93.5	28.5	0.2	113

Figure 3 The drying box was used.

Laboratory Methods

The study was conducted in Rabat, the capital of Morocco, utilizing the previously demonstrated indirect air solar dryer. Rabat experiences a warm and temperate climate, characterized by most rainfall occurring during winter and relatively low rainfall during summer. According to the Köppen-Geiger climate classification, Rabat falls under the *Csa* category **[21]**.

In this study, we also investigated integrating a computer system for data management and storage throughout the drying process, along with using thermocouples to facilitate communication between the computer and the solar dryer. For an indirect solar dryer, it is crucial to consider background meteorological measurements to understand the ambient weather conditions that may influence the dryer's performance. These measurements include temperature, relative humidity, solar radiation, wind speed, and rainfall. Campbell Scientific sensors and CR1000 data loggers were deployed at the solar dryer site to capture these measurements. A computer assembly consisting of a junction box connected to an acquisition box was utilized to collect data hourly, with a computer designated for data storage.

Figure 4a displays the eight T-type thermocouples that constitute the assembly. Each thermocouple is individually connected to the solar dryer elements to measure their temperatures. A junction box (depicted in Figure 4a) consolidates the T-type thermocouples. T-type thermocouples consist of two distinct metal wires: copper (positive) and constantan (a copper-nickel alloy, negative). This type of thermocouple is particularly suitable for low-temperature applications, with a temperature range of -200 °C to 350 °C. The junction box collects temperature readings from all the elements of the dryer and routes them to the acquisition system.

The acquisition system (shown in Figure 4b) serves as the interface between the computer and the junction box. It includes an electronic card and computer software. The laboratory system incorporates a memory module from Campbell Scientific CR10X-2M (up to 1,000,000 data points). All measuring instruments and thermocouples are connected to this acquisition system. The system is controlled by a computer using a data acquisition program. However, there are drawbacks to this procedure: during the assembly of the thermocouples with these wires, some temperature is lost, leading to inaccurate numerical values.

This temperature is used with the temperature of the acquisition box called the reference temperature T_f. The real temperature is given by equation (1):

$$T_r = T - T_b + T_f \quad (1)$$

Where:

T_r – Actual temperature of an element of the dryer ; T – Temperature displayed in the program; T_f – Reference temperature; T_b – Junction box temperature.

Table 4 shows the real location of each thermocouple in the acquisition system.

Table 4 Location of each thermocouple.

Thermocouple	Location
T1	Collector input (At collector level)
T2	On the absorber (At collector level)
T3	Collector outlet (the inlet of the drying box)
T4	The 1st tray (At the level of the drying box)
T5	The 2nd tray (At the level of the drying box)
T6	The 3rd tray (At the level of the drying box)

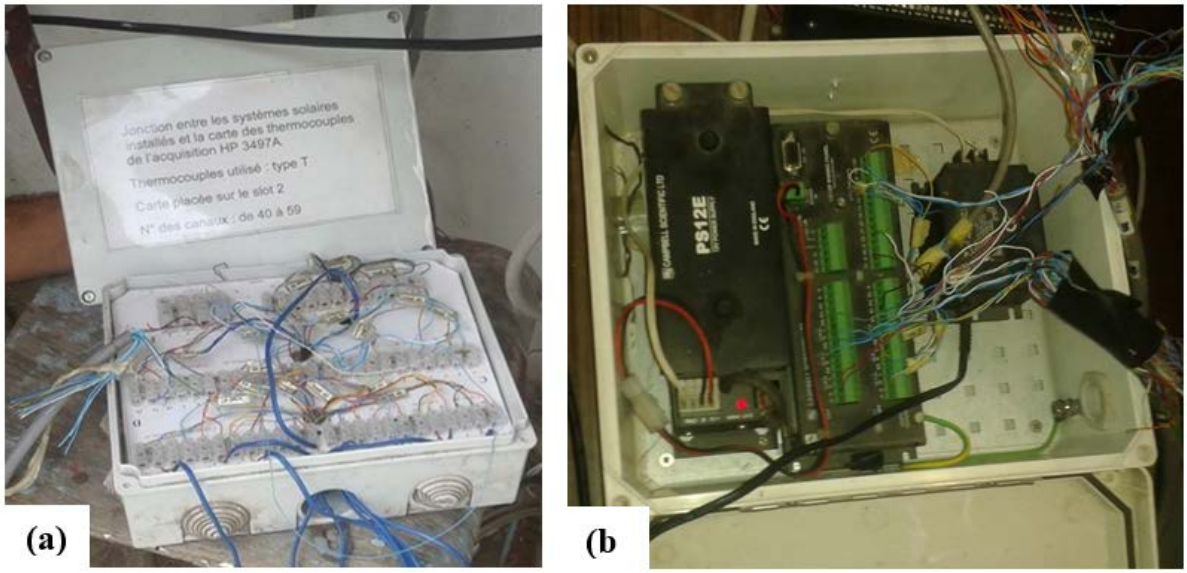

Figure 4 The junction (a) and acquisition box (CR10X) (b).

Description of the Experiment

Sample preparation: The bananas used in the study were purchased on May 1, 2018. They were measured to be between 10 and 13 centimetres in length and 2 to 2.5 centimetres in diameter. Prior to the experiment, the bananas were sliced into rounds with a thickness of 5 millimeters. After removing the waste, which weighed 468.5 g, from the initial mass of the bananas 1130.0 g, we were left with 661.6 g as the starting mass for the drying process.

On May 8, 2018, we introduced another variety of banana (exported banana) to the solar dryer, alongside the Moroccan banana. The Moroccan banana had a length ranging from 18 cm to 20 cm and a diameter ranging from 2.5 cm to 3 cm. For the exported bananas, we imported a total of 1240.0 g. After removing all the imperfect bunches, which weighed 526.4 g, we were left with 661.5 g as the starting mass for the drying process.

It is important to note that while this experiment is from an older timeframe, research on renewable energy and sustainable agriculture remains an ongoing and constantly evolving field. There is still a need for studies exploring the efficiency and effectiveness of solar drying technology. Furthermore, this research may have practical applications and implications for farmers, producers, and other stakeholders in the sustainable agriculture industry. This can be seen in recent publications such as **[22]** (published in 2022) and **[23]**, **[24]**.

To preserve the banana's color and prevent oxidation, we treated all export bananas with lemon juice before drying.

Number of samples analyzed: This study analysed two samples of bananas.

Number of repeated analyses: Data was collected for a duration of 30 days in this study.

Number of experiment replication: Each experiment was repeated 30 times in this study to obtain a single value.

Design of the experiment: The indirect solar dryer was assembled step-by-step within the University of Mohammed V laboratory, specifically in the laboratory of solar energies and the environment. The nutritional composition of the agricultural products, such as moisture, protein, fibers, ash, salt, as well as the content of vitamins, magnesium, calcium, water, and fat, was determined. Furthermore, the products were thoroughly

cleaned, and the exported bananas were mixed with lemon juice before being placed inside the drying chamber. This step was taken to facilitate a comparison between the two products after the experiment.

Statistical Analysis

The data collected from the computer assembly features was analyzed using using Microsoft Excel and Python, a programming language commonly used for data analysis. The analysis included performing descriptive statistical analysis and calculating the Pearson correlation coefficient. Descriptive statistics provided measures such as mean, standard deviation, minimum, maximum, and quartiles, which helped in understanding the data's central tendencies, variability, and distribution. The Pearson correlation coefficient was used to assess the strength and direction of the linear relationship between variables, specifically between the temperature of the trays and the global solar irradiation. By analyzing Python, we leveraged its powerful libraries and functions for data manipulation, analysis, and visualization, allowing for a comprehensive examination of the data and accurate interpretation of the results.

RESULTS AND DISCUSSION

Experimental observation: We have two types of bananas: Banana 1 (Moroccan) and Banana 2 (export). The drying process took place from May 1 to May 28, 2018, with Banana 1 being placed in the dryer from May 1 to May 8, and Banana 2 being added from May 8 until May 28. The total drying period was thirty days. While the temperature varies from day to day on May 1 (the first day of drying), after a week of daily monitoring, we observe that banana 1 is still relatively in the same condition as when we first checked on it. Banana 1 has been slightly overripe, and its high humidity is readily apparent **[25]**; therefore, it was seen that its viscosity reduces during a sunny day around 2 p.m., indicating drying takes place; however, by 8 a.m., the banana had reverted to its initial state.

Statistical results of clear sky day and partially overcast day: Tables 5, 6 and 7 provides a descriptive statistical analysis of the data for the clear sky day and partially overcast day (6 and 5 May 2018 respectively), offering insights into the temperature variables (T4r°C, T5r°C, T6r°C) and irradiation levels. The statistics reveal important information about the dataset, such as the average values, standard deviations, minimum and maximum values, and quartiles. These measures help us understand the typical range, variability, and central tendency of the temperature and irradiation data. Moving to Table 6-8, it presents the correlation coefficients between the temperature variables and irradiation. The correlation coefficients quantify the strength and direction of the linear relationship between these variables. This analysis shows high positive correlations among the temperature variables, indicating that they are closely related and change together. Additionally, a strong positive correlation between temperature and irradiation suggests that irradiation levels also tend to increase as temperature increases. These correlation coefficients provide valuable insights into the relationships between the variables and help us understand the interconnected nature of temperature and irradiation. Figure 5 shows the map of the Pearson correlation of a clear sky day and a partially overcast day.

Table 5 Descriptive statistics of clear sky day (6 May 2018).

	T4r °C	T5r °C	T6r °C	Irradiation W/m^2
count	23.00	23.00	23.00	23.00
mean	24.18	23.45	24.18	344.23
std	12.78	11.78	12.78	376.39
min	10.56	10.54	10.56	0.20
25%	12.45	12.33	12.45	0.39
50%	18.82	18.96	18.82	167.70
75%	35.445	33.30	35.44	704.30
max	44.74	42.280	44.74	951.00

Table 6 Pearson correlation analysis results of clear sky day (6 May 2018).

	T4r°C	T5r°C	T6r°C	Irradiation W/m^2
T4r°C	1.00	0.99	1.00	0.96
T5r°C	0.99	1.00	0.99	0.96
T6r°C	1.00	0.99	1.00	0.96
Irradiation	0.96	0.96	0.96	1.00

Table 7 Descriptive statistics of partially overcast day (5 May 2018).

	T4r°C	T5r°C	T6r°C	Irradiation W/m^2
count	23.00	23.00	23.00	23.00
mean	22.10	21.71	21.01	170.64
std	6.47	6.04	5.26	219.33
min	15.99	15.90	15.75	0.30
25%	17.33	17.30	17.26	0.41
50%	19.02	18.61	18.15	71.60
75%	25.56	25.00	24.05	312.55
max	34.04	33.14	31.25	614.60

Table 8 Pearson correlation analysis results of partially overcast day (5 May 2018).

	T4r°C	T5r°C	T6r°C	Irradiation W/m^2
T4r°C	1.00	0.99	0.99	0.97
T5r°C	0.99	1.00	0.99	0.96
T6r°C	0.99	0.99	1.00	0.95
Irradiation W/m^2	0.97	0.96	0.95	1.00

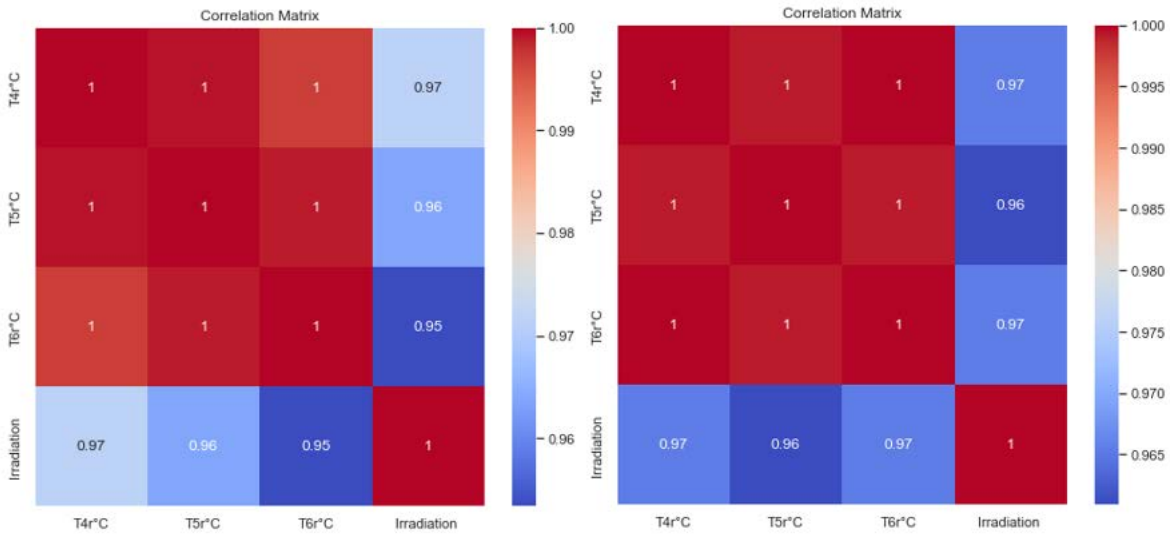

Figure 5 Correlation map of clear sky day and partially overcast day.

Experimental results: The following method is proposed for studying the drying process's evolution: we measured the drying process's temperature evolution over two days, one sunny and the other partially covered, for each period ([1 May-8 May] and [8 May-28 May]), which will allow us to determine our dryer's behaviour and combine the result with the experimental drying result to determine the factors governing the drying process **[26]**. For banana 1 (Moroccan banana): we selected May 5 as a partly cloudy day **[27]**, and May 6 as a sunny day **[28]** over this time frame (May 1-May 8). The selected days during the drying period were chosen to represent average weather conditions, with a mix of sunny and partially cloudy days. Evaluating the thermal performance of an indirect solar dryer for bananas involves assessing its ability to effectively utilize solar energy for heat generation and moisture removal **[29]**. This process is closely linked to meteorology, as it relies on factors such as sunlight intensity, temperature, humidity, and wind speed **[30]**. In this study, ERA5 hourly fractional cloud cover data **[31]** was utilized to identify clear-sky days in the study area, following the approach validated by recent studies in South Africa by Mabasa et al. **[32]** and in Morocco by Mendyl et al. **[15]**. Evaluating the solar energy resource available at the desired location is crucial before implementing solar energy technologies for photovoltaic (PV) or thermal applications. This assessment helps determine the feasibility and suitability of solar energy as a renewable energy source for that specific location **[33]**, **[34]**. The pvlib python library **[35]**, **[36]** can be utilized to configure solar models and assess the hourly global horizontal irradiance (GHI) for the study location prior to

the start of the experiment.Figures 6 and 7 depict the 24-hour temperature evolution in the 3- trays dryer on a sunny and partly sunny day, respectively.

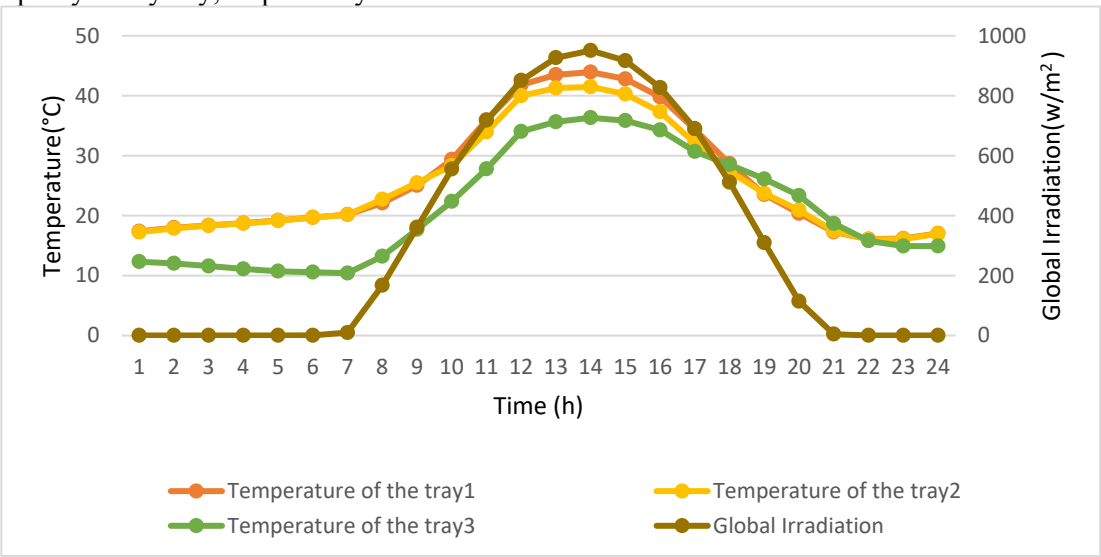

Figure 6 Temperature and global Irradiation evolution during clear sky day(W/m^2).

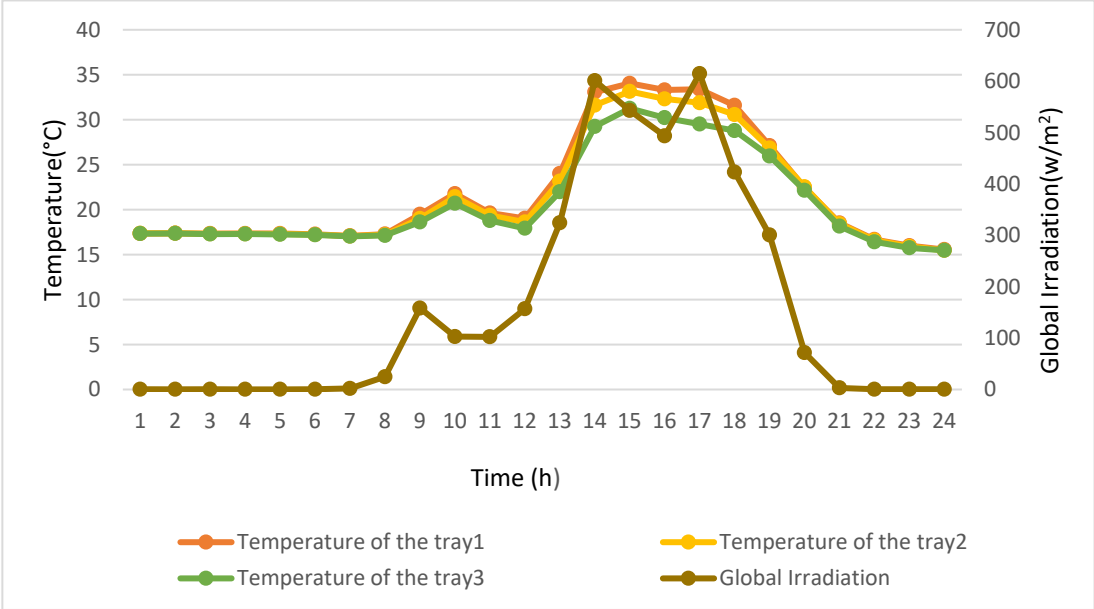

Figure 7 Temperature and global Irradiation evolution during partially overcast day(W/m^2).

The curves generated in Excel and the experimental results demonstrate that global irradiation on partially cloudy days fluctuates significantly but does not exceed 600 W/m^2 for extended periods. On sunny days, global irradiation can reach 950 W/m^2 and remain above 700 W/m^2 for approximately 5 hours. This disparity has a noticeable impact on the trays, as observed by the temperature of the first tray reaching 45 °C around 3 p.m. on sunny days, while staying below 25 °C on partially cloudy days. Throughout the experiment, the temperature of the lower tray (tray 1) consistently exceeds that of the upper tray (tray 2). This temperature difference arises from the airflow path in the indirect solar dryer, where the air initially contacts the lower tray, providing heat and absorbing moisture, as noted by the authors **[37]**.

These data will assist us figure out what's going on with our dehydration **[38]** due to the drying temperature, which does not reach 45 °C even under ideal conditions within the chamber, and the initially ripe stage of banana 1, the drying process took a very lengthy time (May 1-May 8). This product is best dried between 50 °C and 70 °C. The study carried by **[39]** concluded that a higher temperature (80 °C) was ideal for producing dried bananas, as it had the highest global desirability value.

Banana 2 (export bananas): We picked "May 16" as the sunny day for this time frame (May 8-May 28). We choose these days because, on average, they were sunny and bright with consistent daily irradiation, ideal for drying.

The water content of 2 products: The quantity of water extracted from each banana was determined by measuring its mass again after a month (Moroccan and exported bananas) and comparing it to its initial mass (Table 5).

Table 9 The amount of water extracted from each banana type

	Banana from Morocco	Export banana
The initial mass (Q_i) to be dried (g)	631.6	713.6
The final mass (Q_f) of the dried banana (g)	77.5	137.3
The amount of water extracted from the banana (g)	554.1	576.3
The percentage of the amount of water extracted (Q) in %	87.7	80.8

The percentage of the amount of water is calculated by equation 2 according to the American Society of Agricultural and Biological Engineers Standards (ASABE) **[40]**.

$$Q = \left(\frac{Q_i - Q_f}{Q_i}\right) \times 100 \quad (2)$$

These findings reveal that the water content of each product is high, with only a slight variation between the two bananas. The longer a banana from Morocco is allowed to dry, the more moisture can be removed from it. The authors of **[41]** found that during a continuous 8-hour drying process of banana slices, it was noted that the greatest decrease in both weight and moisture content occurred in the lower tray at a higher air mass flow rate. Specifically, the weight of the banana slices reduced from 150 grams to 48 grams, while the moisture content decreased from 78% to 28.1%. These observations were made without any interruptions in the drying process.

The effect of the treatment: We found a significant distinction between Moroccan and exported bananas at the end of drying process. In fact, unlike untreated bananas, those that were treated with lemon juice did not become entirely black. Since the export bananas have preserved a fairly large size compared to Moroccan bananas, the diameter of the bananas played a role as crucial as that of the treatment, especially at the aesthetic level. The study's findings **[42]** indicate that using pre-treatment methods such as freezing and combined blanching and freezing can significantly increase the drying rate of bananas and reduce the drying time. This may offer a cost-effective solution to enhance the efficiency of the drying process. However, it should be noted that the pre-treatment methods that yielded the most notable improvement in drying performance also significantly reduced the quality of the final product.

The smaller banana in Figure 7 is the Moroccan type, whereas the larger banana is the export variety.
The size of each banana in the initial state:

- Export banana Type 1 (diameter [2.5 cm-3cm]).
- Moroccan banana Type 2 (diameter [2 cm-2.5 cm]).

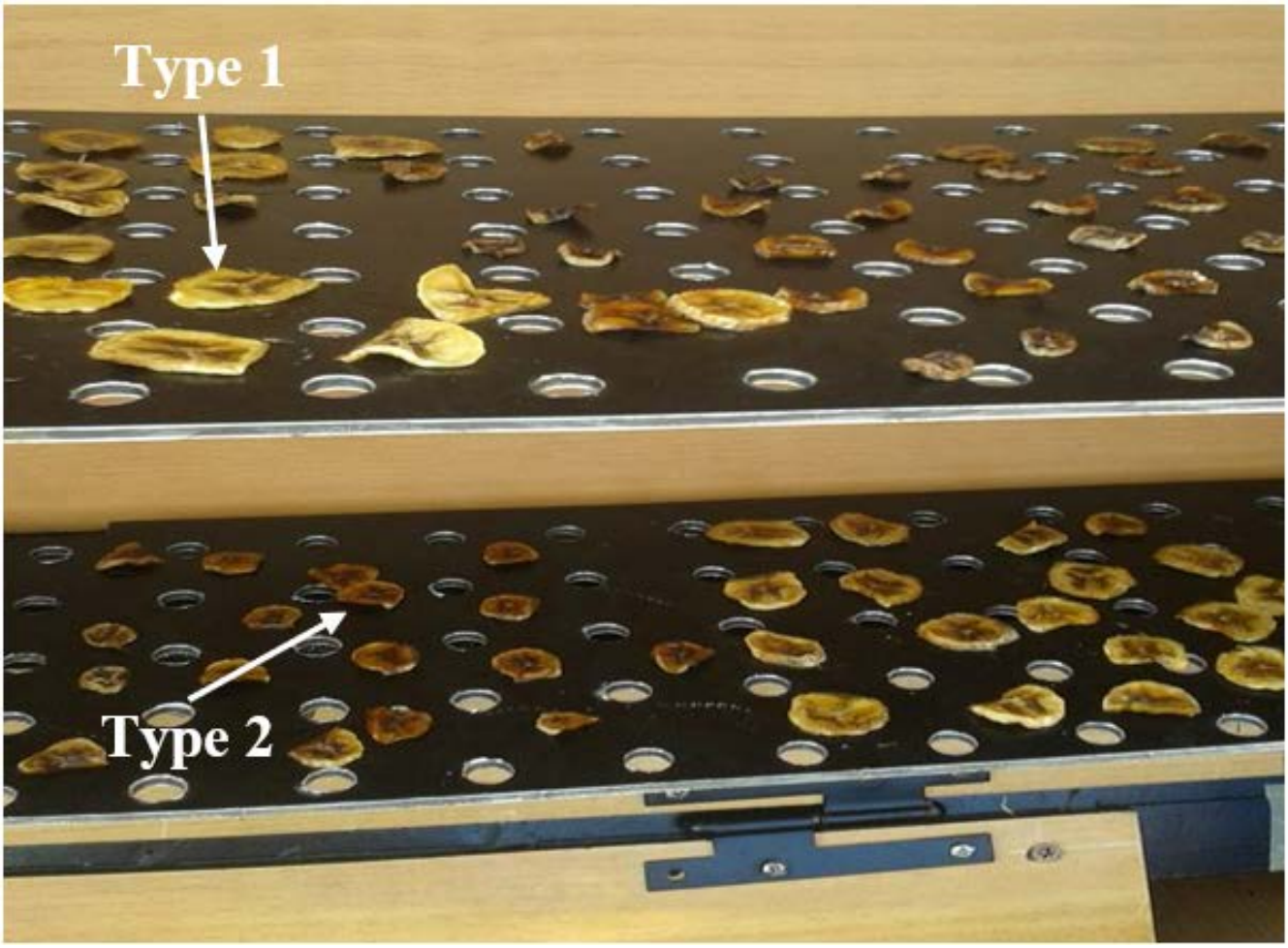

Figure 8 Real image of the two types of bananas placed on the trays in the dryer at the end of drying process.

Problems and Recommendations: One of the biggest issues we had was a dip in temperature during the night caused by the humid air entering via the cavities. Humidity in the drying chamber has increased due to the infiltration of outside air, so the product must spend more time drying. This study by **[43]** examined the impact of changing ambient conditions on the solar drying of mint leaves using two methods under specific dry climate conditions. The study observed how changes in air relative humidity affected product rehydration during the drying process.

Due to the fluctuating weather conditions during the day, we can also say that the drying temperature was unfavourable. It should be remembered that a drying time of 144 hours and a temperature range of 60 °C to 80 °C (without ventilation) is ideal for drying bananas (6 days). This is accomplished by putting the day's production of heat into a storage system so that it can be used to warm the product overnight. This study **[44]** investigated the impact of various variables on the convective drying behavior of bananas, including cultivar, shape, blanching, and heated air conditions (temperatures of 50 °C and 70 °C and velocities of 0.14 and 0.42 m/s). The study used mathematical modelling to analyze the drying process and dehydrated the bananas in a tray dryer, monitoring their weight at set intervals. In **[45]** A drying temperature of 55 °C was found to give superior quality OD banana slices in terms of reduced bulk, improved flavour, decreased aw (<0.60), and reduced dehydration time and energy using HHP as a pretreatment.

Drying viscosity is another issue we've noticed. During the drying process, it was observed that the banana's viscosity increases mostly in the morning and decreases in the afternoon **[46]**. Additionally, we find that the products' viscosity is still noticeable even after drying has reached its final stages.

This difference in viscosity results from the fact that sugar **[47]** adheres to pores when water migrates towards them, creating an appearance of viscosity on the product and slowing down drying kinetics.

It's important to note that products placed on the second and third trays in a drying chamber don't dry as quickly as those on the first try. This is because the products in the first tray are dried by air entering at a specific temperature. Since the humidity in the drying chamber rises as water vapor is taken from the product **[48]**, the process continues to the second and third tray , although at a lower temperature. Because of this, and as evidenced by the daily curves sketched earlier, there is a substantial temperature difference between the trays when the product is present.

It is recommended that the following steps be taken to address these issues when drying bananas:

- Never dry ripe bananas, and install a heat storage system during the day to compensate for the dryer's temperature dropping at night.
- Use a treatment to lower the quantity of sugar in the banana for sweet products, such as sodium or sodium metabisulphite, to eliminate the crusting problem. The study by **[49]** found that the bananas lost sugar during the ultrasonic treatment, so the ultrasonic pre-treatment can be an interesting process to produce dried fruits with low sugar content.
- Close the compressor outlet at night to prevent ambient air from entering the drying box.

CONCLUSION

Through the drying process of bananas, we had the opportunity to observe the behavior of an indirect air solar dryer. During this process, we noticed a significant reduction in the weight of banana slices, decreasing from 150 grams to 48 grams. Additionally, the moisture content of the slices decreased from 78% to 28.1%. These valuable insights allowed us to evaluate the dryer's effectiveness and identify areas requiring enhanced performance improvement. Consequently, we gained a deeper understanding of the significance of certain elements involved in the drying process.:

- When drying a product, it's important to stay within the range of temperatures recommended by the manufacturer.
- Heat loss can be prevented by using an effective and well-made dryer.
- Agricultural products, especially those that have been sweetened, need to undergo treatment to eliminate all the obstacles that slow down the drying kinetics.
- As the temperature drops at night, the dryer needs a way to store the heat it generates during the day.

All of the recommendations are necessary prerequisites for drying food to the appropriate standards and in the allotted time for the product to be presented in optimal nutritional and aesthetic form. However the analysis of the moisture content is crucial in understanding the effectiveness of the drying process and identifying areas that require improvement for a more efficient dryer.

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

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The effect of mechanized shelling and packaging on the quality of melon seeds

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ABSTRACT

A comparative study was carried out between mechanized shelling and manual shelling of Egusi-melon seeds and the effect of different packaging materials on some quality attributes of shelled Egusi-melon seeds(Bara and Serewe). Egusi-melon seeds were manually shelled and mechanically shelled using the National Centre for Agricultural Mechanization (NCAM) mechanical melon Sheller (2018 and 2020 models) and a Rice huller (SB-10D Rice mill). Shelled Egusi-melon seeds were packaged and stored in kraft paper, glass bottles, laminated pouches, and a low-density polypropylene bag for 10 weeks. The Shelling efficiency of different machines compared to manual shelling (control) was evaluated. Manual shelling (control) was the most efficient in terms of output quality. The 2020 NCAM mechanical melon Sheller was the most efficient of the mechanized processes, while the rice huller was the least efficient. The shelled seeds were evaluated for shelling, free fatty acid, and microbial analysis. Results showed that Bara was more efficiently shelled by machines while Serewe was more efficiently shelled manually. Kraft paper seems to reduce seed spoilage over the storage period compared to the other packaging materials, the seeds shelled using the 2020 NCAM mechanical melon Sheller consistently recorded a significantly lower percentage increase in free fatty acid and fungal load. This research has provided valuable insight that selecting a suitable variety, shelling machine, and packaging material is crucial for the overall efficiency and high-quality output of large-scale production of shelled Egusi-melon.

Keywords: Egusi-melon seed, shelling machine, free fatty acid, packaging material

INTRODUCTION

Egusi is a type of watermelon (*Citrullus lanatus, Citrullus vulgaris, or Colocynthis citrullus*) **[1]** that is mostly grown in West Africa for its seed, with Nigeria accounting for about 65% of the total production **[2]**, **[3]**. Egusi-melon seed is characterized by high moisture content (about 90%) and is an excellent source of dietary oil (49.05%-53.10%) and protein (27.60%-33.80%). Its amino acid profile compares favourably with soybean meal **[4]**, **[1]**, **[5]**. Given its rich nutritional profile, Egusi-melon seeds are used as a major ingredient to enrich soups especially, as a soup thickener. Egusi serves as a meat substitute in soups and snacks served on special occasions **[6]**, **[7]**. Its use as a meat substitute follows the formulation process with spices, moulding into different shapes, roasting, smoking, and drying. In addition, Egusi oil is high in unsaturated fatty acids which could be used for different applications (domestically and industrially) **[8]**.

The economic importance of Egusi-melon seed as a trading commodity is growing. Nigeria's export of Egusi-melon seed in 2021 to India, Guatemala, and Brazil was approximately 42,000 metric tons **[2]**, **[9]**, **[10]**. Notably, before the exportation of Egusi-melon seeds, they are processed after harvest into value-added products for easy marketability and acceptance. The unit operations involved in Egusi processing include fermenting the pod (10-

15 days) after harvest to soften the pulp, extracting the seeds from the soft pulp, washing and drying the seeds, followed by removing the shells (hulls) to obtain the cotyledon/kernel **[8]**.

Importantly, the decortication of Egusi-melon seed is a step in its processing that is crucial to produce cotyledons of acceptable quality before being processed for consumption. Currently, Egusi-melon seeds are manually peeled by local women. The process of manual decortication is a slow, tedious, time-consuming, and inefficient process, and increases the risk of contamination due to manual handling. This tends to create a scarcity of products with associated high pricing, especially during the off-season period in non-producing areas. To avert the limitations associated with manual handling, Egusi producers adopted the use of shelling machines which has significantly changed the dynamics of Egusi-melon seed production to meet the capacity for large-scale production. Most researchers have developed different Egusi shelling machines intending to automatically separate kernels from shells, thus saving time and labour requirements **[11]**, **[12]**. However, some of these machines have some identified setbacks as there have been reports of broken seeds and mechanical injuries on the cotyledon (kernels) inflicted by these shelling machines. For instance, Olusegun and Adekunle **[13]** recorded broken seeds that exceeded 20% of the number of seeds being shelled after testing Egusi-melon seed shelling by passing them through two wooden shelling discs, one fixed and the other made to rotate in the clockwise and anti-clockwise direction. Consequently, product quality is lost as spoilage can rapidly set in due to rancidity because of cell rupturing that liberates free fatty acids **[14]**, **[15]**, causing objectionable flavour, taste, and appearance **[16]** observed that when such injured/broken seeds are used as a soup ingredient, the soup quickly acquires a rancid off-flavour. Given these setbacks, the large-scale processing of Egusi-melon seed for export purposes is significantly limited, as consumers tend to prefer the manually shelled seeds to the mechanized shelled Egusi-melon seed. Therefore, it is very fitting to find ways of improving the shelling efficiency of Egusi-melon seed by studying how the types of machines and seed varieties affect the overall output.

Furthermore, following the shelling of Egusi-melon seed is the packaging of the cleaned melon seeds, as it adds value to the final product. Packaging is done to provide the food with an enclosure that protects it from contamination from physical, chemical, or biological sources in its environment **[17]**. The type of packaging material significantly affects the shelf-life and product quality. Glass bottles, paper bags, polyethylene/polypropylene, and laminated aluminium pouches are primarily used in packaging food products because they tend to be inert/unreactive to the food. However, these packaging materials have different barrier properties and would offer varying levels of protection to food **[18]**. Finding the right packaging material for Egusi-melon seed is also vital for large-scale production (especially for the export market) since moisture and gases (air) are responsible for hydrolytic rancidity and oxidative rancidity respectively. Therefore, this study aims to determine the effect of mechanized decortication and packaging on the quality attributes of different varieties of Egusi-melon seed. This work will guide the design of improved equipment for the mechanical shelling of Egusi-melon seeds, reduced cost of shelled Egusi-melon seeds and reduced spoilage of processed Egusi-melon seeds. It will also guide processors on variety selection for optimized efficiency with an increased volume for commercial purposes.

Scientific Hypothesis

Using an improved shelling machine, proper selection of Egusi-melon seed variety, and packaging material can improve the quality of stored shelled Egusi-melon seeds. We expect a higher efficiency for the improved Egusi-melon shelling machine than the old model machine. There would be a reduced percentage increase in free fatty acid and fungal colony count for the seeds shelled using the improved machine with suitable packaging.

MATERIAL AND METHODOLOGY

Samples

Two different unshelled Egusi-melon seed varieties (Serewe and Bara) used in this research were purchased from dealers at Kofar-Gwari market located at Kokona L.G.A in Nasarawa State, Nigeria. The study was done at Emery Research Laboratory (ERL) in Abia state, Nigeria.

Chemicals

All Chemicals used were provided by ERL, Nigeria. Phenolphthalein indicator solution for free fatty acid determination. Sterile peptone water was used in the CFU analysis. Freshly neutralized hot ethyl alcohol was used for acid test. Standard alkali solution for FFA determination. Sabouraud dextrose agar plates (SDA), produced by Dimante Scientific. Streptomycin for SDA medium modification. All solvents and reagents supplied by ERL, including water were of analytical grade quality.

Animals, Plants and Biological Materials

Animals - Animals were not used in this research.

Plants - *Colocynthis citrullus* L. referred to as Egusi-melon in this study.

Biological materials - Special biological materials were not used in this research.

Instruments

Electric blender (Itel-IBL80E1) for easy oil extraction. Soxhlet extractor SE- 6P was used to extract fat from Egusi-melon seed. Mechanical melon shellers (2018 and 2020 Models) sourced from National Centre for Agricultural Mechanization (NCAM), Ilorin, Kwara State, Nigeria. Rice huller, SB-10D Rice Mill (Satake Company, China). An industrial violet sterilizer (model UV-2500, Rio, Italy) was used to sterilize the packaging materials. Mechanical sealing machine operated manually (Super master, Japan) was used to seal the packages hermetically. Stirer, conical flask, Petri dishes, ovens, and filter papers were also used in this research.

Laboratory Methods

The shelling efficiency of both manual and machine-shelled Egusi-melon seeds was determined by calculating the ratio of completely shelled melon seeds to the total weight of unshelled melon seeds fed into each machine type or shelled manually. The shelling efficiency of Egusi-melon seed expressed in percentage is given below.

$$\text{Shelling efficiency (\%)} = \frac{Weight\ of\ wholesome\ melon\ seeds}{Total\ weight\ of\ shelled\ melon\ seeds} \times 100 \quad (1)$$

Where the total weight of shelled melon seeds is given as melon shells + broken shelled melon seeds + unbroken shelled melon seeds + damaged melon seeds.

The free fatty acid of the samples were determined by the standard method as described by **[19]**, The packaged/stored samples were milled using an electric blender (Itel-IBL80E1) for easy oil extraction. Soxhlet extractor was used to extract fat from Egusi-melon seed by weighing 5 g of milled sample into a filter paper, wrapping, and placing in an extraction thimble. After extraction, the solvent was evaporated by drying in the oven. The extracted oil (5 g) was weighed and transferred accurately in a 250m conical flask and 50 ml of freshly neutralized hot ethyl alcohol and 0.5 ml of phenolphthalein indicator solution were added. The mixture was boiled for about five minutes and titrated while hot against a standard alkali solution until colour changed to pink. This was carried out periodically for 10 weeks. The free fatty acid, expressed in percentage is given as;

$$\text{FFA (\%)} = \frac{56.1 \times V \times N}{W} \quad (2)$$

Where:

V – Volume of the standard potassium hydroxide used (ml); N – Normality of the potassium hydroxide solution; W – Weight of the sample (g).

Given the nature of the material and susceptibility of Egusi-melon seeds to be prone to fungal infestation, the fungal colony counts of the samples were determined on Sabouraud dextrose agar plates (SDA) according to the standard as method described by **[20]**. The SDA media was modified with streptomycin to inhibit bacterial contamination. Each sample (1 g) was homogenized in 9 ml of sterile peptone water. Ten-fold serial dilution was done aseptically, and 0.1 ml of the diluted samples were inoculated into a petri dish containing solidified SDA and incubated for 3-5 days at 37 °C. Colonies were then counted and recorded after the incubation period. This was carried out bi-weekly for 10 weeks.

Description of the Experiment

Sample preparation: The Egusi-melon seeds were sorted to remove stones, dirt, and unhealthy seeds. Samples (5kg) were separately shelled using 4 different processing systems (Manual shelling, NCAM mechanical melon Sheller (2018 Model), NCAM mechanical melon Sheller (2020 Model), and SB-10D Rice milling machine (Figure 1). Notably, NCAM mechanical melon Sheller (2018 Model) is made of mild steel (prone to rust), while NCAM mechanical melon Sheller (2020 Model) is made of stainless steel. Each sample was pretreated by tempering with 200 ml of water per 5 kg of the sample at ambient temperature, mixed thoroughly, spread on a paper, and allowed to rest for 1 hour before the shelling operation. This makes the seed coat more pliable and reduces breakage making it more suited for shelling. The pretreated samples were divided into four equal parts, each for a different shelling approach.

Number of samples analyzed: We analyzed 80 samples.

Number of repeated analyses: All measurements of instrument readings were performed three times.

Number of experiment replication: Samples from the same population across the Egusi varieties were analyzed in triplicates.

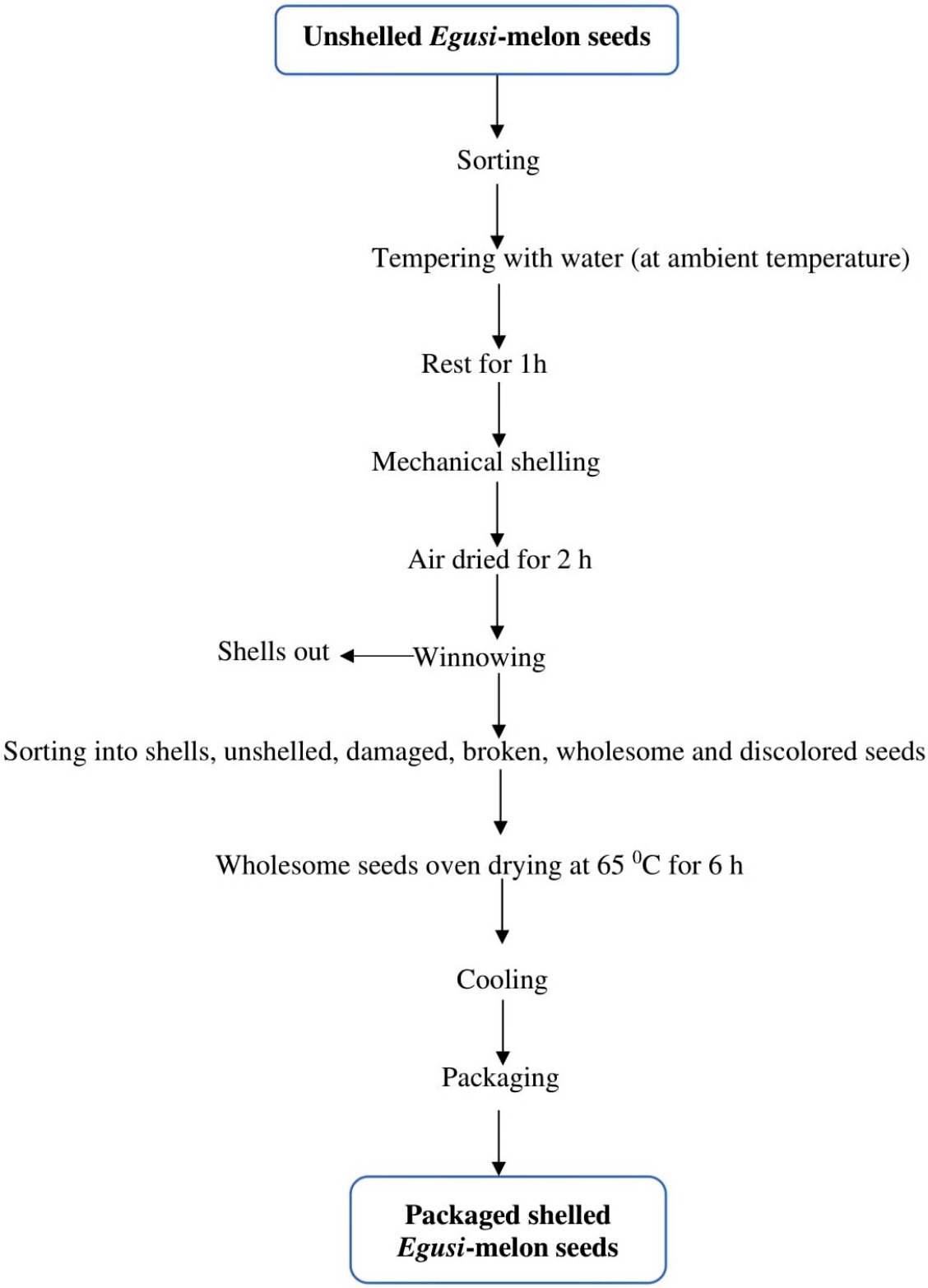

Figure 1 Flow chart for machine-shelled Egusi-melon seeds.

Design of the experiment: The experimental design, showing the various stages from procurement of Egusi samples to laboratory analyses is shown in Figure 2. Briefly, this research was designed to carry out a comparative study between mechanized shelling and manual shelling of Egusi-melon seeds and the effect of different packaging materials on some quality attributes of the shelled Egusi-melon seeds. This was done by shelling Egusi-melon seeds using different machines (Manual shelling, NCAM mechanical melon Sheller (2018 Model), NCAM mechanical melon Sheller (2020 Model), and SB-10D Rice milling machine (Figure 1). The NCAM mechanical melon shellers do shelling by impact and attrition principle and are driven by a 5.5 HP petrol engine prime mover at 650 RPM. The prime mover speed control lever was adjusted to achieve the desired speed.

On the other hand, the SB-10D Rice milling machine is a compact combined rubber roll mill that can rub off the Egusi-melon seed shell as they pass through the space between two counter-rotating rubber rollers driven at 1500 RPM by a 20 HP diesel engine prime mover. Five volunteers shelled a portion of the samples manually, and this served as the control. All shelled samples were dried, winnowed, and sorted into various categories (shells, shelled whole seeds, unshelled seeds, broken seeds, damaged seeds, and partially shelled seeds) followed by packaging as shown in Figure 1.

The packaging materials were sterilized using an industrial violet sterilizer (model UV-2500, Rio, Italy). Shelled Egusi samples (100 g) were placed in different packaging materials made of glass bottles, Kraft paper envelops (thickness 0.01 mm, density 1.4 g/cm^3, and porosity 3.21 g/cm^2), low-density polypropylene bags (thickness 0.02 mm and density 0.45 g/cm^3) and laminated (Aluminum/high-density polyethylene) pouches (thickness 0.5 mm and density 0.82 g/cm^3). All packages were properly corked/sealed hermetically and stored under ambient conditions of about 25-27 ºC in the laboratory for 10 weeks. Analysis (shelling efficiency, free fatty acids, and microbial load of shelled Egusi-melon seeds after being packaged in different packaging materials) was carried out bi-weekly by collecting samples from the packaged/stored samples. The multilevel factorial (2 × 4 × 4) experimental design is shown in Table 1.

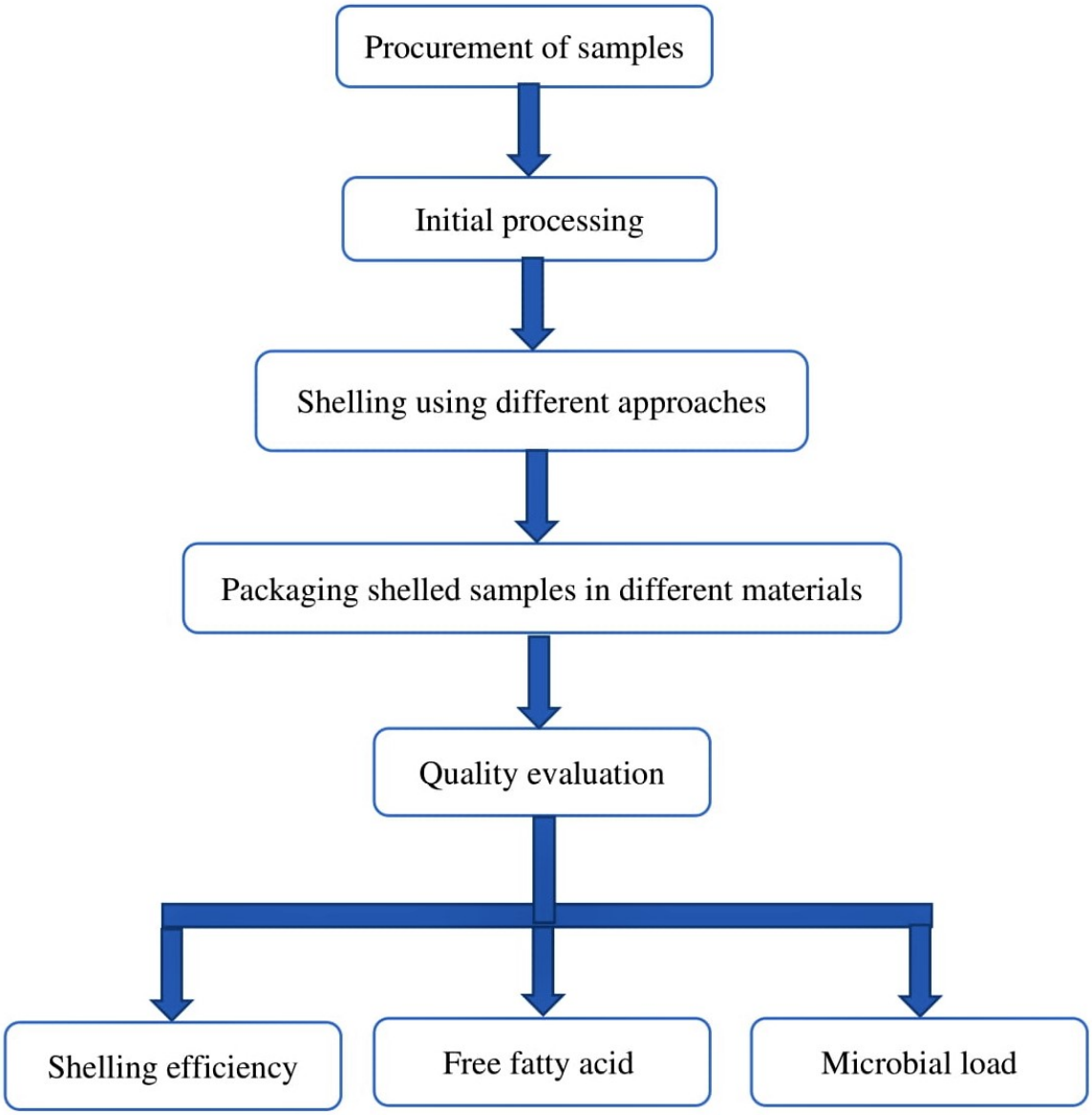

Figure 2 Schematic diagram of the experiment.

Table 1 Multilevel Factorial Experimental Design.

	Factor A	Factor B	Factor C
Name	Melon variety	Shelling equipment	Packaging material
Type	Nominal	Nominal	Nominal
Levels	2	4	4
1	Serewe	Manual	Paper
2	Bara	2018 NCAM Machine	Plastic
3	-	2020 NCAM Machine	Glass
4	-	Rice huller	Laminated pouch

Statistical Analysis

Using the boxplot, Levene's test, and skewness & kurtosis tests, the Analysis of Variance (ANOVA) assumptions of outliers, homogeneity of variances, and normality were examined, respectively **[21]**. As shown in Table 1, data from measurements of melon samples in triplicate were treated to a multilevel (2 × 4 × 4) factorial design. Using SPSS version 26, a three-way ANOVA was conducted, which considered the type of melons, the shelling apparatus, and the packaging material. Mean differences were reconciled using Fisher's least significant difference (LSD). The mean and standard deviation were used to express the results of the dependent variables (measured parameters) (SD). Simple correlation tests were also carried out to identify any connections between the measured shelling analyses. The level of statistical significance was set at 95% (p <0.05) confidence level. IBM SPSS software version 20 (IBM Corporation, New York, USA) was used to do the analysis **[22]**.

RESULTS AND DISCUSSION

The result of the shelling efficiency of two varieties of Egusi-melon seed for the different machines shows that all the mechanized shelling processes were less efficient in terms of output of shelled whole cotyledons than the manual shelled (Control) Egusi-melon seeds. The Bara variety was consistently more efficiently shelled than the Serewe variety for the mechanized shelling processes but less efficiently shelled for the control. The thicker shell of the Bara variety **[23]** may have naturally made it more suitable for mechanized shelling. From the result, the handling for the manually shelled Egusi-melon was more efficient for the smooth, thin-shelled Serewe variety than the thick black edge Bara variety. Therefore, the Bara variety is a preferably better-performing choice raw material for mechanized shelling than the Serewe variety. This result is similar to the findings by **[24]** who reported 55.8% shelling efficiency for mechanically shelled Bara seeds and 50.3% efficiency for mechanically shelled Serewe seeds.

The rice huller was the least efficient machine. This is probably because the rice huller is designed for a more energy-intensive and higher friction task of rice dehusking operation compared to the Egusi-melon shelling operation which may not require as much energy input and friction considering the more delicate cotyledon. This claim is supported by **[25]** who opined that rice processing and milling involve many unit operations that expose the grains to various forces such as impact, shearing, and friction mainly during husking and milling. The thicker shell of the Bara variety was able to resist the damaging effect of the rice dehusking rollers much more than the Serewe variety, and this agrees with **[26]**, who established that the magnitude of the damage caused during the processing depends on the physical and mechanical properties of the grains. Thus, its shelling efficiency was more than double that of the Serewe variety with the rice huller. The Egusi mechanical Sheller (2020 model) machine gave a higher efficiency than the Egusi mechanical Sheller (2018 model) machine for both varieties confirming the design upgrade's improvement.

The shelling analysis of the different varieties of Egusi-melon seeds by different shelling machines is shown in Tables 2 and 3. Manual shelling (control) produced the highest whole-shelled seed output compared to all other shelling (mechanized) processes. This result agrees with the previous research of **[12]** and **[24]** regarding the output of whole/intact shelled cotyledons. The whole-shelled seeds from the mechanized operations were about 50% of the output except for the rice huller, which had 18% (Serewe variety) and 30% (Bara variety). The whole seeds from the manual operation were significantly (p <0.05*)* higher for the Serewe variety (65%) compared to the Bara variety (60%). The Serewe variety produced a significantly (p <0.05) higher whole seed output than the Bara variety for the manual shelling, but the mechanized operations produced significantly (p <0.05*)* higher whole seed for the Bara variety (30%-50%) than the Serewe variety (18%-47%) **[27]**. The thicker shell of the Bara variety **[24]** may have naturally made it more suitable for mechanized shelling. The seed shell was significantly (p <0.05) higher for the Bara variety (7%-22%) compared to the Serewe variety (4%-16%) for all the machine types and control. This is a result of the thicker shell that occurs with the Bara variety, which

corroborates the report by **[28]**, who reported differences in the thickness of the shell between the two varieties of Egusi-melon seed. This thicker shell probably provided greater protection for the cotyledon from being broken. Thus, the Serewe variety suffered significantly (p <0.05) higher breakages in the mechanized operations and control. The lighter shell of the Serewe variety could also cause the occurrence of significantly (p <0.05) higher partially shelled seeds for the Serewe variety for the mechanized operation.

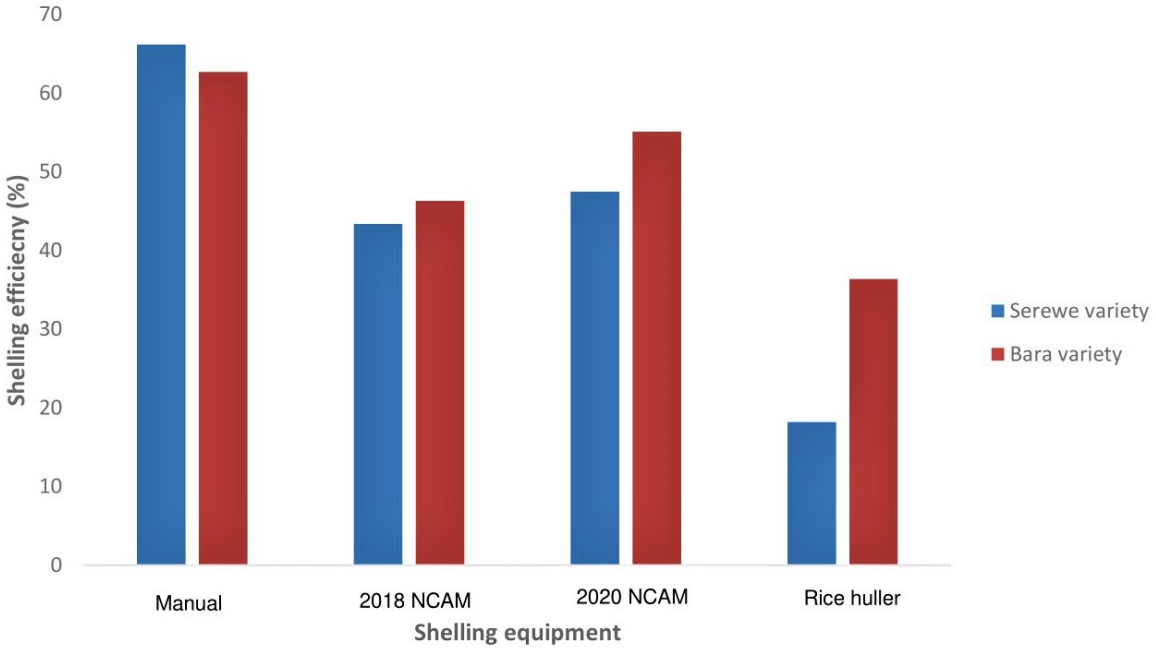

Figure 3 Shelling efficiency of different shelling equipment on Egusi-melon of two varieties.

Table 2 Whole seed, broken seed and seed shell of melon seed varieties as affected by shelling equipment.

Shelling equipment	Whole seed (%)		Broken seed (%)		Seed shell (%)	
	Serewe variety	Bara variety	Serewe variety	Bara variety	Serewe variety	Bara variety
Manual	*65.10^{a} ±0.14	59.90^{a} ±0.39	*13.00^{d} ±0.24	9.80^{d} ±0.29	16.00^{a} ±0.65	*21.57^{a} ±0.77
2020 NCAM	47.00^{b} ±0.21	*49.61^{b} ±0.28	*21.20^{b} ±0.86	16.67^{b} ±0.86	10.00^{b} ±0.53	*13.14^{b} ±0.31
2018 NCAM	40.60^{c} ±0.56	*44.02^{c} ±0.55	*16.20^{c} ±0.56	12.35^{c} ±0.64	8.00^{c} ±0.84	*14.22^{c} ±0.61
Rice huller	18.00^{d} ±0.84	*29.61^{d} ±0.67	*30.00^{a} ±0.75	20.59^{a} ±0.45	3.60^{d} ±0.14	*7.06^{d} ±0.21
LSD	**1.08**	**0.76**	**2.98**	**2.26**	**0.94**	**1.97**

Note: Values are means of duplicate determinations (N = 2). a,b…means with different superscripts along a column for each variety within a parameter is significantly different (p <0.05). *….means with an asterisk (*) within a row and the measured parameters of shelling analysis are significantly different (p<0.05).

The two-factor first-order analysis of variance that shows the interaction between the Egusi-melon variety and shelling equipment is presented in Table 4. There is a high level of interaction between the Egusi-melon variety and shelling equipment in the output of the whole, damaged, and partially shelled seed. This implies that a proper selection of Egusi-melon variety and equipment is important for overall efficiency and high-quality output, mainly to produce whole-shelled Egusi-melon and reduce the damaged seed.

Table 3 Damaged seed, partially shelled seed and density of melon seed varieties as affected by shelling equipment.

Shelling equipment	Damaged seed (%)		Partially shelled seed (%)		Density (g/cm^3)	
	Serewe variety	Bara variety	Serewe variety	Bara variety	Serewe variety	Bara variety
Manual	$0.20^{d} \pm 0.00$	$*0.49^{d} \pm 0.13$	$3.40^{d} \pm 0.28$	$*3.73^{c} \pm 0.09$	$*1.13^{a} \pm 0.00$	$1.11^{a} \pm 0.00$
2020 NCAM	$*10.00^{c} \pm 0.28$	$7.84^{a} \pm 0.45$	$*10.80^{c} \pm 0.14$	$2.75^{d} \pm 0.28$	$*1.13^{a} \pm 0.00$	$1.11^{a} \pm 0.00$
2018 NCAM	$*4.60^{b} \pm 0.53$	$2.84^{b} \pm 0.69$	$*24.10^{a} \pm 0.21$	$22.55^{a} \pm 0.30$	$*1.13^{a} \pm 0.00$	$1.11^{a} \pm 0.00$
Rice huller	$*30.00^{a} \pm 0.82$	$17.65^{c} \pm 0.56$	$*17.50^{b} \pm 0.11$	$8.82^{b} \pm 0.83$	$*1.13^{a} \pm 0.00$	$1.11^{a} \pm 0.00$
LSD	**2.84**	**0.73**	**1.12**	**1.00**	**NS**	**NS**

Note: Values are means of duplicate determinations (N = 2). a,b…means with different superscripts along a column for each variety within a parameter is significantly different (p <0.05). *….means with an asterisk (*) within a row and the measured parameters of shelling analysis are significantly different (p <0.05).

Table 4 Two-factor (first order) ANOVA of Egusi-melon variety and shelling equipment on the shelling parameters of melon samples.

Parameter	Variance ratios (F value)			Mean square error
	Main effect		Interaction	
	A	B	A × B	
Whole seed	85.769*	2279.785*	104.677*	0.451
Partial Eta Squared	*0.915*	*0.999*	*0.975*	-
Broken seed	30.284*	40.471*	2.202^{NS}	3.636
Partial Eta Squared	*0.791*	*0.938*	*0.452*	-
Seed shell	68.554*	98.635*	1.891^{NS}	1.232
Partial Eta Squared	*0.895*	*0.974*	*0.415*	-
Damaged seed	28.623*	192.789*	14.444*	2.229
Partial Eta Squared	*0.782*	*0.986*	*0.844*	-
Partially shelled seed	137.913*	519.543*	35.392*	0.585
Partial Eta Squared	*0.945*	*0.995*	*0.930*	-

Note: A – Melon variety; B – Shelling equipment; A × B – Variety × Shelling equipment; Analyses were done for two melon varieties, four shelling equipment, and four packaging materials. *F value is significant at p <0.05; NS implies not significant (p >0.05).

The percentage increase in free fatty acid and fungal colony count is shown in Table 5.

The largest increase in free fatty acid was observed with the samples packaged in the Laminate packaging materials (410%-3298%) while the least increase was observed for the paper-packaged samples (413%-2290%). This trend was corroborated by the increases in fungal growth, which followed a similar pattern. It is possible that the laminate allowed some level of oxygen transmission across the plastic inner lining that enabled profuse fungal growth and an increase in free fatty acid. Plastics are known to permit the transmission of oxygen **[29]** and aluminium is known to have an oxide layer on its surface **[30]**. The paper packaging on the other hand due to its absorbent nature may have reduced the occurrence of FFAs because it allowed for the drying of stored shelled Egusi-melon seed and because it must have absorbed some of the oil **[31]**. This is supported by **[32]**, who stated that volatile molecules evaporate at the packaging surface. This result makes paper the most suited packaging material for quality retention of shelled Egusi-melon seeds, at least, in the short term. There was appreciable free fatty acid in samples packaged in glass with corresponding fungal colony count. The oxygen in the headspace of

the bottle could have supported the increases. This corroborates the findings by **[33]** who reported an increase in free fatty acid from stored sunflower oil.

The highest free fatty acid increase (410%-3298%) occurred with the Bara variety while the least increase was observed for the Serewe variety (413%-2290%). The increase in fungal growth also followed the same pattern. There may be differences in the genetic disposition among the two varieties in terms of promotion or limitation of fat hydrolysis activity; there could be differences in their inherent antioxidant mechanisms **[34]**. This supports the findings of **[35]** who reported genetic diversity among Nigerian Egusi melon varieties. **[36]** Suggests that the presence of microorganisms is a possible cause of high free fatty acid in the stored seeds, therefore samples of the Bara variety, which recorded a significant (p <0.05) higher microbial load also recorded a higher percentage increase in free fatty acid. The bruises to the cotyledons leading to the exposure of the fat content and possibly lipases in the cotyledons would have promoted the build-up of free fatty acids **[37]**. Therefore, the Bara seeds which recorded a significantly (p <0.05) higher fat content than the Serewe variety, according to the findings by **[38]**, also had a significantly (p <0.05) higher percentage increase in free fatty acid.

The highest free fatty acid increase (3298%) occurred with the Egusi samples processed in the Egusi mechanical Sheller (2018 model) machine. The same sample also had the highest increase in fungal colony count (1636%). The least free fatty acid increase (410%) was recorded for the manually shelled Egusi-melon sample and the least fungal growth (255%) occurred with the manually de-hulled Egusi-melon samples. These results confirm that manual shelling still produced the least damaging effect on quality compared to mechanized shelling. It is also possible that the injuries on the Egusi-melon cotyledons, which exposed oil and othe nutirents, promoted the fungal growth. This corroborates the report by **[39]** which suggests that stored shelled oilseeds are prone to fungal growth. These breakages occurred more with the mechanized shelling. The use of mild steel (which rusts easily) for the Egusi mechanical Sheller (2018 model) compared to the stainless steel of the Egusi mechanical Sheller (2020 model) could have caused more pitting on the contact surfaces of the machine thereby making it a rough surface and could have impacted more injuries on the cotyledons **[40]**. The ANOVA table (Refer to Table 4) indicates a high level of interaction (0.627) between the Egusi-melon variety and packaging material. This implies that the variety and packaging material has a significant (p <0.05) effect on free fatty acid production. There was also a significantly (p <0.05) high level of interaction between the shelling equipment and packaging material for free fatty acid changes and the changes in fungal colony count.

Table 5 Percentage increase in free fatty acid (%) and fungal colony count (cfu/g) of melon of different varieties.

Shelling equipment	Packaging material	Percentage increase in FFA (%)		Percentage increase in fungal growth (%)	
		Bara variety	Serewe variety	Bara variety	Serewe variety
Manual	Glass	*1894^{f} ±2.15	1433^{e} ±7.09	*734^{g} ±6.80	460^{f} ±7.92
	Laminate	*2869^{b} ±3.23	1882^{b} ±9.80	*1207^{c} ±9.51	736^{c} ±9.75
	Paper	*410^{k} ±5.65	413^{l} ±5.77	*493^{j} ±8.70	255^{h} ±4.17
	Plastic	*1472^{h} ±7.03	1026^{j} ±7.16	*528^{i} ±5.09	357^{g} ±6.52
2020 NCAM	Glass	*2415^{c} ±7.44	1542^{d} ±5.24	*1219^{c} ±5.32	694^{c} ±4.91
	Laminate	*2888^{b} ±8.40	1896^{b} ±6.42	*1233^{c} ±6.43	739^{c} ±6.11
	Paper	*814^{i} ±3.83	518^{k} ±2.99	*637^{h} ±3.18	399^{f} ±8.56
	Plastic	*2100^{e} ±8.92	1193^{h} ±7.31	*705^{g} ±7.18	371^{g} ±3.46
2018 NCAM	Glass	*2926^{b} ±7.55	1385^{f} ±9.37	*1439^{b} ±8.22	844^{b} ±8.45
	Laminate	*3298^{a} ±9.14	2290^{a} ±9.88	*1636^{a} ±5.79	993^{a} ±8.12
	Paper	*1683^{g} ±5.49	1067^{i} ±7.89	*809^{f} ±6.57	472^{ef} ±7.73
	Plastic	*2523^{c} ±7.66	1788^{c} ±8.28	*985^{e} ±4.32	513^{e} ±8.10
Rice huller	Glass	*2299^{d} ±8.41	1513^{d} ±6.83	*947^{e} ±8.76	557^{e} ±9.15
	Laminate	*2991^{b} ±9.87	1903^{b} ±8.42	*1157^{d} ±4.32	758^{c} ±6.49
	Paper	*644^{j} ±4.95	538^{k} ±2.38	*566^{i} ±5.55	354^{g} ±5.73
	Plastic	*1963^{f} ±3.22	1275^{g} ±4.95	*636^{h} ±5.16	410^{fg} ±8.16
	LSD	160	32	96	90

Note: Values are means of duplicate determinations (N = 2). a,b,c means with the same superscript along a column for each variety within a parameter is not significantly different (p >0.05). *….means with an asterisk (*) within a row and the percentage increase in free fatty acid and fungal growth is significantly different (p <0.05).

The total fungal growth of the shelled Egusi-melon seed varieties during storage is shown in Figure 4. Fungal activity is the major cause of the problem of melon seeds deterioration **[41]**. The Serewe variety maintained a higher load of fungal colony-forming units throughout the storage period compared to the Bara variety, However, the rate of increase of the fungal colony count of the Bara variety was higher than that of the Serewe variety. This could be a result of the genetic makeup of the variety which enhances the suitability of Serewe as a fungi substrate. But the moisture content of the Bara variety, according to the findings by **[38]**, could have promoted the proliferation of fungi on the cotyledons. According to **[42]**, the storage deterioration of melon seed is significantly influenced by the moisture content, because the microorganisms require moisture for their activities. Also, the mechanical damage on the shelled seed could have facilitated the growth of microbes because nutrients will be available at the point of damage. The pattern of fungal growth followed the same trend of reduction in the rate of increase between the second and the fourth week. This reduction is consistent for both varieties and could be attributed to a systemic phenomenon, like a reduction in the headspace's available oxygen, which could have slowed mould growth. The curves also showed a high level of linearity ($R^2 = 0.9952$ for the Serewe variety and $R^2 = 0.9945$ for the Bara variety).

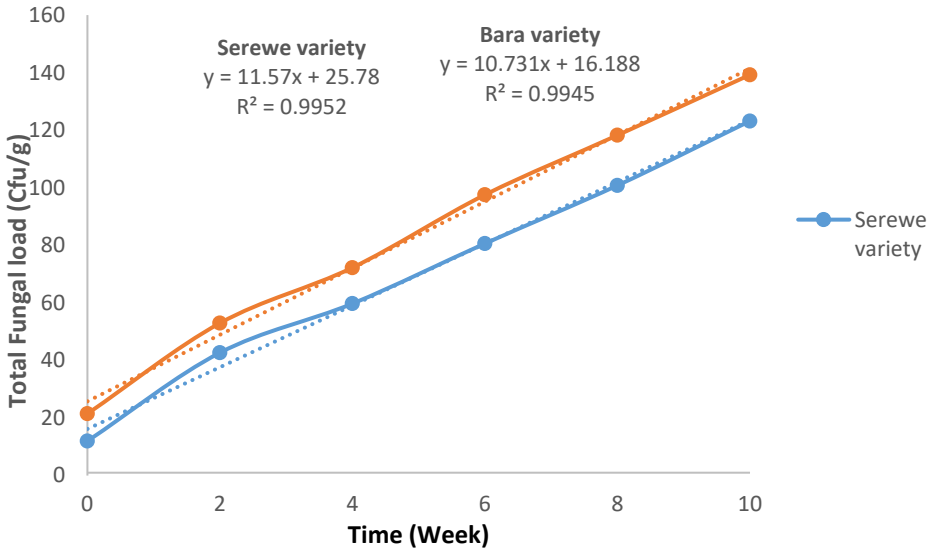

Figure 4 Total fungal load of processed *Egusi*-melon varieties over a period.

The effect of the different shelling equipment on the total fungal load is shown in Figure 5. The Egusi seed samples shelled using Egusi mechanical Sheller (2018 model) had the highest rate of fungal growth followed by the seeds shelled using the Egusi mechanical Sheller (2020 model) shelled samples and the rice huller shelled samples. The control exhibited the least rate of growth. The samples shelled using the Egusi mechanical Sheller (2020 model) had a lower rate of fungal growth than the samples shelled with the Egusi mechanical Sheller (2018 model) indicating that it could have bruised the cotyledon less than the Egusi mechanical Sheller (2018 model). A slight decrease in the proliferation rate of fungi between the second and the fourth week was observed in all samples except for the control. This could result from growth factor like available nutrients or a drop in the oxygen in the head space. It is possible that the oxygen drop occurred more in the samples that had more injuries because they sustained a greater proliferation of fungi. There would have been a greater oxygen requirement from both the exposed oil (for oxidation) **[43]** and the fungi growth **[44]**. The exposed oil will absorb oxygen from the air around it as it gets oxidized. This will be more intense if the oil is exposed from the interior of the cotyledon through an injury. The injured surface could also provide a more nutrient-rich substance than an intact cotyledon. But in the control sample, the injury on the Egusi-melon seed cotyledon was less,minimizing the oxidation oxygen requirement from exposed oil. This is also reflected in the linearity of the slope for the control sample which was the highest ($R^2 = 0.9981$).

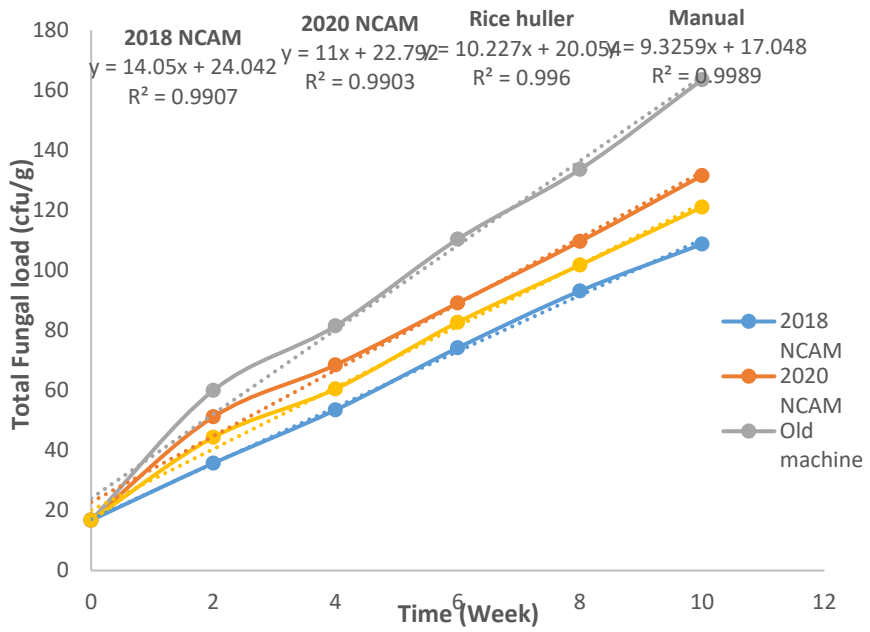

Figure 5 Effect of shelling equipment on the total fungal load over a period.

The effect of packaging materials on the total fungal load of the shelled Egusi-melon is shown in Figure 6. The highest fungal growth rate is observed in the samples packaged in a laminated pouch, followed by glass packaging and plastic packaging. The samples packaged in the paper had the least rate of fungal growth (33 CFU/g for week two and -93 CFU/g for week 10). Although factors such as the nature of the substrate **[45]** and moisture content of the sample **[46]**, are major contributors to the fungal deterioration of stored seeds, the use of improved storage structures/ packages, as mentioned by **[47]** significantly affects the product quality. This suggests that paper packaging material in this study could serve well in packaging shelled Egusi-melon seeds. The slowing of the rate of growth that occurred in the second to the fourth week was not apparent for the paper-packaged samples. The laminated pouch packaging exhibited a further slowing down of the rate of fungal growth beyond the 8th week. Both the plastic and paper-packaged samples exhibited a slight increase in fungal growth rate after eight weeks. The differences indicate the effect of different packaging materials on the quality of the product.

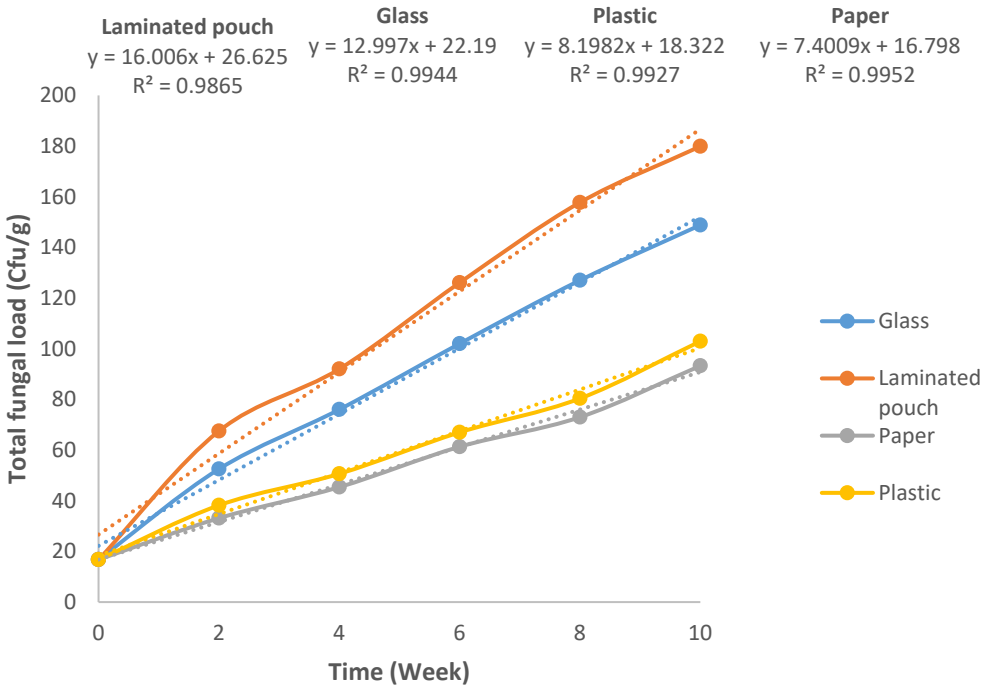

Figure 6 Effect of packaging on the total fungal load over a period.

2020 NCAM Mechanical melon sheller

2018 NCAM Mechanical melon sheller

SB-10D Rice mill

Manual (Hand) shelled seeds

NCAM shelled seeds

Rice huller shelled seeds

Figure 7 Mechanized shellers and shelled Egusi-melon seeds.

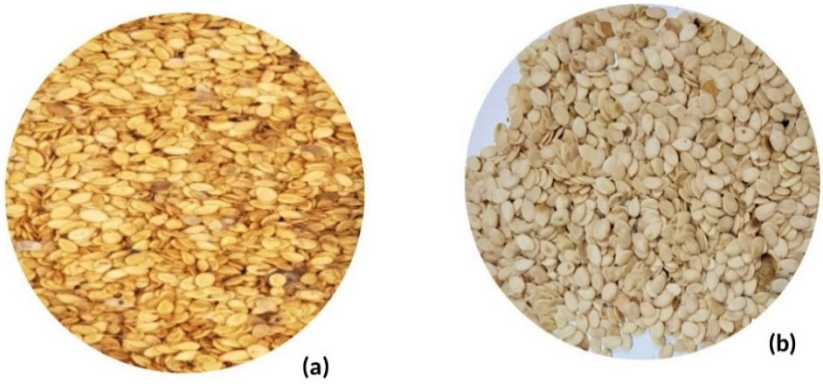

Figure 8 Unshelled (a) and Shelled (b) Egusi-melon seeds.

CONCLUSION

A comparative study was conducted between mechanized shelling and manual shelling of Egusi-melon seeds and the effect of different packaging materials on some quality attributes of the shelled Egusi-melon seeds. The Manual shelling of Egusi-melon produced more whole and fewer broken seeds than the Egusi-melon shelling machines used in this study. Serewe Egusi-melon seeds were more efficiently shelled manually (66% shelling efficiency) Bara variety was more efficiently shelled with machine processing (55% shelling efficiency). The manually shelled Egusi-melon seeds produced a less per cent increase in free fatty acids (410%-2869%) for 8 weeks of storage compared to the machine-processed seeds (518%-3298%). Material of machine construction affected the efficiency of shelling of Egusi-melon and the quality of cotyledons after shelling and during storage. Machines built with stainless steel produced more efficient shelling. Egusi-melon varieties with higher fat content

(Bara) produced more free fatty acids at a higher rate but with lower final free fatty acid content than those with lower fat content (Serewe). Packaging of shelled Egusi-melon seeds using sealed Kraft paper envelops (as given in this study) recorded a reduced per cent increase of free fatty acid (410%-1683%) on the seeds and exhibited lower microbial load. A proper selection of variety is necessary for the optimum performance of any machine design. Different Egusi-melon varieties have different microbial loads and exhibit different rates of microbial proliferation after shelling and during storage. Likely, the output of the shelled Egusi-melon from the rubber roll rice dehuller can still be improved if the manufacturer can make the necessary adaptations.

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Transforming livestock by-products into nutritious extruded feed additives: a sustainable approach for modern agriculture

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ABSTRACT

The search for a solution to the challenge of providing the agriculture industry with complete feeds is relevant to modern animal husbandry in many countries, and protein is a key limiting component in feed. Along with the growth of food production, the waste it produces, which is also a valuable resource of useful components, can be recycled and used. Thus, in slaughter, butchering and meat production, a large amount of waste is generated, including by-products that can be processed further. The extrusion process is one of the best processing methods to improve the nutritional value of ingredients and feed and improve feed efficiency. In modern feed milling operations, extrusion must be considered as the main process for increasing feed profitability. The benefits of the extrusion process in improving the nutritional value and efficiency of ingredients and feeds depend on many factors, such as the structure and chemical composition of the ingredients, the processing conditions and the equipment used in processing. This article substantiates the need to develop technologies for involving production waste in their further use in medium- and long-term growth in the demand for food. A technology for producing an extruded feed additive based on vegetable feed with the addition of slaughter waste is presented. The formulation of a feed additive in which different percentages of slaughter waste of 5.10% and 15% corn was replaced with by-products was studied. The water activity of the obtained extruded feed additives was studied, where, at a content of 15% slaughter waste, it was low compared with that of the other two samples. Further research will allow procuring fodder products with a high biological value and utilising unclaimed by-products of livestock slaughter.

Keywords: cattle slaughter waste, extrusion, heat treatment, technology, chemical composition, protein

INTRODUCTION

At present, the problem of vegetable protein on a global scale is turning into a vital one, and protein is becoming a strategic raw material. Therefore, reducing protein deficiency is one of the most important and complex tasks of world agricultural science and practice, requiring urgent solutions. Increasing feed protein production is the main way to solve the protein problem. The chemical industry contributes to replenishing feed protein resources, but the share in feed protein production is small. The lack of protein in compound feeds, as well as the usefulness of protein itself, is the main factor constraining the intensification of the industry: a protein shortage of 30%-40% increases unproductive costs by a factor of 1.4. Especially acute is the shortage of proteins of animal origin, which are essential for growing young animals and breeding animals **[1]**.

Recently, by-products, including heads, legs and entrails, have become increasingly valuable as feed or feed additives in animal diets **[2]**. The animal nature of these by-products significantly contributes to their energy density; protein and amino acid contents; acid quality, and fat, calcium and phosphorus contents. The by-products could be successfully used as animal feed, but if not properly disposed of, they can damage the environment **[3]**.

With the increase in production at slaughterhouses, the quantity of by-products and technical slaughter products increases simultaneously, and enterprises need help to dispose of waste or process all raw materials without producing residue. In modern conditions, there can be no other answer to this question: to increase its profitability and competitiveness, it is necessary to introduce new, efficient technologies **[4]**.

The effective functioning of enterprises for producing meat from farm animals depends on the competent use of all the resources and by-products of its processing and slaughter waste. Most of the by-products have a valuable chemical composition and can be used to produce food and feed. When processing raw materials of animal origin, for example, at meat processing plants, blood is collected, endocrine-enzymatic raw materials are collected and processed, and intestinal raw materials are collected. Enzyme preparations, feed flour and dry vegetable and animal feed are obtained from waste. Equally important is the proper organization of slaughter sites and compliance with technological, veterinary and sanitary rules. If the rules of processing, transportation and storage are violated, meat products' nutritional value and shelf-life decreases, and losses increase **[5]**, **[6]**.

The approximate percentage of live weight of various animals considered inedible material is as follows: 49% for cattle, 47% for sheep and lambs, 44% for pigs and 37% for broilers **[7]**, **[8]**.

According to the Food and Agriculture Organization of the United Nations (FAO), total meat production worldwide, excluding China, is growing by 1.9% annually **[9]**. Accordingly, the amount of by-products produced by the meat industry is also growing worldwide. The meat processing industry collects and processes part of the slaughter waste, mainly by-products, to obtain raw materials used in animal feed and pet food.

Today, dry food is widely used due to its ease of acquisition, transport, storage and distribution, as well as the positive results of growth and conversion rates. Today, the two most widely used technologies for feed production are granulation and extrusion. The forage produced by these processes is denser, with lower moisture content and provides better preservation **[10]**, **[11]**.

The extrusion process is widespread today as it can be used to produce all kinds of feed, whether floating, fast sinking or slow sinking, depending on the needs of each species. Extrusion is a high-temperature, short-time process that minimizes nutrient loss while improving the digestibility of starches and proteins compared to granulated foods. Extrusion is a process in which food products are not only pressed, as in granulation but are also ‘cooked’, requiring higher humidity, temperature and pressure levels than granulation. All of these requirements must be met to achieve the desired degree of expansion when exiting the extruder. As a result, extruded feed is a higher-quality product that increases the profitability of farms **[11]**.

During the extrusion process, starches are converted into easily digestible forms. The expansion of starches during high-pressure extrusion gives the feed a lighter and bulkier texture, in contrast to the denser granules obtained by low-pressure heat treatment. The extrusion also makes starches water soluble, so extruded feeds easily turn back into a slurry when water is added. Simple sugars and starches found in roughage, such as hay, also become more available to the body due to the breakdown of fibrous material.

The use of feed obtained as a result of extrusion has several advantages:

– High digestibility – about 95% of the feed is easily digested by animals compared to crushed grain (up to 40%). This increases productivity and gives you the maximum benefit from animal husbandry (more milk, meat products, and eggs). After extrusion, the digestibility of legumes (soybeans, peas, vetch, etc.) increases up to 10 times. This will allow the body to get the maximum amount of proteins, amino acids and vitamins that legumes are so rich in.

– Profitability – the extruded product is consumed half as much as conventional whole grains. It effectively replaces food of animal origin (extruded peas completely replace reverse when feeding calves older than a month old).

– Minimum resource costs – grain can be processed without preliminary sorting and drying. The raw materials must be free of earth, straw, stones, etc.

– Efficiency – even damp grain lying in a granary for several years lends itself to extrusion. The processing of grain production waste (buckwheat husks, etc.) makes it possible to obtain nutritious feed for pigs, sheep and goats.

– Good eating by animals due to the pleasant bread taste and aroma.

– Stimulation of growth and strengthening of immunity.

– Providing the body with the necessary sugar without the use of food additives.

– Feeding hygiene – feed can be fed dry without additional processing. Animals do not scatter or burrow in leftover food. As a result, there is no other dust content in the air. And this contributes to improving working conditions for personnel and protecting equipment from premature breakdowns.

– Long shelf life due to low moisture content.

– Reducing the mortality of young animals by 2 times from gastrointestinal diseases due to the sterility of the feed **[12]**.

Water is the main component of all biological systems, since food products, except for dry fruits, contain more water in quantitative terms than any other substances. The quality and safety of products depends on their moisture content and binding energy, which reflects the water activity indicator. The indicator of water activity (aw, aw-water activity) characterizes the relationship between the material's moisture and the microorganisms' ability to use it for their biological activities **[13]**.

The efficient use of by-products directly impacts the country's economy and environmental pollution. The non-use or under-use of by-products leads to potential revenue loss and additional and increasing costs to dispose of these products. Failure to properly use animal by-products can create serious aesthetic problems and catastrophic health problems. In addition to the pollution and hazard aspects, in many cases, meat, poultry and fish processing wastes have the potential to be used to recycle raw materials or to be turned into useful products of higher value. Regulatory requirements are also important as many countries restrict the use of organ meats for reasons of food safety and quality. Offal such as blood, liver, lungs, kidneys, brains, spleen and rumen have good nutritional value **[14]**.

Scientific hypothesis

The replacement of corn for by-products is enriched with a feed additive. The water activity of the obtained extruded feed additives at 15% slaughter waste was low compared to the other two samples. Research makes it possible to obtain fodder products with a high biological value and utilize unclaimed by-products of livestock slaughter.

MATERIAL AND METHODOLOGY

Samples

Waste from the slaughter of livestock, a feed additive obtained from the Almaty region (Kazakhstan), was used for the study. For the study, such waste from slaughter as: bone, skins, intestines, raw fat, contents of the gastrointestinal tract and non-food raw materials were used.

Chemicals

All reagents were of analytical grade and were purchased from Laborfarm (Kazakhstan) and Sigma Aldrich (USA).

Instruments

The following instruments were used for the work: meat grinder MIM-100 (JSC 'Torgmash', Republic of Belarus), hammer crusher H-115 (POM AUGUSTOW, Poland) and grain extruder PE-170 (Agrotechservice, Russia).

Laboratory Methods

When performing laboratory studies, generally accepted and special modern physical and chemical methods were used:

- Humidity was determined on an MX 50 moisture analyser (Japan).
- Crude protein according to GOST 13496.4-93.
- Crude fibre according to GOST 31675-2012.
- Raw fat according to GOST 13496.15-97.
- Raw ash content according to GOST 26226-95.
- Contents of exchange energy using spectroscopy in the near infrared region according to GOST 51038-97.
- The degree of dextrinization according to GOST 29177-91.

Description of the Experiment

The feed additive was prepared in the experimental production workshop of the Kazakh Research Institute of Processing and Food Industry, according to previously developed recipes, using a PE-170 extruder with a 170 kg/h capacity.

Number of samples analysed: Four samples were analysed.

Number of repeated analyses: All tests were performed in triplicate.

Number of experiment replication: Replications were conducted twice.

Design of the experiment: The control recipe, without including by-products was developed according to the recommendations for cattle **[44]**. A mixture of slaughter waste was added to the test formulations, replacing corn with 5%, 10% or 15% slaughter waste. Extrusion was carried out under the conditions of a feed mill at LLP 'Kazakh Scientific Research Institute of Processing and Food Industry.' The pre-cleaned and washed slaughter waste was crushed in a MIM-100 meat grinder. The crushed raw material was further dried in a thermostat at 35-

38°C for 5-6 hours. The components were weighed according to the recipe and mixed on a vertical mixer 'SV-5Sh'. Grinding of grain components was carried out separately for each type on an H-115 hammer mill, and then samples were analysed. Extrusion was carried out on a PE-170 extruder press. Water activity (aw) was determined on an Aqualab 4TEDUO activity analyser.

All by-products were individually crushed, dried and ground according to the extrusion technology. The humidity of by-products was studied at different temperature conditions of drying (Figure 1).

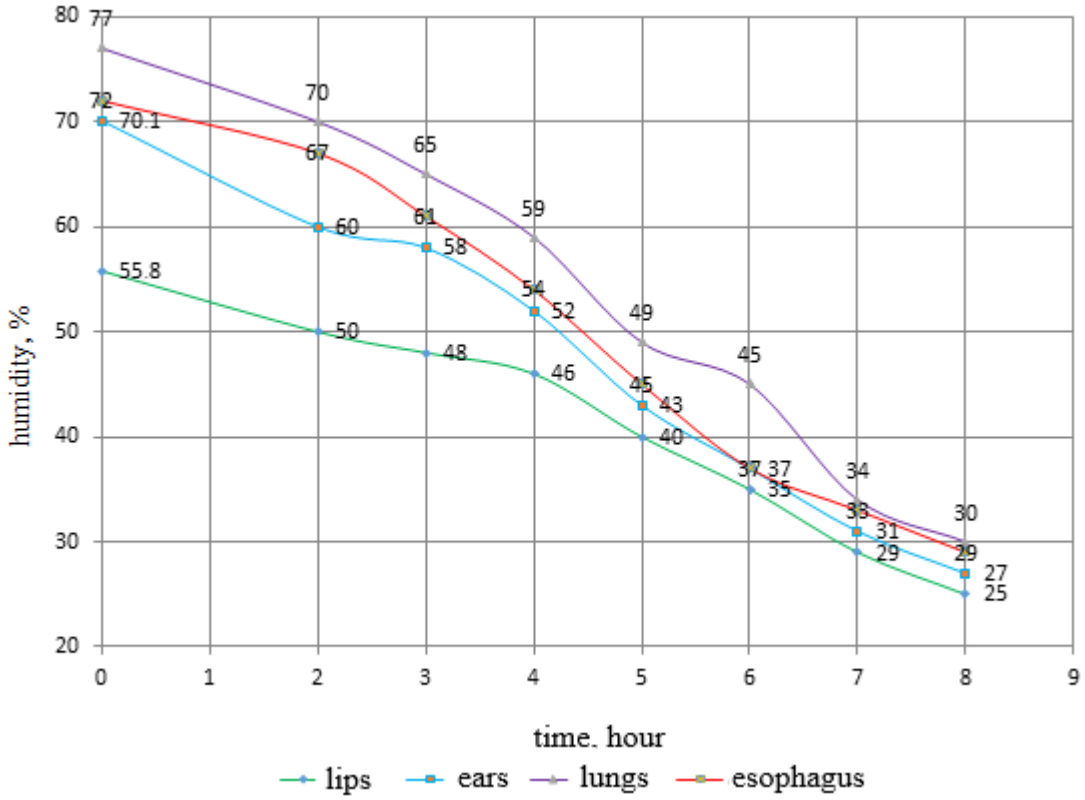

Figure 1 Change in moisture content in beef offal (lungs, ears, lips, oesophagus) during drying.

The final moisture content of offal ranged from 25% to 30%. A recipe was developed from vegetable and animal raw materials to create a feed additive. This recipe replaced 5.10% and 15% corn with by-products obtained after drying. Three recipes were developed using offal at 5%, 10% and 15%. The component composition and chemical composition of the control formulation and three experimental formulations are presented in Table 1.

Table 1 Feed additive formulation.

Components	Content (%)		
Corn (crushed grain)	37.0	32.0	27.0
Waste from slaughter	5.0	10.0	15.0
Wheat bran	40.0	40.0	40.0
Grain waste	7.0	7.0	7.0
Corn bran	5.0	5.0	5.0
Feed zeolite	4.0	4.0	4.0
Salt	1.0	1.0	1.0
Premix	1.0	1.0	1.0
Total	100.0	100.0	100.0
Crude protein	18.2 ±0.14	18.6 ±0.21	18.8 ±0.25
Crude fibre	3.3 ±0.05	3.4 ±0.06	3.4 ±0.04
Crude fat	2.34 ±0.03	2.33 ±0.04	2.34 ±0.05
Ash	2.15 ±0.02	2.14 ±0.04	2.12 ±0.05
Exchange energy (MJ)	12 ±1.06	12 ±1.06	12 ±1.06

As seen from Table 1, the protein content in the extrudates also increases with an increase in the content of by-products. These results are consistent with the results of studies on the increase in protein content, including 5% and 10% beef lungs **[43]**.

During extrusion, the raw material is subjected to a complex baro-hydro-thermal effect; as a result, complex physicochemical changes occur in it, providing sterilization, dehydration and restructuring of polysaccharides and protein. Anti-nutritional compounds such as urease, protease inhibitors and trypsin are entirely or significantly destroyed. The critical point of technologies based on dehydration is the high hygroscopicity of the resulting product, accompanied by its saturation with water vapour from the air with a corresponding increase in humidity, contamination with microflora and a decrease in shelf life. A distinctive feature of the analysed technology is a highly efficient product cooling system, which allows a sharp decrease in the temperature and hygroscopicity of the product within a few seconds after extrusion **[15]**, **[16]**, **[17]**.

In the experimental workshop of the Kazakh Research Institute of Processing and Food Industry, extruders were extruded on a PE-170 brand extruder at a temperature of 130-140 °C and a pressure of 2-3 MPa. In this case, the passage time of the feedstock in the unit was 8-10 s.

The main technological parameters that determine the nature and intensity of the extrusion process and the depth of physical and chemical changes in extrudates are the temperature and pressure of the extruded materials in front of the matrix; humidity of the extruded product; time spent by the product in the working area of the extruder; frequency of rotation of the pressing screw and ratio of starch to protein in the extruded mixture.

Statistical Analysis

The experiments were performed in triplicate. All measurements are given with standard deviation values. Differences in the measurements of the experimental and control groups were calculated using analysis of variation (one-way ANOVA) using Tukey's test. The measurement value $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

The forces of internal friction of the processed mixture and friction on the parts of the body and screw heat the mixture in the extruder. The finished product (Figures 2-5) in the ratio of the feed additive specified in the recipe leaves the forming device in the form of an endless tow.

Figure 2 Extruded feed additive from recycled slaughter (5%).

Figure 3 Extruded feed additive from recycled slaughter (10%).

Figure 4 Extruded feed additive from recycled slaughter (15%).

Figure 4 Extruded feed additive (control sample).

During the experimental work, the measurement and control of the technological parameters of the extrusion process were carried out under laboratory conditions. The temperature was measured using an infrared meter. The results of studies of the grain extrusion process are shown in Table 2.

Table 2 Research results of the grain extrusion process.

Product name	Preparation	Humidity (%)		Degree of dextrinization (%)	Extruded grain temperature (°C)	Extruder capacity (kg/h)	Specific electricity consumption (kW.h/t)	Engine load (A)
		before	after					
Experience 1 (5% by-products)	hydrated	15.0 ±0.14	8.8 ±0.06	38.5 ±0.17	135 ±1.45	148 ±2.5	82.0 ±0.24	55.0 ±0.26
Experience 2 (10% by-products)	hydrated	15.3 ±0.15	8.4 ±0.05	40.6 ±0.21	135 ±1.45	150 ±2.35	80.6 ±0.24	55.0 ±0.20
Experience 3 (15% by-products)	hydrated	14.8 ±0.12	7.6 ±0.04	57.2 ±0.42	138 ±1.46	155 ±2.21	78.8 ±0.23	55.0 ±0.24
Control	hydrated	15.0 ±0.11	8.8 ±0.03	38.1 ±0.39	135 ±1.47	147 ±2.21	82.4 ±0.24	55.0 ±0.23
Significance	-	n.s.	n.s.	*	n.s.	*	n.s.	n.s.

Note: Indicated values: ± – standard deviation calculated from three parallel measurements, * – p <0.05, significant, n.s. – not significant (p >0.05).

During extrusion, a trend towards a decrease in the moisture content in the composition of the extrudates with an increase in the content of by-products was observed. The degree of dextrinization and the productivity of the extruder increased significantly as the offal content increased (p <0.05). In terms of the extrusion temperature, specific energy consumption and engine load, no significant changes were depending on the level of offal in the extrudates.

The indicator aw refers to the most important element of barrier technology since its lower values serve as an effective barrier to developing negative technological and pathogenic microorganisms, as the rate of chemical and biochemical processes, including spoilage, depends on the water activity level. Moisture products are classified according to their moisture content: high-moisture products have aw > 0.9, intermediate moisture products 0.6 < aw < 0.9 and low-moisture products aw < 0.6.

Water activity characterizes the product itself and is determined by its chemical composition and hygroscopic properties. Equilibrium relative humidity characterizes the environment in hygrothermal equilibrium with the product. Water activity characterizes the form of the moisture bond in the product. Of the total amount of water contained in a food product, microorganisms, for example, can use only a certain “active” part of it for their vital activity. And for each type of microorganisms there are maximum, minimum and optimal water activity values. Deviating the value of aw from the optimal leads to inhibition of the vital processes of microorganisms and sometimes to their death. With the help of this indicator, the degree of participation of water in various chemical, biochemical and microbiological reactions occurring in the product both during the manufacturing process and during its storage is assessed: lipid oxidation, enzymatic and non-enzymatic activity, hydrolytic reactions, development of microorganisms. Table 3 shows the water activity of the feed additive with different ratios.

Table 3 Water activity.

Recipe with content	Water activity value
5%	0.3798
10%	0.3440
15%	0.3458

The data in Table 3 shows that the feed additive's water activity is low, with aw < 0.6. In products with low humidity, microbiological processes do not occur, and they retain their qualities for a long time. It follows from the table that the water activity of the feed additive containing 15% slaughter waste is low compared with that of the other two samples.

One of the main problems for the production of livestock products in the Republic of Kazakhstan, as in many other developing countries, is the lack of a high-quality, year-round forage base. The increase in population and the rapid growth of world economies is leading to an increase in demand for animal products. At the same time, the demand for fodder crops for livestock is also increasing. Therefore, in the future, maintaining food security will depend on the expansion and efficient use of non-traditional resources, as well as on innovative technologies for the manufacture of feed and feed additives for animals **[18]**, **[19]**.

By controlling the functional and technological indicators in the product, particularly the aw indicator, it is possible to predict its storage capacity, which will make it possible to create ‘stability maps’ of products and determine the optimal conditions for their storage **[20]**, **[21]**.

Only 60% of a slaughtered pig in Germany ends up on a plate in the form of cutlets or sausages. Parts unfit for human consumption, such as bones, hooves, and some internal organs, are processed into pet or fish food, used in the chemical or fertilizer industry or converted into biofuels **[22]**, **[23]**. Of the 8.6 million tons of total carcass weight in 2019, about 2.6 million tons of these ‘animal by-products’ were used in this way. Additional losses occur during wholesale and retail trade and at the consumption stage, when goods expire or products are prepared for consumption but not eaten. According to the latest available data, 11.9% of global meat production was lost between slaughter and retail in 2016. Meat waste: much less than the whole fog **[24]**, **[25]**.

Due to the relatively high consumption of meat and meat products, consumers' production losses and product waste at the consumption stage becomes significant. It is estimated that up to 23% of production in the meat sector is wasted. The largest share is generated at the consumption level, accounting for 64% of total food waste, followed by production (20%), distribution (12%) and primary production and post-harvest processing (3.5%). Food loss and waste data in the meat sector is very limited **[26]**, **[27]**. At the same time, meat and meat products are characterized by adverse environmental impacts (meat has the highest emissions per kilogram of food compared with other foods), which requires sustainable management with these products in the entire chain (stage of production, processing, transportation and consumption) **[28]**, **[29]**. The increase in food waste has serious negative consequences for the global environment, climate, water and land resources **[30]**.

Food waste and wastage have become an important political issue as the demand for food on a global scale is steadily increasing due to an increase in population and consumption **[31]**, **[32]**.

René Renato Balandrand-Quintana et al. note in their article that agro-industrial waste is an economical source of proteins that must be used, for which it is necessary to improve traditional extrusion methods **[33]**, **[34]**, **[35]**. Emphasizing the potential of agricultural waste, Christiana O. Giola et al. said: 'Today, waste is seen and mentioned as a raw material for producing various products and is well valued for its economic value **[36]**, **[37]**. Technology has significantly increased the physical and nutritional value of many production wastes. Instead of exporting their waste for meagre foreign exchange earnings, many countries are developing the technological capacity to convert more waste into useful products' **[38]**, **[39]**.

One of the universal methods for preparing feed raw materials for feeding animals and poultry, as well as for processing biological waste, is the extrusion method, which allows the use of secondary raw materials for feed purposes with virtually no restrictions. Experts have repeatedly noted the high nutritional value of such processed products, which can acquire many new, initially absent useful qualities and properties, as well as nutritional value **[40]**, **[41]**.

However, the waste's high moisture content (MC) and the specificity and presence of numerous microorganisms make it a highly unstable material. Its disposal, transportation and processing in factories create significant problems **[42]**. Using suitable extrusion parameters (temperature, material holding time and raw material quality) allows us to obtain a completely sterile product with attractive physical and chemical properties and a high nutritional value**.**

CONCLUSION

Examination of finished extrudates showed a trend towards a shift in protein composition with an increase in the content of the offal mixture (lungs, oesophagus, ears and lips – 1:1:1:1). Further study of this direction will make it possible both to obtain extrudates with a high biological value and to utilize unclaimed by-products of slaughter. The final moisture content of offal ranged from 25% to 30%. A recipe was developed from vegetable and animal raw materials to create a feed additive. This recipe replaced 5.10% and 15% corn with by-products. The value of animal waste is determined by the high content of complete proteins in it, which have in sufficient quantities all the essential amino acids necessary for the intensive development and fattening of farm animals, as well as mineral salts, trace elements and fats. As an additive to the diet, they compensate for the lack of protein in plant foods and increase their digestibility.

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Effect of extrusion process parameters on pellet crumbliness in fish feed production

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ABSTRACT

The article presents the results of studying the pellets crumbliness index of a pilot batch of extruded compound feed for pike-perch. The regimes of extruded pellets fish compound feed production with minimal crumbling were defined using a mathematical model. A 3-factorial central composite design was implemented to obtain a mathematical model of the process of extrudate resulting in the second-degree polynomial. The process influencing factors include the feed mixture moisture W (%), extrusion temperature T (°C) and steam pressure P (MPa). Experimental studies were conducted according to the experimental plan. The experimental data obtained were entered into the planning matrix. Experimental data was processed in a program prepared in Microsoft Excel. As a result, an adequate second-order mathematical model describing the dependence of the crumbliness index on the feed mixture moisture before extrusion, extrusion temperature and steam pressure was obtained. The mathematical model adequacy was tested based on Fisher's variance ratio. The Fisher criterion is an important statistical tool for verifying the adequacy of the model and analyzing the variance. The analysis of the obtained model and its graphical interpretation are presented. A good extrusion process mathematical model was developed. The fundamental principles of feed mixture processing on a single-screw extruder and the choice of its rational parameters ensure the production of extruded compound feed pellets for pike-perch with minimal crumbling were studied. A minimal crumbling is provided at the following values of the factors - feed mixture moisture before extrusion is 32%, extrusion temperature is 132 °C, and pressure is 0.4 MPa. In the selected levels of factors, the calculated value of extruded pellets crumbling was 1.02%.

Keywords: aquafeed, extrusion, crumbability, modelling, extrudate

INTRODUCTION

The theory of balanced nutrition has allowed a scientific justification of the need for human food in terms of energy and plastic components to overcome many diseases associated with a lack of vitamins, essential amino acids, trace elements, etc. Various diets have been created for all population groups, considering physical exertion, climatic, and other living conditions. Fish and seafood are widely recognized in terms of their role in nutrition, as they provide protein and a unique source of omega-3 fatty acids and bioavailable micronutrients **[1]**. Aquaculture, as a feature of agriculture, has to play a huge role in implementing the balanced nutrition concept of the population. Apparent advantages include the possibility of fish cultivation organization directly in the places of its consumption and in a wide range according to population demand – from traditional to delicatessen species. The specificity of aquaculture as a technological process guarantees production transparency and controllability, which is the basis for improving efficiency, safety, environmental performance and quality of the final product **[2]**. However, the aquaculture of the Republic of Kazakhstan is developing slowly enough and its growth opportunities have not yet been exhausted. The intensive development of industrial aquaculture is currently

holding the limited range of fish feed proposed by domestic feed manufacturers **[3]**. Developing full fish feeding is the paramount consideration of scientists from many countries with a growing aquaculture. Different species and ages fish feed formulation changes all the time, new components and feed additives, reflecting the latest data on the study of fish physiology and metabolism, are introduced into their composition **[4]**.

Kazakh Research Institute of Processing and Food Industry LLP is actively working to find new raw materials for the mixed feed production to improve fish compound feed's technological and structural-mechanical properties. The production of compound feeds is a complex technological process that allows obtaining a final feed product with the required characteristics from several types of raw materials. Each type of compound feed is produced according to a specific recipe developed, taking into account the breed, direction of cultivation, age and type of animal, bird or fish for which it is intended. At the moment, scientists are working on developing formulation and technology for the domestic extruded feed production for several promising aquaculture facilities for breeding fish, such as pike-perch, sharp tooth catfish, jade perch, tilapia and pike in recirculating aquaculture systems (RAS).

Pike-perch export to Europe has increased over the last 15-20 years. In this regard, there is a depletion of biological resources in fisheries waters – rivers, lakes, and ponds, which today remain the main sources of fish production in the background of extremely poor development of culture-based fisheries in the republic **[5]**.

Work on pike-perch artificial reproduction for fish stocking of water reservoirs and cultivation of marketable products in the farms of Kazakhstan has recently begun. Therefore, studies are actively conducted toward increasing efficiency in pike-perch breeding within industrial conditions and developing the physiologically complete domestic starter and production extruded feed for them.

Currently, a significant part of plant and animal raw materials used in the production of feed for aquaculture is extruded **[6]**. The main advantage of this technological operation is the transformation of the raw material structure. For example, during the temperature treatment of the feed mixture under pressure, starch is broken down to dextrin and sugar, proteins are denatured, enzyme inhibitors are inactivated, some toxins are neutralized and their producers are destroyed. At the same time, nutrients become more available for fish to digest **[7]**. Extrusion makes it possible to change the properties of the finished feed over a wide range by varying the parameters of the technological process **[8]**. Many scientific publications outline the results of studies on the extrusion of one type of raw material, less often, a two-component mixture. However, the process of multicomponent mixtures extrusion, which are compound feeds for aquaculture, has not yet been studied sufficiently. At the same time, the established principles of the compound feed extrusion process need further clarification **[9]**. The composition of fish compound feed includes poorly pelletized components, namely: fish-flour, salt, chalk, whey, which affects the energy intensity of the extrusion process, as well as structural and mechanical properties of the pellets, such as hardness and crumbling. Compound feed pellets crumbling can be reduced by optimising the extrusion regimes without the formulation changing **[10]**.

The pellets crumbling is a quality factor which characterizes the degree of dependence of particles making up the pellets. During feed transportation, particularly over long distances, and in transshipping from one mode of transport to another, or in transportation under unsatisfactory conditions, the pellets can degrade, losing their consumer quality characteristics and reducing in volume. Fish compound feed crumbling index affects the depletion of feed nutrients in the water, the swelling characteristic, water resistance, safety during transportation and dispensing. During pneumatic transportation, collisions the pellets with the pipe walls can lead to damage to a particular part of the pellets, which is primarily a problem for large pellets (> 8 mm). As a rule, many different mechanisms are associated with the degradation of pellets. Abrasion of pellets or its surface leads to the formation of small particles. This is mainly because of the interaction of pellet with the pellet or pellet with the pipe wall.

The crushing of larger particles from the pellets is considered as shearing. In particular, ribs and corners are weak points and subject to shearing. As for the physical quality, during pneumatic transportation, the abrasion of pellets occurs in the feeder mechanism at various flight speeds (25, 30 and 35 m/s) and feed rates (9, 18 and 36 kg/min). The physical quality of the 12 mm feed pellets was measured as present quality, DORIS value, hardness coefficient and durability. Significant differences in fine particle formation between feeds were observed during pneumatic transportation. Increasing the air velocity (m/s) increased cracking (particle size 2.4-10 mm) and small particles (particle size < 2.4 mm). Increasing the feed rate (kg/min) had the opposite effect, causing the reduction of cracking and small particles **[11]**.

Thus, crumbled small feed particles generated during transportation and dispensing can lead to water pollution, and oxygen deficiency in water due to their bacterial decomposition. Suspended feed particles can settle inside the fish gills and cause tissue irritation with inflammatory processes.

Studies using special machinery and equipment for testing the strength of compound feed, where the mechanical impacts are made on the pellets, are carried out for evaluation of the pellets' strength with various methods and quality assessments **[12]**. In our country, the crumbling of feed pellets is usually determined

according to GOST 28497-2014. The essence of the method lies in the degradation of the tested product pellets, the separation of nondegraded pellets from the fines and crumbs by their sieving and weighing, followed by the crumbling calculation **[13]**.

Scientific Hypothesis

By varying the parameters of the technological extrusion process, it is possible to affect the physical properties of the obtained finished pellets of fish compound feed. We expect that developing an adequate extrusion process mathematical model, the study of the fundamental principles of multicomponent mixtures processing on a single-screw extruder and the choice of its rational parameters will help obtain extruded compound feed pellets with minimal crumbling.

MATERIAL AND METHODOLOGY

Samples

Extruded pellets of compound feed for fish (compound feed for pike perch). They are cylindrical brown granules with a diameter of 6 mm and a length of 5 mm. The composition contains raw materials of animal origin (fish meal, meat and bone meal), vegetable origin (corn gluten, wheat, rapeseed meal, oil, betaine), feed yeast, mineral additives and oils.

Chemicals

No chemicals were used.

Instruments

CAS SW-2 bench scales (CAS Corporation, Seoul, South Korea), Model N: MW-113000, it 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 – 4.75 mm with a diameter of – 300 mm (IP Sedov A. B., Moscow, Russia) they are used to separate destroyed granules from unresolved ones. Glass container for pouring the analyzed sample and weighing.

Laboratory Methods

The pellets crumbling was determined following GOST 28497-2014 "Feed, compound feed. Method of crumbling properties granule determination" on the U17-EKG **[13]**. The essence of the method consists in destroying the extruded pellets, separating undisturbed pellets from the fines and crumbs by sieving, weighing them, and calculating the crumbliness index.

Description of the Experiment

A total of 24 samples were analyzed, with two repeated analyses and two experiment replications for each sample.

Design of the experiment: The production of experimental compound feed for pike-perch according to the developed formulations and testing of the production technology modes were carried out using feed components of domestic production by extrusion at the Golden Fish.kz LLP plant located in the Belbulak village, Almaty region. An extrusion line assembly of the plant is from the Chinese "HENAN RICHI MACHINERY CO.LTD". A single-screw extruder was used for the extrusion of feed. The working body is a screw rotating in the chamber, during its rotations, the crushed feed raw materials with a humidity of 12-16%, are heated to 120-150 °C, at a pressure of 2.8-3.8 MPa, plasticized and loomed through the holes of the circular cross-section matrix with a diameter of 5 mm, a soft knife fitting form to realize stepless speed change, which can cut the discharge arbitrarily into a product of the required length. Then the process quickly passes from the area of high pressure to the area of ambient pressure, the homogeneous mass expands, resulting in the formation of a product of a porous structure.

Compound feed for pike-perch is high-protein and high-energy. Respectively, raw materials for compound feed production had to be selected with a high protein content and digestibility.

From raw materials of animal origin, fish meal has a complete set of amino acids necessary and easily accessible for fish, but its use is limited due to its high cost and shortage in our region. As a result, it is necessary to use alternative sources of vegetable and animal protein to partially replace fishmeal. Meat and bone meal is also a good source of animal protein, it contains essential amino acids – arginine and histidine. The high content of saturated rapidly oxidizing fats limits its use to 10%. In the extrusion processing of the raw materials mixture, the fat content of it must be less than 6%, otherwise, the pellet will not form. Therefore, the raw material should be low in fat. It is established that such raw materials are various isolates and concentrates, protein meal and seed cake, and wheat and corn gluten. For example, soy meal can be used up to 40% as part of fish feed, it contains up to 40% protein and up to 1.5% fat. Soy isolate contains 86.6% protein and 0.5% fat. Wheat gluten contains up to 75% protein and 1.2% fat **[14]**.

The formulation of grower compound feed for pike-perch developed by us contains raw materials of animal origin – 40%, vegetable origin – 39%, microbiological origin (feeding yeast) – 15%, mineral and other additives – 2.75, oils – 3.25, which applied to the extrudates after the output of pellets from extruder by coating.

The pellets crumbling was determined following GOST 28497-2014 "Feed, compound feed. Method of crumbling properties granule determination" on the U17-EKG. The grinder is a two-chamber box, each chamber is 100 mm x 350 mm x 350 mm in size, on the side of the chambers there is a metal protruding plate (divider) that affect the compound feed sample during chamber rotation.

In this regard 500.0 ±0.1 g double-finished extruded feed weight was taken and placed in the device's chambers. Then device was run, and the chamber began to rotate. The chamber with the extrudate is rotated for 10 minutes at a speed of 50 rpm. Then the device automatically shut off, the contents are poured, sifted through a sieve with a cell size equal to 0.75 of the diameter of the analyzed granules, the remaining granules on the sieve are weighed with an accuracy of ±0.1 g.

The crumbling was calculated using the following formula:

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

Where:
m1 – pellets weight before testing, g; m2 – pellets weight after testing, g.

The duplicate arithmetic mean was taken as the final test result. The mathematical model was carried out using a multifactorial experiment. The following factors were chosen: feed mixture moisture before extrusion, extrusion temperature, steam pressure. The crumbling index was taken as a quality criterion. A 3-factorial central composite design was implemented to obtain a mathematical model of the process of extrudate resulting in the second-degree polynomial **[15]**.

Statistical Analysis

In order to obtain a three-factor mathematical model, a Box-Behnken rotatable design B3 was implemented. Experimental studies were conducted according to the experimental plan. The experimental data obtained were entered into the planning matrix. Experimental data was processed in a program prepared in Microsoft Excel. Calculations of the coefficients of the mathematical model and verification of its adequacy were carried out according to the standard methodology for a Box-Behnken rotatable three-factor design. Calculations of the coefficients of the regression equation (mathematical model) in coded and natural values of the variance of reproducibility and adequacy, calculated values of Fisher's criterion to check the adequacy of the model were carried out in a Microsoft Excel spreadsheet according to the program prepared by the authors. As a result, an adequate second-order mathematical model describing the dependence of the crumbling coefficient on the feed mixture moisture before extrusion, extrusion temperature and steam pressure was obtained. The mathematical model adequacy was tested based on Fisher's variance ratio **[16]**. The Fisher's criterion is an important statistical tool for verifying the adequacy of the model and analyzing the variance. It compares the significance of factors and their interactions in the model and determines whether the model adequately describes the data.

RESULTS AND DISCUSSION

The variables changing the extrusion process and the properties of the finished extruded feed, are usually the following: raw material feed rate, extruder screw rotation speed, its dies diameter, extrusion temperature, and extruder output pressure **[17]**, **[18]**, **[19]**. In this study, the process influencing factors include the feed mixture moisture W (%), extrusion temperature T (°C) and steam pressure P (MPa). An experimental matrix design was prepared – Table 1.

Table 1 Matrix plan of the experiment in decoded form.

Experience option	Parameters			y_1 crumbability,%
	X_1 feed mixture humidity,%	X_2 extrusion temperature, °C	X_3 pressure, MPa	
1	28	128	0.3	1.48
2	28	132	0.3	1.34
3	28	138	0.3	1.11
4	28	140	0.3	1.08
5	32	128	0.3	1.33
6	32	132	0.3	1.08
7	32	138	0.3	0.98
8	32	140	0.3	0.95
9	36	128	0.3	1.08
10	36	132	0.3	1.05
11	36	138	0.3	0.99
12	36	140	0.3	0.83
13	28	128	0.4	1.11
14	28	132	0.4	1.08
15	28	138	0.4	1.05
16	28	140	0.4	1.01
17	32	128	0.4	1.01
18	32	132	0.4	0.96
19	32	138	0.4	0.93
20	32	140	0.4	0.72
21	36	128	0.4	0.82
22	36	132	0.4	0.79
23	36	138	0.4	0.75
24	36	140	0.4	0.69

The selected variability intervals of influencing factors and their levels are shown in Table 2.

Table 2 Variability intervals of influencing factors and their levels.

Factors		Levels of variation				
Natural	**Encoded**	-1.68	-1	0	+1	+1.68
The humidity of compound feeds *W*, %	x_1	16	20	26	32	36
Extrusion temperature *T*, ^{0}C	x_2	116	120	126	132	136
Steam pressure *P*, MPa	x_3	0	0.1	0.25	0.4	0.5

According to B3 three-factor design, the number of experiments is $N = 24$, the number of zero points is $n^0 = 6$. Experimental data processing was carried out on a program developed in Microsoft Excel. Pellets crumbling mathematical models are in coded values.

$$y1=2.1964-0.2806_{x1}-0.3736_{x2}-0.3395_{x3}+0.30375_{x1x2}-0.16625_{x1x3}-0.07625_{x2x3}-0.076_{x1}^2-0.1907_{x2}^2-0.129_{x3}^2 \quad (2)$$

Pellets crumbling mathematical models are in natural value:

$$Y1=-44.9464-0.9539W+1.032T-5.27P+0.0084WT-0.1847WP+0.0847TP-0.0021W^2-0.0053T^2-5.7312P^2 \quad (3)$$

The mathematical model adequacy was tested based on Fisher's variance ratio. Dispersion of reproducibility:

$$S^2(\bar{y}) = \frac{0.1442}{6-1} = 0.0288$$

Dispersion of adequacy:

$$S_{ad}^2 = \frac{0.8659 - 0.1442}{20 - 10 - (6 - 1)} = 0.1444$$

Calculated F-value:

$$F_p = \frac{S_{ad}^2}{S^2(\bar{y})} = \frac{0.1444}{0.0288} = 5.01 < F_T = 5.05.$$

Degrees of dispersions freedom:

$$f_E = n_0 - 1 = 6 - 1 = 5$$

$$f_{ad} = N - \lambda - (n_0 - 1) = 20 - 10 - (6 - 1) = 5.$$

Tabular value of Fisher's variance ratio at $f_E = 5$ and $f_{ad} = 5$ is $F_T = 5{,}05$. Where, $F_p < F_T$ - a hypothesis for mathematical model adequacy.

Response surfaces and two-dimensional section contours $y=f(x, x_2)$, $y=f(x_1, x_3)$ and $y=f(x_2, x_3)$ at $x_3=0$, $x_2=0$ and $x_1=0$ (Figures 1-3).

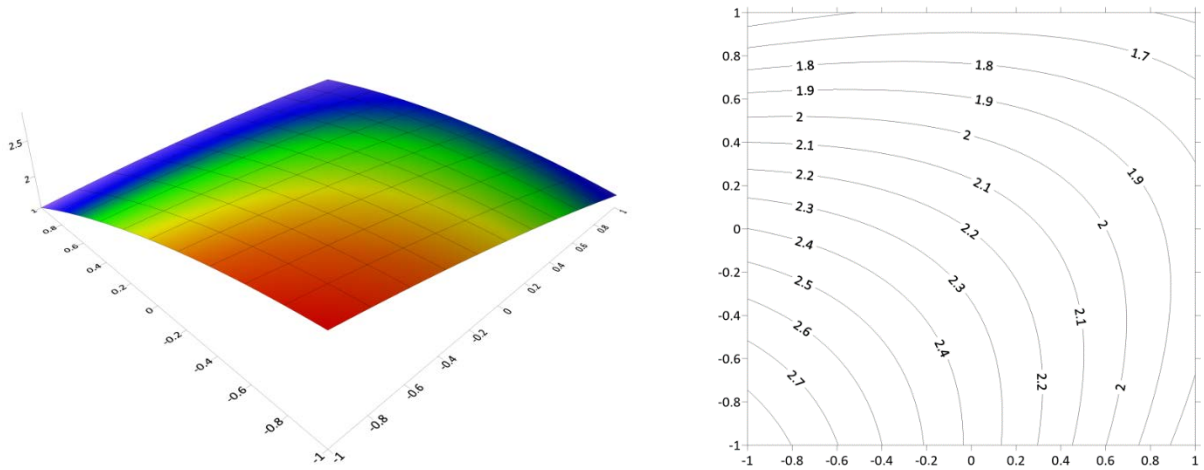

Figure 1 y_1 dependences of pellets crumbling on x_1 compound feed moisture and x_2 extrusion temperature at $x_3 = 0$.

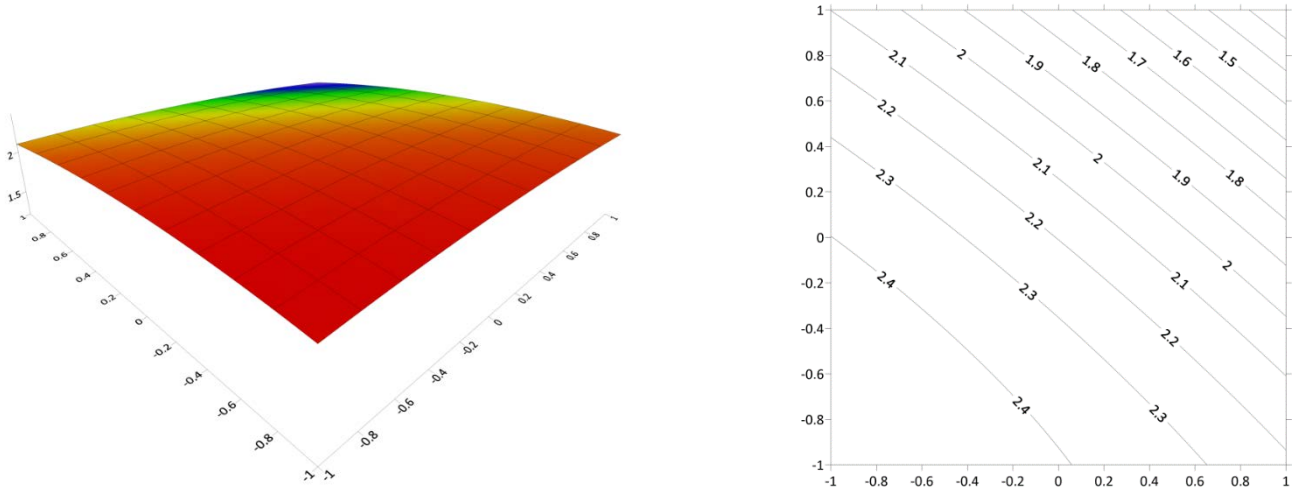

Figure 2 y_1 dependences of pellets crumbling on x_1 compound feed moisture and x_3 steam pressure at $x_2 = 0$.

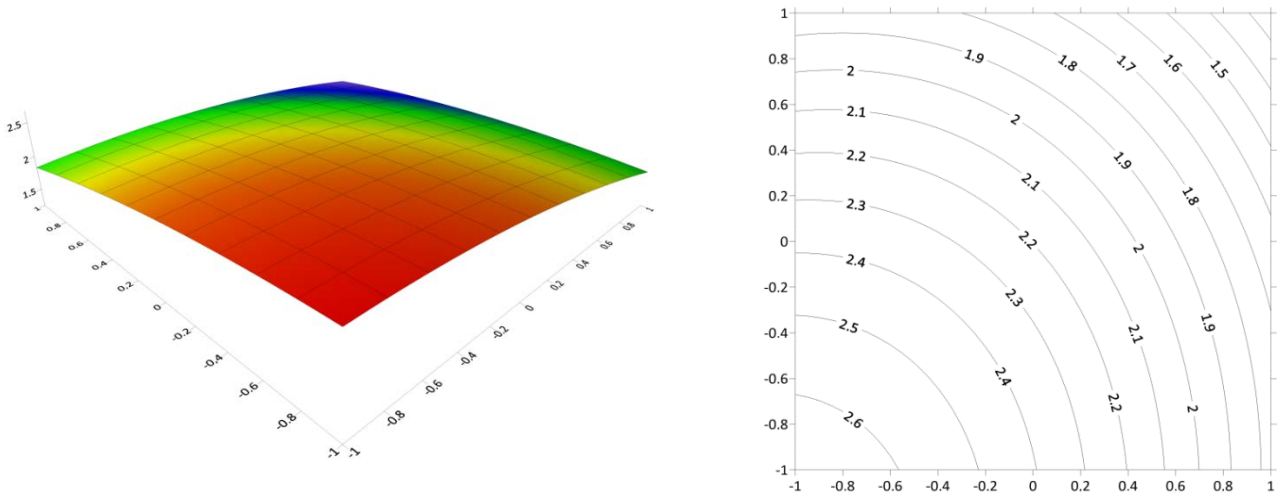

Figure 3 y_1 dependences of pellets crumbling on x_2 extrusion temperature and x_3 steam pressure at $x_1 = 0$.

Analysis of the two-dimensional section of the response surface showed the following:

- x1 and x2 influence study at x3 = 0, where the minimal pellets crumbling is provided at x1 = +1 and x2 = +1;
- x1 and x3 influence study at x2 = 0, where the minimal pellets crumbling is provided at x1 = +1 and x3 = +1;
- x2 and x3 influence study at x1 = 0, where the minimal pellets crumbling is provided at x2 = +1 and x3 = +1;

Summarizing the obtained results, it is possible to draw the following conclusion - a minimal pellets crumbling is provided at the following values of the factors:

- in coded values of the factors: x1 = +1, x2 = +1 and x3 = +1;
- in natural values of the factors: compound feed moisture is W=32%, extrusion temperature is T = 132 °C, and the steam pressure is P = 0.4 MPa.

In the selected levels of factors, the calculated value of extrudate pellets crumbling from equation (2), is y = 1.02%.

Calculations of the variation of these three technological parameters in the extrusion process made it possible to obtain an adequate mathematical model of processing a multicomponent mixture on a single-screw extruder.

Achieving the minimum crumble of granules is important, since dust and smaller particles formed as a result of high crumble have no nutritional value, lead to a loss of feed and, consequently, increase production costs. The interaction of feed with the aquatic environment creates problems that are not encountered when feeding terrestrial animals. This makes the physical properties of the feed more important for aquaculture than for terrestrial animal **[20]**. The presence of lost nutrients and uneaten feed in the wastewater of fish farms has been a major obstacle to the expansion of commercial aquaculture **[42]**.

The physical properties of the finished feed pellets by their characteristics directly depend on the conditions of extrusion processing, preparation and composition of the formulation. Process variables, such as temperature, humidity, screw rotation speed, raw material type, etc., cause different reactions depending on their interaction, where physical parameters make it possible to make decisions about optimal operating conditions, being a very useful tool when evaluating a new formulation. Calculating rational parameters will help to obtain pellets of extruded compound feed with minimal crumbling.

Factors such as moisture content and temperature profile used in the extrusion process affect the molten materials' viscosity and the finished product's characteristics **[43]**, **[44]**. The properties of raw materials such as particle size distribution and chemical composition (protein, lipids, carbohydrates content, etc.) are also important **[19]**.

Several authors have pointed out that adding a vegetable protein (soy and wheat) to fish-meal results in extrudates with sufficient porosity for maintaining a balance between the ability to absorb oil and enough durability making it possible to store, transport and feed product pneumatically **[21]**, **[22]**, **[23]**.

In the production of extruded feed products, it is important to provide the conditions of the lowest possible total stress in the process material of the extruder in order to prevent the mechanical destruction of the material. And also, at the same time, create the highest possible density of the processed material to obtain a finished product at the output of the extruder with a denser and more durable structure providing the required quality of

the extruded feed product. This can be achieved by quickly changing the impact parameters on the processed material, depending on its structure **[24]**, **[25]**.

In addition to processing the feed mixture in the extruder, the pellets crumbling index is influenced by the components included in the formulation, and the fineness modulus of grinding the compound feed before extrusion (limit 0.2-1 mm) **[26]**. During the extrusion process, every powdered protein ingredient can be considered as a single phase, requiring a different moisture content and temperature conditions for plasticization into dough during the extrusion process **[27]**. Achieving these conditions for all ingredients in the feed mixture is essential for obtaining new intermolecular binding networks and an acceptable physical quality of the product **[28]**. Studies by Oterhals et al. **[44]** show that the critical moisture level for plasticizing soy protein concentrate was 233-306 g/kg. The same study determined a critical moisture level of 138 g/kg for fishmeal. The moisture content used in this study (235 g/kg) is at the lowest level for plasticizing soy protein concentrate. Pellets of pure mixture of soy protein concentrate had significantly lower hardness and durability compared to pure mixture of fishmeal and may result from incomplete plasticization of the soy protein concentrate mixture at this stage. This is in line with other studies conducted on extruded food foams and fish feeds **[10]**, **[29]**, **[30]**, **[31]**.

It is known that premoistening significantly stabilizes the extrusion process. Therefore, special attention was paid to the preliminary moisture-heat treatment of grain in the studies of the extrusion process. It was found that heat treatment significantly affects the carbohydrate complex of grain. Heating it at high temperatures causes starch degradation, followed by the creation of easily soluble carbohydrates, which has a positive effect on feed digestibility **[32]**, **[33]**, **[34]**. It was established that starch dextrinization and digestibility increased intensively with an increase in grain moisture content up to 18%. For example, in extruded corn, the degree of starch dextrinization and digestibility at this moisture value reached 65% and 140 mg/g, in grain mixtures – 45% and 108 mg/g, in wheat – 2% and 90 mg/g, respectively. The grain moisture content above 18 % does not have a noticeable effect on the growth of grain starch degradation. Starch degradation in it increases at the same value of grain moisture content (18%) with an increase in the heating temperature of the extruded grain **[35]**.

The pressure generated by the screw significantly influences the extrusion process. This indicator affects the mixture treatment temperature and the quality of obtained extrudate. Chevanan N. reports a significant decrease in pressure at the output of the extruder (from 13.5 to 3.7 MPa) with increasing temperature from 90 to 160 °C **[36]**. Similarly, as moisture content of the raw material increased from 15 to 25%, the pressure decreased from 12.8 to 5.4 MPa. To calculate the mixture treatment temperature during extrusion, it is necessary to deduce the mathematical dependencies of pressure changes along the length of the screw. Mathematical dependencies will allow at the designing stage of the extruder to set the design parameters thereof under which the pressure necessary for obtaining high-quality extrudate should be provided **[37]**, **[38]**.

Many studies have found that the pressure of the raw materials processed in the pre-matrix zone of the extruder can be controlled by its supply in the loading area, rate speed of the applied screw, and the diameter of the moulding channel **[39]**. It should be noted that these factors concerning the pressure are interdependent. Therefore, correct results in studies can only be obtained by considering this fact **[40]**, **[41]**.

CONCLUSION

The regime of extrudate production with minimal fish compound feed pellets crumbling was established using a mathematical model. This mode is provided with the following natural factors values - feed mixture moisture before extrusion is 32%, extrusion temperature is 132 °C and pressure is 0.4 MPa. In the selected levels of factors, the calculated value of extrudate pellets crumbling was 1.02%. This is a good achievement since, according to GOST 10385-2014, the crumbling of extruded feed should not exceed 3%. The obtained rational extrusion parameters, established using the mathematical, experimental design method, could serve as a basis for the fish feed production. Physical properties according to its characteristics are directly dependent on extrusion and compound feed formulation conditions. Process variables such as temperature, moisture, screw speed, as well as the type of raw material used in the formulation cause different reactions depending on their interaction, so analysis of physical properties in industrial processes should be carried out regularly, mainly when new ingredients are added or if processing conditions change.

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

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Study of quality and technological parameters for the storage of greens using ionized water

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ABSTRACT

During the storage of agricultural products, in order to maximally preserve quality indicators and to prevent spoilage, the creation of a special temperature and climate regime in the cold storage boxes, harmless to health and environmentally friendly preparations are used, which along with a temperature environment, allow for long-term, high-quality storage of products, as well as complex sanitary-hygienic measures, the main one of which is disinfection and deodorization of the cold storage boxes. The article discusses the studies on the treatment of agricultural products by ionized water and their further storage, in particular, for the greens storage, there was selected their treatment by „alkaline ionized water" while „acidic ionized water" is used for disinfection of the cold storage boxes. The method of obtaining ionized water and the research object were selected, the storage conditions were established, and the quality and technological indicators of the greens were determined. The reduced density of glucose under optimal storage conditions varies from 28 to 19, and the reduced density of water ranges within 445 to 404 after 10 days of storage. The change of technological indicators after 3 and 10 days of storage amounts to: mass losses – 0.70% and 0.85%, water binding capacity - 70% and 61%, and water holding – 65% and 57%.

Keywords: acidic ionized water, alkaline ionized water, reduced density, greens, storage

INTRODUCTION

Storage and transportation of agricultural products and raw materials have always been and are relevant when solving their processing, production and sale issues. For carrying vegetables and herbs, canvas bags are currently being used, in which the storage and transportation of agricultural products and raw materials have always been and are among the relevant areas when solving issues of their processing, production and sale. For carrying vegetables and greens, canvas bags are currently being used, in which the products to be transported and pieces of ice are stacked layer-by-layer. This method cannot ensure the storage and transportation of herbs even for 2-3 days. Besides, it requires a large amount of ice, which may exceed the mass of greens and vegetables to be transported. This method is very inconvenient and costly. In the best case, the vehicle must have a special refrigerator or container with a suitable microclimate, associated with technical difficulties and large expenditures.

The analysis of scientific studies and labor market research revealed relatively low scientific studies on processing agricultural products using safe and environmentally friendly drugs and towards long-term storage. Therefore, the studies included in the project are highly relevant because the methods and regulatory documents (recommendations) for refrigeration storage enterprises that have been designed based on these studies outline a strategic plan for their effective functioning and for high-quality long-term storage and transportation of agricultural products. It should be noted that these methods are continuously updated in order to reflect modern advances in science and technology.

In modern conditions, the ozonation method of cold storage boxes is used to store vegetables and fruits **[1]**. It should be noted that, despite the significant positive results of the ozonation process, according to the literature data, there are various contradictory opinions regarding its use, in particular, measurements of ozone concentration in the air using the iodometric method cannot provide sufficient accurate measurement of its concentration. Also, the high air humidity in the box causes fast ozone depletion, making it necessary to measure ozone concentration frequently. There also seems to be disagreement about the effects of ozone on microbial cells, so it becomes imperative to consider the effect of the ozone-air mixture on bacteria. It should be remembered that ozone belongs to the first category of danger. According to the sanitary standards of the European countries, the acceptable limits for the ozone content in spaces where people work is 0.1 $mg.m^{-3}$, so, before ozonating the cold storage boxes, it is necessary to seal them and shut down the air distribution fans. Before operation, it is necessary to check the correctness of technological and electrical schemes **[2]**, **[3]**.

The existing method of disinfection of the cold storage boxes involves the following stages: a) disconnection of the refrigeration unit from power supply; b) the release of the cold storage box from the product; c) warming up the cold storage box and the release from ice and snow; d) „wet" processing of the internal surfaces of the cold storage box; e) ozonation of the cold storage box with a concentration of 12-14 ∂ẓ ∂$^{-3}$ and the duration of 10-12 hours **[1]**.

We tried to find such means for the sanitary-hygienic treatment of the cold storage boxes and agricultural products, which would not have the mentioned negative properties and could be used by any staff member. We considered ionized water to be such a preparation for the treatment of the cold storage boxes, in particular, „acidic ionized water", because it has several advantages compared to other disinfectants, while for the processing and transportation of agricultural products, in order to maintain their quality indicators optimally, we chose „alkaline ionized water".

„Acidic ionized water" is a transparent liquid free of sediment with an acid reaction and a slightly stinging taste **[4]**. If the concentration is selected correctly, it has antiseptic, anti-allergic, drying, anti-parasitic, and anti-inflammatory properties **[5]**. Its antiseptic effect corresponds to the iodine, diamond greens, hydrogen peroxide and other treatments, but unlike them, it does not cause chemical burning of living cells and their colouration **[6]**. „Alkaline ionized water" slows down metabolic processes in living cells and destroys microflora and microorganisms **[7]**.

„Alkaline ionized water", on the contrary, is a blue liquid in the first minutes of preparation with an intense snowflake-like sediment, which settles entirely in 20-30 minutes. It has an alkaline reaction and a mild taste of baking soda. With its oxidizing properties, „alkaline ionized water" is among the antioxidant drugs, acting as an immunostimulant **[8]**. It is a radioprotective agent, a strong simul and for biological processes and metabolism, which has high extractive and solvent properties, and easily removes slags from the body, including radionuclides. It heals wounds quickly and, accordingly, has antibacterial and antimicrobial properties, but its antiseptic effect is inferior to „acidic ionized water" **[9]**, **[10]**.

Based on the properties mentioned above, we chose „acidic ionized water" as the environmentally friendly and effective means for the sanitary-hygienic processing of the cold storage boxes and containers for carrying fruits and vegetables because its use is not harmful to human health and the environmental safety of natural environment. On the contrary, after taking it, blood pressure is regulated, metabolic processes in living cells slow down, joint pain decreases, and so on. When applied to the skin, it promotes wound healing by destroying microbes **[11]**, including with the flu, preventing food poisoning and restoring cell immunogenesis and the fluid medium pH **[12]**. Accordingly, unskilled staff can perform this work **[13]**.

As for „alkaline ionized water", due to its positive properties, in order to maintain the quality indicators of agricultural products, additional studies are needed on their treatment with this drug, which is a novelty of our research **[14]**. Treating seed material with „alkaline ionized water" is also relevant and needs to be studied to improve its biological and vegetative indicators. At this stage, we studied the storage and transportation technology of agricultural products using ionized water, particularly the issues of maintaining its quality indicators after treating greens with ionized water.

Vegetables and fruits are living organisms, so they are characterized by the exchange of substances with the environment **[15]**. This process consists of two interrelated processes - assimilation and dissimilation. After the collection of products of vegetable origin, important life processes occur, such as: physical processes, processes of biochemical decomposition (reactions) and respiration **[16]**.

Thus, based on the analysis, we can conclude that in order to extend the storage period of the collected fruits and vegetables, it is necessary to stop the respiration process of the collected fruits and vegetables in time, which will lead to a sharp reduction in the process of heat release and, accordingly, to a reduction in mass loss.

The research aims to determine the optimal conditions for maintaining the quality indicators of agricultural products during their storage or transportation using ionized water.

Scientific Hypothesis

Due to its numerous properties, in particular, due to its antibacterial and antimicrobial properties, the „alkaline ionized water" can be used for the treatment of greens under storage conditions in order to maintain its quality indicators because it restores the damaged tissue and prevent the process of moisture evaporation from the inner volume of greens. As a result, it is expected to reduce the loss of mass of greens and maintain its quality for up to ten days. Due to its antiseptic properties, the „acidic ionized water"can be used for the sanitary-hygienic treatment of cold storage boxes, excluding dangers. This will allow us to perform the work by any ordinary low-skilled service personnel, which will have a significant economic effect.

MATERIAL AND METHODOLOGY

Samples

In order to achieve this goal, as the research object, we have selected perishable vegetables – the greens – parsley (*Petroselium Sativum*) and fennel (*Anethum graveolens*) grown in the Imereti region of Georgia, which we bought at the farmers' market in Kutaisi. The results of the research can be used in different environments and on greens grown in different countries because greens cultivated in any country have chemical compositions that are virtually identical. In the market in Kutaisi, we have also bought purified drinking waters „Bakuriani" (Brand: Bakuriani, Borjomi, Georgia; product type: drinking water: Calcium: 25-80 $mg.L^{-1}$; Magnesium: 20 $mg.L^{-1}$; Sodium: 20 $mg.L^{-1}$; Hydrocarbonate: 150-300 $mg.L^{-1}$; Chlorides: 20 $mg.L^{-1}$; Sulfates: $mg.L^{-1}$; Total composition of minerals: 0.20-0.5 $g.L^{-1}$; pH 6.0-8.0; StateSt 85:2019) and „Sno" (Brand: Sno, Kobi-Kazbegi Reg. Georgia; product type: drinking water: calcium: 43-45 $mg.L^{-1}$; Magnesium: 6-10 $mg.L^{-1}$; Sodium: <15 $mg.L^{-1}$; potassium: 0.7-1.2 $mg.L^{-1}$; Hydrocarbonate: 150-200 $mg.L^{-1}$; Chlorides: <15 $mg.L^{-1}$; Sulfates: 15-16 $mg.L^{-1}$; Total composition of minerals: 0.20 $g.L^{-1}$; pH 6.0-8.0; ISO 9001:2015) to obtain ionized water („living" water and „dead" water).

Chemicals

We use it to fill the inner container for storing greens by gas Carbon dioxide (CO2) (Carbon dioxide, LTD Penguin (GE), 99%) and to fill the outer container – by gas Nitrogen (N2) (Gas Nitrogen, LTD Penguin (GE), 98%).

Equipment

We used the „shock" freezing laboratory device ATT05 - Blast chiller/shock freezer 5x GN 1/1, (Thermotechnika Bohemia s.r.o., Brno, Czech Republic) as a disinfection object.

We obtained ionized water using the scheme shown in Figure 1, by electrolysis of drinking water.

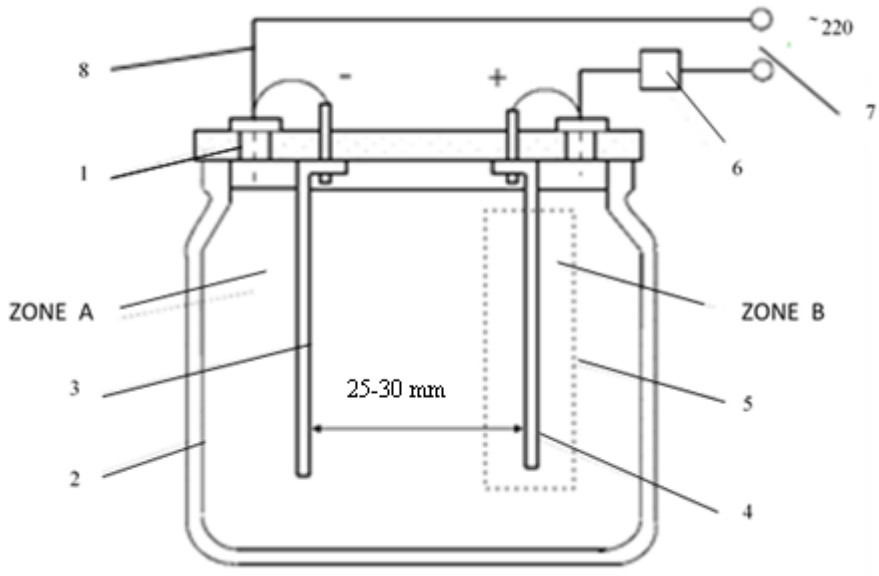

Figure 1 Equipment scheme for obtaining ionized water. Note: 1 – Glass jar cover; 2 – Glass jar; 3 – Electrode (cthode); 4 – Electrode (anode); 5 – Dense canvas bag; 6 – Diode, current rectifier; 7- AC source; 8 – Wire. Zone A – „alkaline ionized water" area; Zone B – „acidic ionized water" area.

Electrodes (cathode and anode 3, 4) made of stainless steel are attached to the plastic jar cover. They are connected to the alternating current source (7) using a current-controlling diode bridge (6). We poured purified

drinking water into a glass jar (2) and closed this jar tightly with a cover. Anode (4) is placed in a dense canvas bag (5), which was previou¬sly inserted into the jar and plays the role of a filter. After turning on, the electrolysis process begins.

As is known, water is to be a poorly dissociable substance, and a constant electric field forces water to dissociate. At the cathode, the dissociated water is negatively charged and „alkaline ionized water" is accumulated nearby, while at the anode, the dissociated water is also charged and „acidic ionized water" is accumulated in the canvas bag. We used the resulting ionized waters, known as „alkaline ionized" and „acidic ionized waters" for experiments.

For the transportation of herbs, we have developed a scheme of the container, the working principle of which is shown in Figure 2, and the general view of the container is shown in Figure 3.

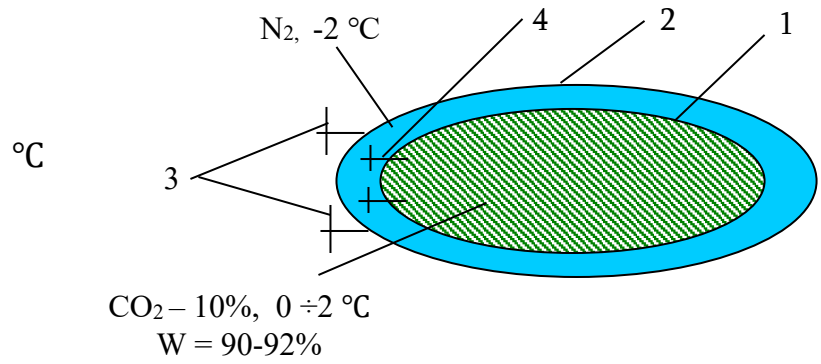

Figure 2 Schematic of a container for transporting greens. Note: 1 – Inner polymer container with herbs; 2 – External polymer container with nitrogen; 3 – Nitrogen inlet-outlet valves; 4 – Carbon dioxide inlet-outlet valves.

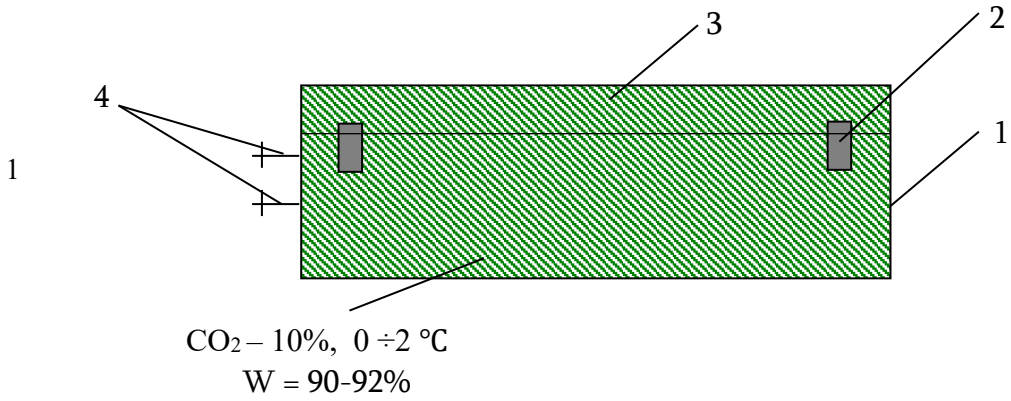

Figure 3 General view of the container. Note: 1 – Polyethylene container with greens, 1.2 x 0.6 x 0.4 m; V = 0.288 m^3; 2 – Locks; 3 – Lid of the container; 4 – Carbon dioxide inlet-outlet valves.

Instruments

Of physicochemical indicators of ionized water, we determined pH **[17]** through the pH Meter of the HM Digital COM-360 models (HM Digital Inc, Rodendo Beach, USA). To determine the mass of the product, we used an electronic digital analytical balance SF-400C model (Toms, Qilin, China) with a weighing accuracy of 0.01 g.

Laboratory Methods

From technological indicators, we determined specific glucose and water content, ionised water's pH value, water-binding capacity, water-holding capacity, and mass losses of greens during storage. We obtained ionized water by the method of electrolysis of drinking water. Of the physicochemical indicators of ionized water, we determined pH value. We determined the specific content of glucose and water by the ratio of the densities in the unit of greens mass relative to the total mass **[18]**. We determined the technological properties of greens as

follows: water-binding capacity **[19]** – by pressing, using the method by Grau-Hamm. Water-holding capacity **[20]** - by the difference between the amounts of released moisture of the newly collected products and the moisture released after storage; mass losses during storage **[21]** were determined by the mass difference between the newly collected and stored products. To determine the effect of „alkaline ionized water" on greens, we soaked freshly harvested herbs in a bath of „alkaline ionized water" and left them in atmospheric conditions for some time.

Description of the Experiment

Sample preparation: We collected samples of freshly harvested herbs and temporarily stored them at 10 °C. Later, we treated them with „alkaline ionized water" and took 100 g of each sample for the experiment.

Number of samples analyzed: We analyzed 3 different 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. The experiment was repeated three times in different days. We bought greens of the same origin and different batches on different days. The average values of the results are given in the article.

Design of the experiment: We washed the newly-harvested greens in a living water bath, delayed them for 1-1.5 min, took it out of the bath, shook them out to remove water drops, delayed them for 10 min and then put them on the table with a layer thickness of 0.10 m for sale, while for long-term storage, we put them on the shelf of the cold storage box with a layer thickness of 30 cm.

Statistical Analysis

To analyze the test parameters of product, a statistical analysis of the obtained data was conducted, and the reliability of the obtained data was assessed by method of mathematical statistics T-test, using the Windows IBM SPSS Statistics version 20.0 program, (IBM, Armonk, New York, USA). We used statistical functions of the average arithmetic value and the average standard error to describe the ordered sample. We chose a reliability value of $p < 0.05$.

RESULTS AND DISCUSSION

During our experiments, we tried to determine the metabolic conditions of herbs, characteristic of them with the environment **[15]**. As mentioned above, after harvesting the products of plant origin, important life processes occur, such as physical processes, processes of biochemical decomposition (reactions) and respiration **[16]**. Of the physical changes, we can distinguish the processes of moisture evaporation, density and color change **[22]**. Evaporation of moisture from vegetables and fruits depends on their type, quality, morphological and chemical composition. The rapid process of moisture evaporation is facilitated by: the large size of cells and intercellular spaces in the products; the insignificant thickness of the upper layer of cells; the relatively weak water-holding capacity of protoplasm due to the small protein content in the colloidal state; large evaporation specific surface area and so on **[23]**. Moisture evaporation accompanies the product from its picking through the storage process until the end of ripening **[14]**. Intensive evaporation of moisture occurs at the beginning of storage, then there is a period of minimal evaporation, and finally, evaporation increases again in connection with over-ripening/wilting **[15]**. Loss of moisture by-products has a great impact on its durability and affects its appearance, so the more moisture the product loses, the faster it is infected and deteriorates **[14]**, **[15]**, **[23]**. Chemical changes in fruits and vegetables are associated with the respiration and ripening processes. The essence of respiration lies in the enzymatic oxidation of complex organic substances (fermentation process) and the release of energy. The external expression of respiration is the absorption of oxygen from the air and the release of CO_2. Along with this, a large amount of heat is released, which was not used by the cell during its vital activity **[16]**. In general, taking into account external factors, plant products produce not only respiration with oxygen (aerobic), but also oxygen-free respiration (anaerobic). Monosaccharides, disaccharides, polysaccharides, fats, organic acids, tannins, glucose and so on are primarily spent on respiration **[15]**, **[16]**, **[22]**. The intensity of respiration depends on many factors, of which one of the main factors (parameters) is temperature. Its reduction drastically reduces the intensity of respiration **[16],** so we should direct the storage process to this point because respiration is the process of decomposition of substances in the products.

The internality of respiration is also affected by the atmosphere's composition, which is considered when storing fruits and vegetables in a regulated environment.

As we mentioned above, respiration is accompanied by the release of heat. The largest amount of heat is released by greens. For example, at 20 °C, greens release about 0.12 $W.kg^{-1}$ of energy per hour, while fruits release – 0.013-0.020 $W.kg^{-1}$ of energy **[22]**.

We performed the following works:

- Analysis of neutral media and temperatures **[17]**, conducted in previous studies that we conducted, during which the quality and structure of the greens were preserved as much as possible **[18]**, **[19]**.
- We developed a process that practically produced ionized water („acidic ionized water" and „alkaline ionized water") in the laboratory and determined its quality indicators **[18]**.
- We selected the research objects and determination of methods of their treatment methods with „acidic ionized water" and „alkaline ionized water" **[18]**.
- We studied the qualitative and technological indicators of herbs treated „alkaline ionized water" water under different storage conditions and determined optimal storage parameters **[18]**, **[24]**, **[25]**.
- We compared the wet treatment method of the cold storage boxes with ionized water **[1]**, **[15]**.

Through early research **[24]**, **[25]**, **[26]**, **[27]**, **[28]**, we examined issues of storage and transportation **[26]** of the herbs in various ambient conditions, in particular: for two different temperatures (0 - + 5 °C) of the gaseous and inert media at different concentrations of carbon dioxide and nitrogen (8%, 10%) **[27]**, for different velocities of conditioned air (0.00145 $m.s^{-1}$, 0.0029 $m.s^{-1}$ and 0.0145 $m.s^{-1}$) **[25]** and finally in combined ambient conditions **[24]**. We selected the values of the specific contents of glucose and water as qualitative parameters of greens, because they are the two major components of the chemical composition of greens, which determine the quality of the products. (The physical process of respiration of herbs is determined by the reaction of glucose decomposition, which ensures its quality). Based on the above experimental studies **[24]**, **[25]**, **[26]**, **[27]**, **[28]**, we determined the optimal parameters for storing and transporting greens in the mentioned ambient conditions **[28]**.

The optimal storage parameters in the collection point:

- Air temperature – 0 °C,
- Air circulation speed – 0.0145 $m.s^{-1}$,
- The thickness of the layer of herbs – 0.3 m,
- Relative humidity – 90-92%,
- Carbon dioxide (CO_2) concentration – 10%.

We have developed the construction of the container for transporting greens (Figure 2, Figure 3), the type of inert gases to be filled in the container, and the optimal storage parameters (results are given below):

Inner container:

- Carbon dioxide (CO_2) – 10%,
- Temperature – 0-2 °C,
- Layer thickness – 0.3 m,
- Relative humidity – 90-92%.

Outer container:

- Nitrogen (N_2) – 90-93%,
- Nitrogen (N_2) entering temperature – (-2 °C).

We have further studied the treatment and storage of the greens using ionized water, particularly „alkaline ionized water".

We developed the process of obtaining ionized water. We determined the quality parameters of „acidic ionized water" and „alkaline ionized water".

We have determined:

- Tap water – pH = 7
- „acidic ionized water" – pH < 7
- „alkaline ionized water" – pH > 7

In the case of living water, we conducted tests in two directions: 1 – in order to maintain the quality of the greens on the shelves of the trading network, we treated a 10 cm-thick layer of the herbs on the shelf with only „alkaline ionized water", and during 10 days we calculated the values of the specific contents of glucose and water therein. For longer-term storage of the herbs, we placed the herbs treated in „alkaline ionized water" in the cold storage box, where the air temperature was 0 °C, the air circulation speed was 0.0145 $m.s^{-1}$, the thickness of the greens layer was 0.3 m, and the relative humidity was 90-92%, and for 10 days, we calculated the values of the specific contents of water. We used a control sample – ordinary greens without any treatment for comparison. We placed 0.3 m thick greens under normal environmental conditions and measured the values of reduced densities of glucose and water (Figure 4, Figure 5).

As can be seen from the figures, when treating greens only with „alkaline ionized water" (Figure 4, Figure 5, curve 3), the values of reduced densities of glucose and water during the first three days and almost the fifth day

(Figure 4, Figure 5 curves 1, 2) coincide or are very close to the values of these qualitative indicators of storage of greens in the combined mode, while during the storage of the greens treated with „alkaline ionized water" in the air-cooled to 0 °C for 10 days, these values (Figure 4, Figure 5, curve 2) are close to the storage quality parameters of the greens under optimal conditions in a combined gaseous medium (Figure 4, Figure 5, curve 1). In this case, we also used a control sample - ordinary greens without any treatment. We placed greens with a layer thickness of 0.3 m under normal environmental conditions and measured the values of reduced densities of glucose and water. The control sample completely lost its quality after the first three days (Figure 4, Figure 5, curve 4).

Figure 4 and Figure 5 illustrate the convergence of the results of curves 1 and 2, indicating that the costs incurred to obtain the descriptive results of curve 1 are inappropriate compared to the costs incurred to obtain the descriptive results of curve 2.

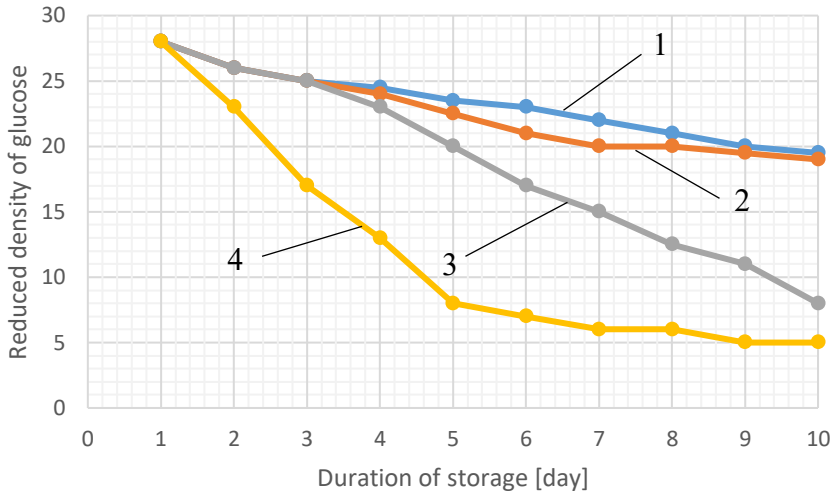

Figure 4 Changes in the reduced density of glucose in the herbs over time under different storage conditions. Note: 1 – combined gaseous medium; 2 – „Alkaline ionized water"-treated and cooled to 0 °C medium; 3 – treated only with „alkaline ionized water"; 4 – Control sample – ordinary greens without any treatment.

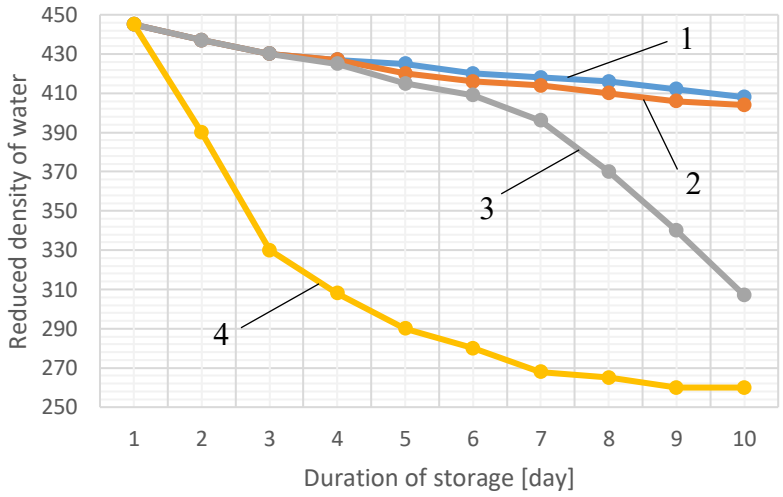

Figure 5 Changes in the reduced density of water in the herbs over time under different storage conditions. Note: 1 – Combined gaseous medium; 2 – „Alkaline ionized water"-treated and cooled to 0 °C medium; 3 – Treated only with „alkaline ionized water"; 4 – Control sample - ordinary greens without any treatment.

Based on the above-described experiments, we have determined the optimal storage parameters for the greens in the collection point (cold storage box).

Treatment with „alkaline ionized water"; air temperature – 0 ºC, air circulation speed – 0.0145 $m.s^{-1}$, The thickness of the layer of herbs – 0.3 m, relative humidity – 90-92%.

Losses in the mass of greens (Table 1), values of moisture binding capacity (Table 2) and values of moisture retention capacity (Table 3) were determined after three, ten and twenty days of storage using different storage methods, as these parameters do not provide decisive values and this accuracy is sufficient to confirm the quality.

Table 1 Greens mass losses when using different methods to store.

	Mass losses in different storage conditions, [%]		
Greens storage duration, [days]	Traditional mode T = 2-5 ºC v = 0.1m.s^{-1} relative humidity up to 90%	Treated only with „alkaline ionized water"; The thickness of the layer of greens – 0.3 m.	Treated with „alkaline ionized water"; Air temperature – 0 °C, Air circulation speed - 0.0145 $m.s^{-1}$, The thickness of the layer of greens – 0.3 m, relative humidity – 90-92%.
Three [days]	1.5	1.55	0.70
Ten [days]	2.1	2.50	0.85
Twenty [days]	4.0	6.80	1.50

Table 2 Greens water-binding capacity when using different methods to store.

	Water-binding capacity, [%] of the total weight		
Greens storage duration, [days]	Traditional mode T = 2-5 °C v = 0.1 $m.s^{-1}$ relative humidity up to 90 %	Treated only with „alkaline ionized water" The thickness of the layer of greens – 0.3 m.	Treated with „alkali- ne ionized water"; Air temperature – 0 °C, Air circulation speed – 0.0145 $m.s^{-1}$, The thickness of the layer of greens – 0.3 m. relative humidity – 90-92%.
Three [days]	60	57	70
Ten [days]	55	39	61
Twenty [days]	40	34	50

Table 3 Greens water-holding capacity when using different methods to store.

	Water-holding capacity, [%] of the total weight		
Greens storage duration, [days]	Traditional mode T = 2-5 °C; v = 0.1 $m.s^{-1}$; relative humidity up to 90%	Treated only with „alkalin ionized water"; The thickness of the layer of greens – 0.3 m.	Treated with „alkali- ne ionized water"; Air temperature – 0 °C, Air circulation speed – 0.0145 $m.s^{-1}$, The thickness of the layer of greens – 0.3 m, relative humidity – 90-92%.
Three [days]	52	51	65
Ten [days]	47	40	57
Twenty [days]	33	30	51

As shown in the tables, the samples treated with „alkaline ionized water" had the best technological parameters; air temperature – 0 °C, air circulation speed – 0.0145 $m.s^{-1}$, the thickness of the greens layer – 0.3 m, relative humidity – 90-92%. It is also worth noting the fact that after three days, the technological parameters of the herbs stored in the cold storage boxes by the traditional method and under conditions of storing the sample treated only with ionized water are close to each other, which confirms the correctness of the above studies (the specific cotents of glucose and water) and once again demonstrates that the greens can be stored cost-effectively for 3-4 days only if they are treated by „alkaline ionized water".

Disinfection of the cold storage boxes is important for preserving quality indicators of greens during storage **[29]**. Here, too, ionized water can be used, only in this case, it is a matter of „acidic ionized water". The bactericidal effects of ozone have been documented on various organisms, including Gram-positive and Gram-negative bacteria and spores and vegetative cells. In this review, ozone's chemical and physical properties, its generation, and antimicrobial power with two suggested mechanisms were explained and many advantages of ozone use in the food industry **[30]**. The multifunctionality effects of ozone in food processing, in both gaseous and aqueous form, have promoted its use in the food industries to meet the increased consumer preference for a healthy diet and ready-to-eat products. However, ozone may present undesirable effects on physicochemical characteristics on certain food products at high concentrations. The combined uses of ozone and other techniques (hurdle technology) have shown a promotive future in food processing. It can be concluded that applying ozone technology to food requires increased research; specifically, treatment conditions such as concentration and humidity for food and surface decontamination **[31]**.

Ozone avoids and controls biological growth on vegetables, keeping their attractive appearance and sensorial qualities, assuring nutritional characteristics' retention and maintaining and increasing the shelf-life. However, if ozone is improperly used, it causes deleterious effects on products, such as losses in their sensory quality. For effective and safe use of ozone, specific treatment conditions should be determined for all kinds of vegetables **[32]**.

The review discusses research related to pathogen inactivation and DBP formation by chlorine and ozone during the washing of produce, meat and seafood. In particular, the research highlights the difficulty of inactivating pathogens on food but the efficacy of these disinfectants for controlling pathogen cross-contamination through the wash water. This review highlights the need for research on the initial transformation products of disinfectant reactions with biomolecules since these products may present a risk for consumer exposure by remaining within the food **[33]**.

The main focus of this review is on the effects of ozone on the fresh produce quality, defined by the maintenance of texture, visual quality, taste and aroma, and nutritional content. Furthermore, ozone has been found to be efficient in reducing pesticide residues from produce. The treatments that can reduce microbial contamination of the product without adversely hurting its visual, textural and nutritional quality can be recommended and subsequently incorporated into the supply chain. A good understanding of all the benefits and limitations related to the use of ozone is needed, and relevant information has been reviewed in this paper **[34]**.

The use of ozone has been identified as a feasible solution to reduce microorganisms present in food, in this way extending the shelf-life of fresh produce. Several factors that may affect the efficiency of ozone treatment have been identified, e.g. microbial populations, ozone concentration and time of exposure, type of produce, temperature, relative humidity and packaging material **[35]**.

The food industry is interested considerably in using ozone to enhance the shelf-life and safety of food products and in exploring new sanitiser applications. This interest was recently accompanied by a US governmental approval of ozone for the safe use, in gaseous and aqueous phases, as an antimicrobial agent on food, including meat and poultry. Ozone has a strong microbicidal action against bacteria, fungi, parasites and viruses when these microorganisms are present in low ozone-demand media **[36]**.

The effect of ozone on post-harvest garlic bulbs was evaluated. The data collected showed that ozone treatment did not affect the aromatic profile of garlic. A significant detrimental effect of ozone treatment on garlic decay was observed. Our results encourage using gaseous ozone treatment to contain garlic fungal decay during its storage **[37]**.

The effect of ozone treatment on total phenol, flavonoid, and vitamin C content of fresh-cut honey pineapple, banana „pisang mas", and guava was investigated. The fresh-cut fruits were exposed to ozone at a flow rate of 8 ±0.2 $mL.s^{-1}$ for 0, 10, 20, and 30 min. Ozone treatment significantly decreased the vitamin C content of all three fruits. The study shows promising results for enhancing antioxidant capacity of some fresh fruits by ozone treatment, although the positive effect is compromised by a reduction in vitamin C content **[38]**.

Rinsing or dipping vegetables in water saturated with ozone could be an alternative environmentally friendly and safer process since no harmful by-products or residues are formed. Immersing vegetables in water pre-saturated with ozone (0.5 $mg.L^{-1}$) did not make any difference because the total microbial count decreased by

approximately 0.5 log simultaneously. Sanitation treatments were most effective when vegetables were dipped in continuously ozonated (0.5 mg.L^{-1}) water, leading to a decrease of 2 log of microbial load in the first 15 min and 3.5 log after 30 min of exposure **[39]**.

Employees of the State Technical University of Georgia **[1]** investigated that ozonation of the cold storage boxes for 12 hours with a 10%-concentration of ozone and every 4-hour break ensures a reduction in the bactericidal composition in the air and on the walls of the cold storage boxes by 93-97%. According to our research, it was revealed that during the wet treatment of the walls, ceiling and floor of the cold storage boxes by „acidic ionized water", 80% of bacteria are killed on average, the same effect according to findings of the research is 86.5% **[1]**, which is acceptable from an economic point of view (Table 4).

Table 4 Indicators of sanitary-hygienic treatment of the cold storage boxes.

Type of sanitary treatment	Indicators		
	Duration of operation [hr]	Duration of operation, [week]	Reduction of bactericidal composition [%]
Ozonizing	12	1	86.5
„Acidic ionized water" treatment	-	1	80.0

CONCLUSION

Experimental studies show that the treatment of greens with only „alkaline ionized water" is enough to preserve their quality indicators and appearance on the shelves for 3-5 days at the lowest cost, while as for longer-term storage, at this time, less costs are achieved to preserve better quality indicators through the use of a cooled air medium along with „alkaline ionized water" treatment (Treated with „alkaline ionized water"; Air temperature – 0 °C, Air circulation speed – 0.0145 m.s^{-1}, The thickness of the layer of greens - 0.3 m. relative humidity – 90-92%). Sanitary treatment of the cold storage boxes for the greens should be carried out with „acidic ionized water" at weekly intervals, which, with less energy consumption, produces about the same effect (Reduction of bactericidal composition – 80%) as ozone generators. Experimental studies show that the treatment of greens with only „alkaline ionized water" is enough to preserve their quality indicators and appearance on the shelves for three days at the lowest cost, while as for longer-term storage, at this time, less costs are achieved to preserve better quality indicators through the use of a cooled air medium along with „alkaline ionized water" treatment. Sanitary treatment of the cold storage boxes for the greens should be carried out with „acidic ionized water" at weekly intervals, which produces about the same effect as ozone generators but with less energy consumption. In our hypothesis, we have expressed the opinion that „alkaline ionized water" and „acidic ionized water" can be used to store agricultural products without taking precautions, and they give almost the same results as ozone treatment which was confirmed by the results of our research. The obtained results agree entirely with our opinions in the hypothesis.

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

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Young leaders as implementers of neuroscience innovations in family food businesses

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ABSTRACT

Neuroscience and its implementation in work with human resources is an important part of managerial work. It helps to understand people and the processes of motivation, learning, and adaptation to new situations and reactions to changes in human resource management. Implementing new trends in work with human resources is also very important for ensuring the sustainability of family businesses as an irreplaceable part of national economies. Their implementation is helped by the fact that many of the family food businesses are going through the process of generational change, and family business leadership is being taken over by a generation of young managers - leaders. The contribution aimed to discover how the younger generation of managers perceives neuroscience and where they see the opportunity for its application in human resources management. Our research focused on the younger generation of managers -leaders in Slovakia's small and medium-sized food family businesses. A structured controlled interview was used for qualitative data collection, which was statistically evaluated using the Text mining method. As we discovered, some new neuroscience-based practices are already gradually being applied. By focusing our research also on a different view of the implementation of neuroscience into managerial work by gender, the conclusion is that female, young managers focused on using neuroscience to improve the working environment and in the area of human leadership. Young men as managers, were more focused on the growth of employees who already work in the company to be even more efficient and better manage the learning process. It is a very positive finding that young managers of small and medium-sized food enterprises in Slovakia have already begun actively introducing innovative methods of working with human resources using neuroscience knowledge.

Keywords: neuroscience, human resources, young manager, SME

INTRODUCTION

Despite the frequent belief that managers make decisions based on facts and logical arguments - their decisions are based on their unconscious feelings. Brain aims to look for patterns and automatize human decisions and actions **[1]**. Due to new trends in direction and management, defining the present quality manager's profile is necessary. One of the common problems in managerial work is the so-called student syndrome. Student syndrome is an unexpected and surprising delay in performing managerial tasks. It is based on a typical student problem – postponing uncomfortable learning and last-minute work before the exam.

On the other hand, it emphasizes vigilance and attention to mindfulness, which is based on not dealing with details, but maintaining insight and perception of ourselves and the situations in which we find ourselves **[2]**. Mindfulness is an approach carried out by meditation, which aims to appropriately draw attention to the status quo. Neuromarketing research recommends practicing mindfulness because it affects brain structures and functions. Mindfulness practice focuses on brain networks related to emotion regulation, self-recognition and attention **[3]**. Dr. Rock created the SCARF model to improve relationships between managers and employees.

SCARF is an acronym for the five social qualities with which this model works: S – status, C – certainty, A – autonomy, R – relatedness, F – fairness **[4]**. This model is based on research that suggests that the brain always tries to minimize threats and maximize benefits. It is based on brain activity, uses cooperation and the influence of others. It outlines several domains of human experience, such as condition, certainty, autonomy, connection or justice, around which our perception activates different brain regions. This determines how individuals respond to rewards or threat **[5]**. Using neuroscience, the manager can learn more about creating change, controlling learning, controlling learning, and understanding moral reasoning **[6]**. The techniques used in neuroscience help to understand employees. This makes employee satisfaction and performance identifiable **[7]**. However, it is important to remember that even in neuroscience, there is no one size fits all approach. Different cultures, communities, and different demographic groups of employees will respond differently to the same stimuli **[8]**. Research has shown that there is a significant difference between the academic definition of work assignment and its practical application in enterprises **[9]**. Businesses should carefully handle so-called mental motivation, as this can significantly impact employees' internal motivation **[10]**.

In our research, we focused on how young managers (under the age of 30) of small and medium-sized food family businesses in Slovakia perceive innovative approaches in human resources management with the use of neuroscience. We were also interested in the difference in perception of these approaches based on gender. We paid attention to the perception of neuroscience by a new generation of managers who were personally and professionally brought up in the market economy and are not significantly influenced by Slovakia's former centrally planned policy. Based on several published sources, we assume that they are the ones who can correctly implement new methods from human resources management using neuroscience knowledge.

While business research and scientific research adhere to high ethical standards, using neuroscientific methods involving the human subject raises various ethical issues that business scientists need to be used to **[11]**. Neuroscience is a science that deals with examining the nervous system **[2]**. To understand the functioning of nerve cells and brain, it uses approaches of anatomy, molecular biology, mathematical modelling and psychology **[12]**, **[13]**, **[14]**. It also examines nerve tissue relationships and individual behavior **[15]**.

Human personality can perceive their environment and absorb a lot of information. Scientists have discovered more about neuroscience and brain functioning in the past 10 years than they have since the beginning of human existence. People needed less information in the past, but as time progresses, we need more information today **[16]**. There has been tremendous progress in understanding basic brain processes in management, marketing and consumer behaviour **[17]**. One of the most important neuroscience knowledge areas is that the brain constantly changes in use, depending on the learning processes **[18]**. The brain is programmed to survive. He responds to social threats in the same way as physical threats – he tries to avoid it. When assessing whether a situation is dangerous, the brain trusts past experiences. In response to social threats, the brain creates so-called avoiding emotions – e.g. fear, anxiety, anger and shame. Thanks to such emotions, it prevents the threat. Possible responses are defense, attack, and departure. Conversely, when the brain evaluates the situation as "safe", it generates so-called approach emotions such as trust, enthusiasm, joy and love. These emotions are a prerequisite for successful project implementation, enabling cooperation, creative problem solving and rational decision-making **[19]**. So far, economic theory has assumed that a person is happy when he gets what he wants, the more, the better. However, neuroscience has shown that the "emotional brain" quickly gets used to new stimuli and calls for new and new stimuli. This explains why happiness levels in developed countries do not rise proportionally with rising living standards. Not the level, but the change is important **[20]**. The human brain has two ways of thinking: fast and slow **[21]**. Fast thinking is an automatic fast operation with little effort and no sense of voluntary control.
On the other hand, slow thinking uses much energy – which means that the brain does not want to use it unless necessary, but it is very good when solving complex problems **[22]**. Slow and fast thinking may not always work independently of each other. Indeed, quick thinking constantly creates suggestions (e.g. impressions, intuition, intentions and feelings) that slow thinking considers and confirms. Slow thinking is part of controlled processes be-cause (e.g. text learning, financial market analysis) it requires awareness, concentration, effort and deliberate action **[20]**. According to Dr. Davidovich, applying basic neuroscience knowledge to business is a breakthrough in improving the organization's performance. It helps individuals better understand what is happening in their brains and provides approaches that can help deal more effectively with people and create sustainable change at the level of individuals and the whole organization **[23]**. By applying neuroscience to management, neuromanagement is created. This can be described as the art of human resources management to achieve better organizational performance **[24]**, **[25]**. It contributes to better relations between managers, employees, teams, partners, idea-making and their implementation into business practice **[26]**. Neuroscientific studies show three of the most important principles: 1. understanding the learning and re-learning process and how to manage it successfully; 2. redefining the resistance, how to identify its different types and how to overcome it effectively; 3. Facilitating the adoption of changes **[27]**.

Leading people: There is a relationship between the level of intelligence and the type of guidance **[28]**. Self-awareness and self-control are fundamental requirements for sustainable organisational leadership **[29]**. Leadership is becoming more and more important in organizations and team management. Neuroscience can provide many insights into how leaders can be more efficacious **[30]**. The neuroscience perspective also has some important implications on the links between leaders' emotional intelligence and followers' results through the innovation process **[31]**. Neuroleadership is as an application of neuroscience knowledge to leadership and neuroscience can be used to educate new leaders **[32]**. Neuroscience studies could advance research on entrepreneurial leadership because it explores the neurophysiological substrates of mental processes and corresponding behaviors **[33]**. The Entrepreneurial Leadership represents one of the most important fields of innovation management that has become increasingly multifaceted and interdisciplinary with its evolution **[34]**. This concept is a crucial aspect in all the organizations that deal with innovation management strategies as the study of neuroscience can also support the study of the emotions and cognitions of leaders. Zwaan et al. **[9]** found that neuro leadership impacts employees' workload and is one way of increasing the workload. Neuroleadership improves work engagement through psychological, neurobiological, sociological and organizational dimensions **[9]**.

Neuroscience in employee education: Self-awareness is needed in the development and education of employees, i.e. a realistic assessment of employees' abilities because development and training respecting the authenticity and characteristics of the person can produce significant results if the principles of neuroscience are naturally applied **[35]**. The neuroscience-based learning approach attempts to create new brain patterns of subconsciousness, which require time to be built and strengthened **[36]**. Key teaching practices supported by neuroscience:

- An experience-learning environment that allows people to get into a "flow" of deep engagement and creativity. According to neuroscience studies, learning is most effective when an individual is in positive emotional state **[36]**.
- Active learning – a cognitive process that involves three parts cerebral neo-cortex (evaluation and analysis), hippocampus (consolidation of information from short-term memory to long-term memory) and amygdala (helps the brain to identify the main points of new inputs) **[36]**. In active learning, teachers act only as knowledge-gathering intermediaries, not as their one-way providers **[37]**, **[38]**.

Economic and educational activities indicate that the human brain is actively involved in the economy and educational processes and controls human behavior. Modern neuroscience helps to understand what is happening in the human brain and how internal mental processes can affect human skills and behavior **[39]**, **[40]**. The brain controls our thinking, learning and memory **[41]**.

New concepts are emerging – neuroeducation and the so-called "brain learning theory". Neuroscience is a new discipline that proposes to take over knowledge from neuro-scientistic techniques to improve learning processes and thus optimize learning **[40]**. The essence of "brain learning theory" lies in understanding why and how learning takes place and how teaching and learning should be as successful as possible. The results led to the idea that learning should be placed in the brain, or the activity of learning attributed to the brain **[42]**. Classical learning usually does not lead to better organizational performance, because people will soon return to the old ways in which they did things. Learning based on the principles of neuroscience is based on stimulation of dopamine centers and active participation of participants **[43]**. Dopamine is a so-called neurotransmitter – generating positive emotions, self-esteem and energy **[20]**, it manages positive strengthening and motivation **[44]**. According to experts, roughly 20 days a month is needed to turn newly learned behavior into a routine (habit) and the brain has accepted behavior as its own **[43]**. Today, the latest neuroscience research and behavioral approaches are increasingly coming to the awareness of company management through trainings, workshops and presentations. They can directly apply project management tools to effective project management and complex tasks from a unique neuroscience perspective. Above all, it is about eliminating stress, more effective solutions to operational tasks and problems, or better adaptability to change. New possibilities and inspiration include an out-of-box view, initiative and activity, or a better working environment – a better atmosphere and joy of work **[1]**. With learning strategies ranked from least to most effective by cognitive neuroscience: images to text; keyword–mnemonics; summary; word highlighting; re-reading; self-explanation; text listening; exercises; practice; practical testing **[45]**. Most individuals and organizations worldwide are trying to get the most out of their educational programs. But they must change some initial understandings about it to increase learning effectiveness. Research shows that to adopt information with a long-term effect successfully; it is much more beneficial to spread education into several study blocks. The latest neuroscience findings have been summarized in a four-phase model that ensures that learned things are not forgotten – ages: attention, generation, emotion, sleeping **[46]**, **[47]**.

Stress in the workplace: Stress, health or arousal are often studied to understand various other phenomena. Most studies use measures of the autonomic nervous system or biological indicators of various physiological

subsystems such as cardiovascular, metabolic or immunological **[48]**. If the brain gets into stressful situations, logic is immediately switched off and passes into subconscious and instinctive functioning **[16]**. The mindfulness method has also shown a modification of some physiological indicators associated with stress response, such as cortisol release and change in heart rate **[49]**. Mindfulness can demonstrably reduce employee stress levels and increase personal empathy **[3]**. A positive approach brings up to 23% higher energy levels under stress, 31% higher productivity levels, 37% higher sales levels, 40% more likelihood of promotion and 3x higher levels of creativity **[35]**. When you feel physically or socially threatened, cortisol is released, which affects your creativity and productivity. We literally can't think **[50]**. Symptoms of stress in the workplace include depression, fear (panic attacks), difficulty sleeping, loss of interest and motivation, forgetfulness and poor concentration. In 2015, the work-life balance ratio for employees in Slovakia was worse than the sample from all over the EU **[49]**. According to Aboiron, leaders must cultivate a healthy corporate culture by stimulating beneficial neurochemicals in the workplace. Although this needs to be done individually, the collective stimulation of Oxytocin and Serotonin in each employee collectively cultivates a good and healthy corporate culture in these conditions, employees are not under much stress and know their leaders have a higher acceptance of failure or mistakes. With a proper mindset and attitude, an employee can carry out tasks more effectively **[30]**.

Scientific Hypothesis

Young managers of family food businesses perceive neuroscience in HRM positively and are also ready to implement it in business practice. The topics that young managers of family food businesses deal with in the field of neuroscience in HRM are different from the topics that female managers deal with.

MATERIAL AND METHODOLOGY

Samples

The contribution aimed to evaluate the approach of the young generation of family food business managers - leaders in Slovakia- to the potential use of neuroscience in working with human resources. The research was focused on the younger generation managers of small and medium-sized family food businesses in Slovakia. The reason was that many of the family food businesses are going through the process of generational change and a generation of young managers is taking over family business leadership to ensure sustainability. Implementing new trends in work with human resources should be helpful in this difficult process.

Description of the Experiment

Sample preparation: The stated condition was: that the respondent is a manager in a food family business in Slovakia. As a criterion for classing a business as a family business, we were based on the definition of Poza and Daugherty **[51]**, who claim that a family business is a business in which family members have ownership. The survey involved 134 respondents (80.5% female managers and 19.5% male managers). According to Wilson et al. **[52]**, there are more managers in family businesses than in nonfamily businesses, which may cause them to be more represented in research. Our results do not correspond to Furik, who claims that there are 35% of female managers in the world, but our sample corresponds to the assertion of Meroño-Cerdán and López-Nicolás **[53]** that women work more often in managerial positions in small family businesses.

Number of samples analyzed: The structure of the respondents was:

- Total number of respondents: 134.
- Man: 26.
- Woman: 108.

Education: Managers' education was irrelevant to our research.

Questionnaire preparation: Number of questions analyzed: 2 identification questions, 2 closed scale questions, 1 open question.

- Questions: 1. Respondent's gender (male, female), 2. Were you born between 1985 and 2000? (Yes No), 3. I perceive neuroscience in HRM positively. (Agree, Disagree, Don’t know), 4. I am ready to implement neuroscience in my business practice. (Agree, Disagree, Don’t know), 5. In which topics of HRM do you see the application of neuroscience? (Open question).
- Conducting a questionnaire survey: The survey was conducted between October 2020 and December 2021. Since there is no database of family businesses in Slovakia, we searched for businesses in the finstat database and based on the matching of surnames in the administrative authorities in the commercial register, we tracked down their contact details on their websites. To cooperate on research, we identified and then contacted 186 companies electronically. Of them, 134 agreed to cooperate, whose answers we further processed.

Number of answers: 134

Creating a dataset: The respondents' answers in electronic form were translated into English as an unstructured text. Subsequently, the responses were encoded in MS Excel according to the requirements of the Text Mining method **[54]**.

Processing the answers: We performed data extraction through Statistica and Data Mining. For a higher denunciation value, we have chosen a TF-IDF function that revealed the importance of individual expressions in respondents' responses **[55]**. We extracted the terms into concepts and decided to work with the first two concepts with the highest termination value **[56]**. Based on singular values, we displayed extracted concepts in a scatter chart **[57]** where we focused on extremes. Individual groups were formed based on the gender of the respondents and the terms most frequently used by them (focused on extremes). Groups 1 and 2 determine the extremes in the responses of young female managers, and groups 3 and 4 determine the extremes in the responses of young male managers.

Number of repeated analyses: 0.

Number of experiment replication: 0.

Design of the experiment: The survey was conducted between October 2020 and December 2021. We chose short questionnaire for collecting quantitative and qualitative data. Structured interview was chosen as a qualitative data collection method, which were statistically evaluated using the Text mining method. Qualitative research **[58]** was devoted to analyze the issue deeply. At the beginning of the research, we gave a lecture on "Neuroscience in the work of the manager", which was provided to all respondents in video form throughout the research. It lasted about 30 minutes and contained basic information and options for using neuroscience in HRM. The lecture was sent to respondents along with an online questionnaire, where we focused only on the gender of the respondent. The questionnaire was started with the question, “where do they see the use of neuroscience in human resources in their family business”. They should have prepared their answers based on the problems they often face. We evaluated the responses using the Text Mining method.

Statistical Analysis

Based on data from the questionnaire, analysis of dependence was performed by $\chi 2$ test. The p-value for the $\chi 2$ test was compared with alpha = 0.05. If the p-value exceeds alpha, there is no statistically significant dependence between the categorical variables. If the p-value is lower than alpha, a dependence exists, and its tightness was verified by Cramer's V – coefficient, which takes values from the interval <0.1>. Values between 0 and 0.3 indicate weak dependence, values between 0.3 and 0.8 indicate moderate dependence, and values between 0.8 and 1 are classified as strong dependence between the studied traits **[59]**.

RESULTS AND DISCUSSION

Human beings are embedded in various organizations. Organizational cultures can promote prosocial behaviors such as trustworthiness or antisocial behaviors **[60]**. Success in any organization may depend on changing the behavior of stakeholders to meet new challenges. But humans have brains designed to register change as a threat; thus, they often cling to old habits and mindsets. Recent breakthroughs in brain research provide a fresh alternative to both behavioral and humanistic approaches to organizational development. Neuroscience principles are now transforming leadership in business enterprises, and these concepts have relevance to any organization or program **[61]**.

Despite increased attention on neuroscience discoveries and its methodologies in the social sciences, there need more research among HRD scholars incorporating neuroscience approaches. Relatedly, HRD practitioners and scholars often view reflection as critical for developing human resources and leaders **[62]**. The special province of coaching a leader using applied neuroscience understands that perception controls create the neurochemistry that controls behavior; the leader can quite easily understand how his or her brain functions. Concepts of leadership have typically been founded on masculine models. Pragmatic business leaders generally love seeing knowledge turned into added value. Leaders thereby make sense of their own behavior and can direct attention to what is significant in the observable decision-making of others. Trust is the interrelationship mechanism by which others’ energies will flow in the direction the leader wishes them to flow **[63]**.

According to Berčík **[64]**, neuroscience is gradually coming into the attention of Slovak companies. Also, Smerek **[65]** states that new approaches to human resources management (including neuroscience) are gradually being introduced in Slovak companies. These draw attention to the perception of human resources as their most important input. One of the **[66]** research findings is that people can consciously change and even overcome their instinctive responses to those that will be more effective for them. When things around us change, it's an opportunity to create new habits that will help us to respond better and manage new environment **[67]**. According to Teacu et al. **[26]**, new approaches with neuroscience knowledge can bring creative changes and innovative ideas that can turn economic mechanisms into more efficient ones. Also, Bilevičienė et al. **[68]** wrote that we can

make the business more efficient by applying innovation management principles and managing human resource management changes. For example, the neuroscience research of Hills **[69]** explains how we can better implement talent strategy and why adopting certain policies will get the business better results. By applying the ideas in the article, talent leaders can be more successful in executing their talent strategy and meeting business goals.

Grzywacz and Smith **[70]** wrote suggestions for promising research areas wherein family scientists and social neuroscientists could build collaborative research to address gaps in the work–family literature.

On the base of the aim of contribution "to evaluate the approach of young generation of family food business managers in Slovakia to the potential use of neuroscience in working with human resources" a questionnaire was applied which started by the question "where the young managers in small and medium-sized family food businesses in Slovakia see the use of neuroscience in human resources in their family business".

In the interest of exploring and determining perspectives of young family business managers within the use of neuroscience by leading people, we investigated their perceptions of neuroscience in the field, whether they are already implementing any of the neuroscience methods in their businesses, and where they see the greatest potential for the application of neuroscience in their businesses in the future. Organizational cognitive neuroscience draws together all the fields of business and management, including their operation in the wider social world. It does this to integrate understanding about human behaviour in organizations and, consequently, to more fully understand social behavior **[71]**.

Based on the respondents' answers, we will offer suggestions and recommendations for practice.

Table 1 presents the most important genderless terms expressed by individual managers in their answers. In the "Expression column", we see a summary of the most frequently used expressions in the answers of all our respondents. The "Importance column" shows the results of the TF-IDF method and the individual values represent the weight of the importance of the words used by the managers in the structured interviews. On this basis, we can state that managers mostly associate neuroscience in their businesses with people, employees and a positive future for their business. These allegations show that managers see neuroscience as improving the business's functioning concerning their employees. Importantly, neuroscience is perceived positively in managers' awareness, not as an unnecessary burden. By building solutions informed by the science of how the brain works, author Rock believes organizations can bridge the intention behaviour gap and create lasting behaviour change **[72]**.

Table 1 Important terms in managers' statements.

Expression	Importance
people	116.02
staff	105.08
future	104.08
enterprise	103.47
positive	93.55

Following figure 1 presents the terms that young family business managers used most often in their statements about neuroscience (without taking gender into account). We show only the result of TF-IDF method.

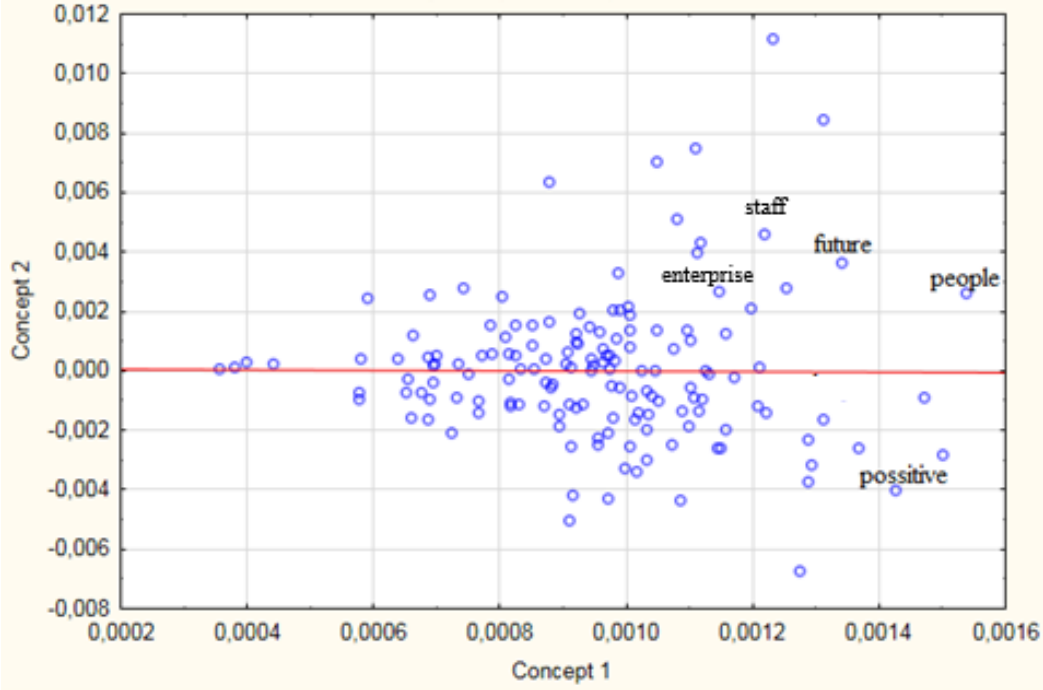

Figure 1 The most often used terms by young family business managers.

Based on the distribution of individual expressions, we could classify them into 4 groups with various areas in which respondents perceived the application of neuroscience in the company. Group 1 was mostly devoted to the place of work and the leadership of the people. Respondent's answers in Group 2 were focused on people and developing their capabilities that can be used to increase the efficiency of the business. Butler et al also wrote about greater attention to bear on the place of mental processes in explaining human behaviour and effectiveness **[73]**.

We assessed these two duties based on the statements of young women working in managerial positions. Group 3 and Group 4 displays expressions of male managers with focus on education and opportunities to improve the functioning of the business. Neuroscience also studies how education changes the brain, and what the mechanisms are that lead to behavioural change (or the absences thereof) through education **[74]**.

We can see that all the statements are positive, which we rate very positively.

Implementing the neuroscience paradigm for public organizations is expected to enhance inspiration and innovation for the employees, transforming the organization into a best working place for employees that witness a feeling of integration and accomplishment in realising organizational success.

In the next part of our research, we focused on finding out if there are some differences in young family business managers´ views on neuroscience in human resources management by leading people –on the base of gender. From the managers' interview responses and the terms, they used most frequently concerning neuroscience in human resources. We found out that women were focused on the working environment (color, feelings, and perception) and men were focused on working with employees (development and education of employees). Modern organizations could seek actions that stimulate the reward and pleasure centers of the brain while making the person experience feelings of acceptance and recognition. In the same direction, neuroscience is decoding the societal engagement and exploring the human behavior in working places supporting the overall struggle for maximization of performance **[75]**.

Table 2 shows the first 6 terms that each gender assigned the most importance. In the “Expression columns”, there is a summary of the most frequently used expressions in our respondents' answers, females and males separately. The “Importance column” shows the results of the TF-IDF method and the individual values represent the weight of the importance of the words used by the managers in the structured interviews. Women placed the greatest importance on people in the workplace – employees. They focused on working with human resources, improving the working atmosphere and an emotional approach. Workplace happiness is one of an organisation's most valued and pursued goals. Researchers, scholars and practitioners have acknowledged the benefits that a happy workforce brings to the table and its enormous contributions to business outcomes. Learning from neuroscience teaches us that happiness is a state of mind resulting from the complex interplay of hormones and neurotransmitters and that the release of neurochemicals and neurotransmitters has a role to play in making us happy **[76]**.

In contrast, men paid attention to increasing performance through creativity, development and education of employees. According to Abraham, several neuroscientific approaches have been adopted to relate creativity to brain function **[77]**.

The most interesting fact is that no manager has dealt with the use of neuroscience in the selection of employees.

Table 2 Comparison of terms used by managers concerning neuroscience.

Females		Males	
Expression	**Importance**	**Expression**	**Importance**
people	97.75	**Creativity**	22.84
enterprise	93.47	**People**	21.94
staff	88.82	**Development**	21.69
resources	87.66	**Use**	20.90
improvement	83.05	**Resources**	20.83
environment	76.09	**Education**	18.95

Perception of neuroscience by managers – female: The involvement of women in a family firm´s board is an important theme to explore **[78]**.

In our research, we found out that women perceive neuroscience in two areas. Both areas are oriented towards the internal employees of the company. They focus on working with human resources and applying neuroscience knowledge to create a better working environment, so that employees have better working conditions. The results of Swedish study show that workplace distress is a major problem for neuroscience, and respondents found it difficult to influence their working conditions **[79]**.

Another study considers how scientifically valid neuroscience could boost the functioning of organizations, improve working conditions for employees, and help individuals achieve goals **[80]**.

As part of the group 1 interpretation, we can conclude that women have placed particular emphasis on the place of work and leadership. Changing and improving the working environment positively affects employees who often spend long hours in the office. This is also stated in Berkman's **[62]** research: "when things are changing around us, it is an opportunity to create new habits that will help us better respond and manage the new environment". This finding is also matched by Teacu's et al. **[26]** research. As part of the interpretation of Group 2, women also focus on people and developing their skills so that the business operates better. People are the key factor that can change or be adapted to the business conditions in a certain way. Placing the right leadership, can foster innovation or competitive advantage. It is also stated in Freedman's **[66]** research about the conscious change of human behavior to one that is more beneficial for the individual and his surroundings. The biggest problem in the current situation was seen in the uniform management style of all employees.

Family businesses are a specific form of business, so family members are often at the top **[81]**. This also affects the way of leading people and the business working environment. Family members may not have always the necessary competence to work with human resources. Since women are more often in management positions in family businesses than in non-family enterprises **[82]**, the management of employees in family businesses is characterized by emotional reflection by leaders **[83]**. According to research, family businesses use a transformative management style **[84]**. Studies show that family businesses have leadership styles that can hamper a business, e.g. sibling leadership teams **[85]**.

Perception of neuroscience by managers – male: Young men tend to focus on using neuroscience to grow employees, which the business already has. In the overall approach, they see its application in improving human capital in the company. In the interpretation of the Group 3 results, we see it focused mainly on using neuroscience in employee education and stress management. Managers see the possibility of neuroscience application in their employees´ education, they look for more effective ways to manage the issues. This is also seen by Gocen **[32]**. In his research he points to the use of neuroscience in the education of good leaders. A very positive finding is that managers are also interested in dealing with their employees' stress level. Slovak employees´ stress is higher than in other EU countries **[49]**. Group 4's results focus mainly on the ways of improving the operation of the business. As Zhang **[10]** states, business managers must handle their employees' motivation carefully, so the result is not counterproductive to anyone. Therefore, very positive finding is that they want to use the knowledge of neuroscience to improve the company's functioning. Neuroscientific evidence has the potential to uncover new insights and refine the conceptual ideas of intrinsic motivation by articulating the granular processes of motivation that behavioral methods alone cannot afford **[86]**.

In family businesses, stress affects business and relationships. This all affects the blending of family and work life **[87]**, which is the young men result in our research. Although Sirotková et al. **[88]** claim that there is a more pleasant way of cooperation in family firms, there is a lack of strategic planning. This also affects management stress. Education is important for family businesses from the point of view of introducing innovations into business. This topic is most often associated with succession **[89]**. However, whether of successors or ordinary employees, training plays an important role in the company's development, and neuroscience offers new perspectives on training that these companies can use.

Successor attributes related to emotional intelligence, such as establishing trust by demonstrating integrity, a genuine commitment to the business, and commanding respect from employees, are highly desired in family organizations **[90]**. Next-generation leaders, who have ambitions of entering the family business, should build deep, profound, and symbiotic relationships that grant them the essential background and the necessary tools to develop into effective leaders **[91]**.

The historical aspect of SME in Slovakia influences the fact that it is difficult to perform neuroscience in Slovak conditions. However, times are changing. Modern technologies have evolved, and it is now possible to explore or display facts more easily and at the same time more minutely and accurately than a few years ago, when the talks about it only started in our conditions **[59]**. Studies on neuroscience research in human resources are steadily increasing **[92]**.

The Scientific hypothesis was formulated: Young managers of family food businesses perceive neuroscience in HRM positively and are also ready to implement it in business practice. The topics that young managers of family food businesses deal with in the field of neuroscience in HRM are different from the topics that female managers deal with.

Based on the questionnaire survey results, we tested the dependency between statements. In the table there are the detected values as test results for both cases are in the following Table.

Table 3 Dependency testing.

Statistic	Value	Prob	Statistic	Value	Prob
Chi-Square	269.1030	<.0001	**Chi-Square**	324.2142	<.0001
Likelihood Ratio Chi-Square	241.1845	<.0001	**Likelihood Ratio Chi-Square**	193.2273	<.0001
Mantel-Haenszel Chi-Square	191.1121	<.0001	**Mantel-Haenszel Chi-Square**	225.1054	<.0001
Phi Coefficient	0.4881		**Phi Coefficient**	0.5142	
Contingency Coefficient	0.5115		**Contingency Coefficient**	0.5371	
Cramer's V	0.3412		**Cramer's V**	0.3847	

Analysis of dependence was performed by $\chi 2$ test. Based on a Prob value lower than alpha (0.05), we confirmed that the dependence between statements exists in both cases. Its tightness was verified by Cramer's V – coefficient. Values 0.3412 and 0.3847 in the previous table indicate moderate dependence.

As we found out, young leaders of family food businesses do not see neuroscience as a burden, but rather as a way of doing things more efficiently and better. They see the opportunity for its application mainly in creating an acceptable and more suitable working environment. It is essential in their employees´ development. Employees can receive new information (education) better through the use of neuroscience knowledge and make it easier to accept changes. Managers can communicate better with employees thanks to knowledge and neuroscience methods, which is the first step to company success. Dürrbeck found that human resource executives are well aware of neuroscience with business applicability, while leaders' awareness was identified as rather low. However, relevance for corporate success and application within leadership development programmes were shown to be low or non-existent. Here neuroscience principles were shown to bear valuable potential to tackle future challenges of leaders and organizations. Therefore, the prospects for increasing the application of neuroscience content within leadership development are promising **[93]**.

CONCLUSION

Based on neuroscience knowledge, if an individual feels well and his brain evaluates the current situation as "safe", the so-called approach emotions (trust, enthusiasm, joy, love) begin to form. These emotions are a prerequisite for successful project implementation, enabling cooperation, rational decision-making and creative problem-solving. Managers should therefore strive for a friendly atmosphere in the workplace and a pleasant working environment for employees. Young managers have a relatively easy way to become good managers and good leaders. By applying neuromanagement, they can gain better relationships at the workplace but also with business partners. Managers should also focus on the training they provide to their employees. It is more effective from the neuroscience point of view to use active learning forms more effectively than traditional forms of education. One of the first and simple step to apply neuroscience knowledge to business practice is to divide tasks for employees into smaller projects. We know that the brain is satisfied after completing the task and tries to achieve this feeling repeatedly – therefore it will try to complete other partial goals-tasks. In the end there will be a comprehensive fulfilment of the task. The next step may be a gradual transition to experiential learning. Research shows that by applying the knowledge of neuroscience to the learning process that is brought about by experiential learning, employees acquire new knowledge much faster. Managers should also strive to apply a positive attitude. By using the mindfulness method, they can create a better work environment and employees are more productive in such an environment. A stress-free work environment will have a better impact on employees, who can make better use of their potential. Young generation of family business´ leaders has a positive attitude towards neuroscience. They are already trying to apply it in human resource management or inclined to this management direction. We consider this a very important conclusion, as the fact that they are not afraid of neuroscientific methods and do not shy away from them creates favorable conditions for implementing these methods in practice.

There are still opportunities and space for improvement. Our findings can be used in the future for comparative analysis of how neuroscience perception and its implementation in SMEs in Slovakia have progressed. The aim will be to compare managers' opinions from the research currently being carried out with those within the range of 5 years. Based on our research, it can be assumed that there is room for increasing managers' awareness of the possibilities of using neuroscience in human resources management. Managers should be more exposed to the possibilities of using neuroscience knowledge in practice. A very good and suitable alternative to this is free-of-price workshops, where the basic advantages of these methods are presented to them. Neuroscience will also come to managers' attention by working more closely with universities on research focused on innovative methods and neuroscience in human resources management. This research was deficient because it was carried out during strict anti-pandemic measures due to covid-19, and many businesses preferred work from home. For this reason, some of the answers could only take the form of an ideal idea of a manager.

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Crosslinking methods for improving the properties of soy-protein based films for meat packaging: a review

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ABSTRACT

Crosslinking methods have been used to improve the properties of soy protein-based films for various applications, such as meat packaging. Some of the crosslinking methods that have been reported in the literature include boiling soy milk, baking soy protein isolates, adding canola and sorghum proteins, incorporating Plantago major seed mucilage and Anethum graveolens essential oil, adding pine needle extract (PNE), incorporating montmorillonite and citric acid, using xylose as a crosslinker, and crosslinking with glutaraldehyde. The incorporation of additives such as canola and sorghum proteins, Plantago major seed mucilage and Anethum graveolens essential oil, and pine needle extract (PNE) has also been reported to improve the properties of soy protein-based films. In conclusion, soy protein-based films have excellent film-forming properties and many functional characteristics, making them a promising material for food packaging applications. However, their poor moisture barrier properties must be improved to make them more suitable for food packaging applications. Crosslinking methods have been used to improve the properties of soy protein-based films for various applications, such as meat packaging. The incorporation of additives such as canola and sorghum proteins, Plantago major seed mucilage and Anethum graveolens essential oil, and pine needle extract (PNE) has also been reported to improve the properties of soy protein-based films.

Keywords: Soy protein-based films, meat packaging, crosslinking, meat

INTRODUCTION

Packaging materials are widely used in the meat business to safeguard meat products from possible dangers such as infection, spoilage, and oxidation. On the other hand, traditional packaging materials, such as metals, plastics, and glass, have substantial environmental problems, such as poor biodegradability, high carbon emissions, and limited renewability **[1]**, **[2]**. Packaging materials have become a major concern due to their environmental impact. The accumulation of plastic waste in the environment has led to the need for sustainable food packaging. Despite safety concerns, the low cost of materials and functional properties of plastics have led to their continued use in food packaging. There is a need to critically evaluate the various materials used for packaging due to their environmental influence during production and after their end-of-life **[3]**, **[4]**, **[5]**.

Protein-based films and coatings have gained attention recently due to their advantages over synthetic films, including their use as edible packaging materials. Soy protein-derived films have evolved as an eco-friendly option for meat packing in response to these issues. These films, made from soy protein derived from soybeans by extrusion or casting, are renewable, biodegradable, and biocompatible. Soy protein-based films, on the other hand, have challenges with mechanical strength, thermal stability, and moisture resistance, limiting their applicability for meat packing applications. Crosslinking techniques for increasing the properties of soy protein-based films have been developed to overcome these limits **[6]**, **[7]**, **[8]**.

Figure 1 summerizes the scheme of different agent involved in meat packaging.

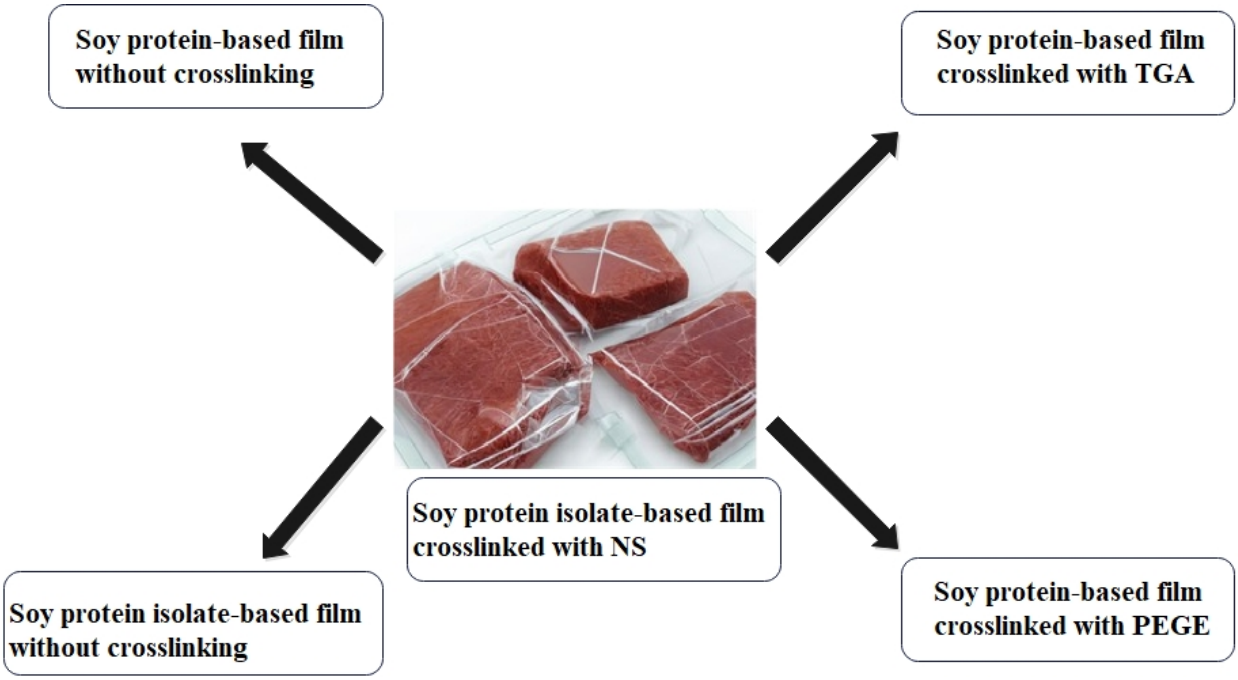

Figure 1 Scheme of different types of crosslinking agent (TGA – triglycidylamine; NS – nanosilica; PEGE – Pentaerythritol Glycidyl Ether).

Several methods have been adopted to enhance the strength of soy protein-based films, with crosslinking being one of the most commonly employed techniques. Crosslinking is widely recognized as an effective approach to improving the mechanical properties of these films. Crosslinking can reinforce the film structure and enhance its overall strength by creating chemical or physical bonds between soy protein molecules. Various chemical crosslinking techniques have been extensively investigated to enhance the performance of films derived from soy protein. Glutaraldehyde, a frequently employed crosslinking agent, is known for forming stable covalent bonds with the amino groups present in soy protein molecules. The process of crosslinking serves to improve the mechanical robustness, water resistance, and barrier characteristics of the films. Formaldehyde has been utilized as a crosslinking agent in various applications. However, its utilization is constrained owing to its potential safety and environmental risks. Genipin, obtained from the gardenia fruit, exhibits potential as a natural crosslinking agent, enhancing the film characteristics and reducing environmental impact. Polyfunctional acids, including citric acid and succinic acid, have been employed as crosslinking agents in order to develop a network structure that improves the mechanical and 7barrier characteristics of films **[7]**, **[8]**, **[9]**.

Physical crosslinking approaches present as a plausible method for enhancing the quality of films composed of soy protein. The application of heat treatment has been demonstrated to result in protein denaturation, facilitating subsequent reassembly, ultimately conferring enhanced film properties, including augmented tensile strength and attenuated water vapor permeability. The solvent evaporation process entails using volatile solvents for dissolving the protein and forming a film, which solidifies through the solvent's dissipation. This technique has the potential to enhance the mechanical robustness and hydrophobic properties of the film. The phenomenon of freeze-thaw cycles plays a crucial role in the development of physical crosslinks among protein molecules, thus leading to enhanced mechanical properties of the film. This process involves subjecting the film to multiple instances of freezing and subsequent thawing **[9]**, **[10]**, **[11]**.

This comprehensive overview provides a detailed analysis of the various crosslinking techniques used to improve the properties of soy protein-based films for meat packaging. The review evaluates the impact of these techniques on mechanical strength, thermal stability, water resistance and barrier properties. In addition, the potential industrial applications and regulatory considerations related to using soy protein-based crosslinked films in the meat packaging industry are explored. A thorough understanding of the strengths and limitations of different crosslinking methods aims to facilitate the development of sustainable and high-performance packaging materials that meet the stringent requirements of the meat industry while minimizing the impact on the environment.

Importance of packaging in the meat industry

Packaging plays a crucial role in extending the shelf life of meat products. Vacuum packaging and modified atmosphere packaging (MAP) are two techniques used to achieve this goal. Vacuum packaging removes air from the package, which slows down the growth of bacteria and other microorganisms that cause spoilage. MAP involves replacing the air inside the package with a mixture of gases that inhibit the growth of bacteria and extend the product's shelf life. In addition to extending shelf life, packaging also provides important information to consumers about the meat product they are purchasing. Labels on meat packaging provide information about the product's nutritional value, ingredients, and cooking instructions. However, it is important to note that meat industry packaging can also negatively impact the environment. The use of non-biodegradable materials, such as plastic, can contribute to pollution and waste. Therefore, the industry needs to explore and adopt sustainable packaging solutions that minimize their environmental impact. Some studies have explored the use of biodegradable packaging materials, such as xanthan-based films, which have positive effects on sensory, physicochemical, and microbiological parameters, as well as on ecological safety of the raw materials **[12]**.

Soy protein-based films as a sustainable alternative to traditional packaging materials

In the meat industry, films made of soy protein have been identified as a sustainable substitute for conventional packaging materials. These films offer several environmental advantages over traditional packaging materials like plastics, metals, and glass because they are renewable, biodegradable, and biocompatible. Soy protein can be extracted from soybeans and used to create soy protein-based films through casting or extrusion procedures. Films made of soy protein using renewable and biodegradable materials have a lower carbon footprint than traditional packaging materials. This means that they can be disposed of in an environmentally friendly manner and do not contribute to the accumulation of non-destructive wastes in landfills. Marine Soy protein films require less energy some than conventional packaging materials, significantly reducing the environmental footprint **[13]**.

However, one of the challenges associated with soy protein-based films is that they often exhibit high mechanical strength, water resistance, and thermal instability, which limits their use in meat packaging limits. To overcome these challenges, crosslinking methods have been proposed for soy-protein-based films to improve their mechanical properties, barrier properties and thermal stability **[14]**, **[15]**, **[16]**, **[17]**.

Film-forming methods for soy protein

Various film-forming methods have been reported for soy protein, including heating, extruding, spinning, casting, and thermally compacting. Heating has been used to form soy protein-lipid films in ancient China, where a creamy yellow film formed after the soy milk heating to near boiling was removed and dried, and there finally formed the soy protein film. Soy protein films can also be produced by extruding soy protein isolates with polyethylene oxide (PEO) and low-density polyethylene (LDPE). Spinning is another method for film-forming of soy protein, producing soy protein fibres by spinning a soy protein solution. Casting is the most popular method for film-forming soy protein, where a thin layer of soy protein solution is cast onto a flat surface and dried to form a film **[18]**, **[19]**, **[20]**.

Thermally compacting is another method for film-forming soy protein, where soy protein powder is compacted at high temperature and pressure to form a film. The processing conditions, such as temperature, pH, and concentration of the protein solution influence the film-forming properties of soy protein. The film-forming properties of soy protein can also be improved by adding plasticizers, crosslinking agents, and other additives **[20]**, **[21]**.

Soy protein-based films have been produced using various film-forming methods such as heating, extruding, spinning, casting, and thermally compacting. Heating is a common method used to produce soy protein-based films, where soy milk is boiled in a thin pot until the film is formed. Another method for obtaining soy protein film is based on baking soy protein isolates on pans for 1 h at a temperature of 100 °C. In a study, canola and sorghum proteins were added to soy proteins to improve adhesion. In another study, a coating based on Plantago major seed mucilage enriched with Anethum graveolens essential oil inhibited bacterial growth **[16]**.

Pine needle extract (PNE) has also been incorporated into soy protein-based films to improve their properties. Incorporating montmorillonite and citric acid into whey protein isolate films has been shown to preserve fresh-cut apples by reducing enzymatic browning and loss of apple quality and increasing shelf-life. Soy protein-based films have also been functionalized by incorporating antioxidants, antimicrobial agents, and other bioactive compounds to improve their functionality **[16]**, **[21]**, **[22]**.

Crosslinking Methods

Boiling Soy Milk Boiling soy milk was investigated as a crosslinking method for soy protein-based films. Soy milk was heated to a boiling temperature, and the films were immersed in the boiling soy milk for a specific

duration. The heat treatment was expected to induce denaturation and aggregation of soy proteins, leading to crosslinking within the film matrix. The crosslinked films exhibited improved mechanical properties compared to the untreated films, attributed to the formation of intermolecular bonds between protein chains **[8]**, **[16]**, **[23]**.

Baking Soy Protein Isolates Baking soy protein isolates was explored as an alternative crosslinking method. The soy protein isolates were dispersed in a suitable solvent and then subjected to a baking process at a specific temperature and time. The heat-induced reactions during baking promoted the formation of covalent bonds between protein molecules, resulting in enhanced film properties. Baked soy protein films exhibited increased tensile strength and reduced water vapor permeability compared to the control films **[16]**, **[24]**.

Adding Canola and Sorghum Proteins Incorporating canola and sorghum proteins into soy protein-based films was investigated as a means of crosslinking. To optimise the crosslinking effect, canola and sorghum proteins were mixed with soy proteins in varying ratios. The presence of these additional proteins provided reactive functional groups that could participate in crosslinking reactions with soy proteins. The resulting films showed improved mechanical properties and reduced water uptake, suggesting successful crosslinking between different protein sources **[25]**, **[26]**.

Incorporating Plantago Major Seed Mucilage and Anethum Graveolens Essential Oil The incorporation of Plantago major seed mucilage and Anethum graveolens essential oil was studied as a crosslinking method for soy protein-based films. Plantago major seed mucilage acted as a film-forming agent, while Anethum graveolens essential oil provided antimicrobial properties. The mucilage formed a gel-like matrix, and the essential oil acted as a crosslinking agent within the film structure. The crosslinked films exhibited improved mechanical strength, enhanced water resistance, and antimicrobial activity **[27]**, **[28]**.

Adding Pine Needle Extract (PNE) The addition of pine needle extract (PNE) was explored as a crosslinking method for soy protein-based films. PNE contains natural polyphenolic compounds that can undergo crosslinking reactions with soy proteins. The PNE was incorporated into the film-forming solution, and the films were subsequently dried to form a crosslinked structure. The resulting films demonstrated increased tensile strength and reduced water vapor permeability, indicating successful crosslinking between PNE and soy proteins **[29]**.

Using Xylose as a Crosslinker Xylose, a monosaccharide, was explored as a crosslinker for soy protein-based films. Through condensation reactions, Xylose has reactive hydroxyl groups that can form crosslinking bonds with soy proteins. The xylose solution was mixed with the film-forming solution, and the films were cast and dried. The resulting films exhibited enhanced mechanical properties and reduced water vapor **[14]**.

Properties of Soy Protein-Based Films for Meat Packaging

Mechanical properties

It is crucial to evaluate their mechanical properties to ensure the strength and durability of soy protein-based films used for meat packaging. The mechanical properties that are typically assessed include tensile strength, elongation at break, and puncture resistance. These properties are essential in determining the film's ability to withstand stretching, deformation, and puncture. By evaluating these mechanical properties, it is possible to ensure that the soy protein-based films provide adequate protection and preservation of the meat product. The term "tensile strength" denotes the upper limit of stress that a given material can endure prior to its failure in a state of tension. The mechanical property of resistance to deformation or tearing during handling and transportation is important for packaging materials. Soy protein-based films often manifest reduced tensile strength compared to conventional packaging materials. Nevertheless, the application of crosslinking techniques has the potential to enhance their mechanical properties **[30]**, **[31]**, **[32]**.

The elongation at break measures the extent of deformation that a substance can sustain prior to rupture when subjected to tensile forces. The attribute mentioned above holds significant pertinence in the realm of packaging materials, owing to its inherent capacity to depict the film's proficiency in terms of elastic deformation and energy retention, sans any rupture or fissure formation. Soy protein-derived films have demonstrated elevated elongation at break when compared to conventional packaging materials.However, this characteristic can be enhanced by implementing crosslinking techniques **[32]**.

Puncture resistance is the film's resistance to puncture or penetration by sharp objects. This is an important property for packaging materials as it indicates the film's ability to protect the product from damage during handling and transportation. Soy protein-based films are typically less puncture-resistant than conventional packaging materials, but cross-linking can also improve this property **[33]**.

In addition, Soy protein-based films have emerged as a promising alternative to petroleum-based films for meat packaging applications. However, to be effective, these films require additional mechanical properties beyond those mentioned earlier. Two crucial properties that soy protein-based films must possess are flexibility and foldability. These properties are essential for films that need to conform to the irregular shapes of meat products. The ability of the film to flex and fold allows it to conform to the product's shape, providing complete

coverage and protection. Soy protein-based films can be formulated to possess excellent flexibility and foldability, making them ideal for various meat packaging applications. Another critical mechanical property of meat wrap films is tearing strength. This property refers to the film's ability to resist the propagation of tears that have already started. Films with good tear strength are less likely to develop minor tears or punctures during handling and transportation, which can compromise the package's integrity and lead to spoilage and contamination. Cross-linking technology can be used to enhance the tear strength of soy protein-based films. This technology improves the tear strength of the films, making them more suitable for meat packaging applications. I need references related to these paragraphs **[34]**, **[35]**.

Thermal properties

Packaging materials used in the meat industry must have good thermal properties as they may be exposed to varying temperatures during storage, transportation, and cooking. However, soy protein-based films have been found to have poor thermal stability compared to commonly used packaging materials like polyethylene and polypropylene. Various methods can be employed to address this issue to improve the thermal properties of soy protein-based films. One approach is to add plasticizers to the films. Plasticizers are substances that enhance the flexibility and processing properties of polymers. By adding plasticizers to soy protein-based films, their glass transition temperature can be lowered, making them more flexible even at low temperatures. Another way to enhance the thermal qualities of soy protein-based films is to add antioxidants. Antioxidants scavenge free radicals and reduce the oxidation rate, preventing film breakdown at high temperatures. For example, vitamin E has been used as an antioxidant to increase the thermal stability of soy protein-based films. Crosslinking is another method that can improve the thermal properties of soy protein-based films. Crosslinking increases the temperature at which film degradation begins and improves overall thermal stability. Formaldehyde has been used as a crosslinking agent in soy protein-based films, improving thermal stability **[36]**, **[37]**, **[38]**, **[39]**.

Optical properties

Visual qualities such as clarity and color are also important packaging considerations in the meat industry. Transparency is important because it allows consumers to inspect the product and determine its quality. In addition, some packaging, such as vacuum packs, rely on transparent materials to monitor for signs of deterioration. Color can also be important for aesthetic reasons, as it can enhance a product’s appearance and help set it apart from competing products. soy protein-based films have poor optical properties compared to common packaging materials like polyethylene and polypropylene. Various methods can be used to improve the transparency of soy protein films, such as adding additives like glycerol or sorbitol. These additives can increase the thickness of the film, reduce light scattering, and increase its refractive index. However, they can also reduce the mechanical strength of the film **[32]**, **[40]**, **[41]**.

Crosslinking Methods for Soy Protein-Based Films

Chemical crosslinking agents

Glutaraldehyde

Glutaraldehyde is commonly used as a crosslinking agent in producing soy protein films. It reacts with amine groups on soy protein molecules to form covalent cross-links that improve the mechanical and barrier properties of the membrane. Glutaraldehyde has been shown to produce films with high tensile strength and good water resistance. However, the use of glutaraldehyde has certain disadvantages. It is a volatile and toxic substance that poses a risk to workers and the environment. Additionally, residual glutaraldehyde in the film can migrate into food products, raising concerns about potential health risks. Therefore, alternative crosslinking agents are sought to be safer and more environmentally friendly **[42]**, **[43]**, **[44]**.

Genipin

Genipin is a natural cross-linking agent that has been investigated for use in soy protein-based membranes. It is derived from the fruit of the gardenia plant and is considered a safer and more environmentally friendly alternative to chemical crosslinking agents. Genipin reacts with amino groups on soy protein molecules, forming cross-linkers that improve the film's mechanical properties and barrier properties. Studies have shown that genipin can effectively improve soybean protein-based films' tensile strength, water resistance, and thermal stability. In addition, genipin has been shown to have antimicrobial properties, which may be beneficial in meat packaging applications to reduce the risk of contamination. However, using genipin as a crosslinking agent also has certain limitations. It has been found to have a slower curing rate than chemical curing agents, which can lead to longer production times. In addition, the use of genipin may discolor the film, which may affect the appearance and marketability of the film **[45]**, **[46]**, **[47]**.

Epichlorohydrin

Epichlorohydrin is a pass-linking agent used in the production of soy protein-primarily based movies. This compound reacts with amino and hydroxyl groups on soy protein molecules, forming covalent go-links that improve the mechanical and barrier residences of the membrane. The use of epichlorohydrin as a crosslinking agent has been proven to provide movies with appropriate tensile power, water resistance and thermal balance. However, there are a few concerns regarding the usage of epichlorohydrin in food packaging packages. This compound is understood to be carcinogenic and can pose a health hazard to people and customers. Additionally, epichlorohydrin left in the movie can migrate into food products, leading to capability health problems. Therefore, there may be a growing interest in locating safer and more environmentally pleasant alternatives to epichlorohydrin for meal packaging **[48]**.

Formaldehyde

Formaldehyde is a chemical move-linking agent used in the manufacture of diverse materials, including movies crafted from soy protein. It reacts with amine agencies on soy protein molecules, forming covalent move-hyperlinks that enhance the mechanical and barrier houses of the membrane. However, formaldehyde has been determined to have sure hazards as a cross-linking agent. It is a poisonous substance that could motive fitness problems, which includes breathing infection and most cancers. In addition, formaldehyde can release risky organic compounds (VOCs), contributing to indoor air pollution. Therefore, there may be increasing hobby in finding opportunities crosslinking agents that are safer and more environmentally pleasant **[45]**, **[49]**, **[50]**, **[51]**.

Transglutaminase

Transglutaminase is an enzyme discovered obviously in some of meals, along with meat, fish, and dairy merchandise. It catalyzes the formation of covalent bonds among proteins, improving meals' feel and balance. Transglutaminase has also been used as a move-linking agent in manufacturing soy protein movies. The use of transglutaminase as a crosslinking agent has been proven to enhance the mechanical homes and barrier houses of soybean protein-based totally membranes. It forms covalent bonds among membrane protein molecules, creating a more stable and durable cloth. In addition, transglutaminase is non-poisonous and non-volatile, making it a safer alternative to numerous move-linking sellers. Transglutaminase can inadvertently move-link proteins, main to the formation of allergens or other unwanted compounds. Therefore, it's important to cautiously examine the protection and effectiveness of transglutaminase and different crosslinking agents before using them in meals packaging or different applications **[52]**, **[53]**, **[54]**, **[55]**.

Physical crosslinking methods

Heat treatment

The heat treatment process represents a physical crosslinking technique that can be effectively utilized to induce modifications in the properties of films based on soy protein. During heat treatment, denaturation of soy protein molecules occurs, resulting in the loss of their original structure and the establishment of fresh bonds with adjacent molecules. The consequence of this phenomenon manifests in the creation of physical intermolecular connections among the protein entities, which subsequently enhances the film's mechanical and barrier characteristics. The degree of crosslinking susceptibility observed during thermal processing is contingent upon a range of variables, predominantly including temperature, duration, and moisture levels. Elevated temperatures and prolonged heating durations may contribute to an overabundance of crosslinking, eventually inducing a decrease in film pliability and an elevation in fragility. Conversely, the induction of significant crosslinking may not be accomplished by low temperatures or brief heating durations **[56]**, **[57]**, **[58]**.

UV irradiation

Radiation crosslinking is a method used to improve the properties of soy-protein based films for meat packaging. This method involves exposing the films to high-energy radiation, such as gamma rays or electron beams, which causes the polymer chains to crosslink and form a three-dimensional network. This network improves the mechanical properties of the films, such as their tensile strength and elongation at break, as well as their barrier properties, such as their water vapor permeability and oxygen transmission rate. Radiation crosslinking can also improve the films' thermal stability and antimicrobial properties. However, the process can be expensive and requires specialized equipment, and there are concerns about the safety of using radiation on food packaging materials. Therefore, further research is needed to optimize the process and ensure its safety and effectiveness **[40]**.

Enzymatic crosslinking

Enzyme crosslinking is a method of crosslinking proteins that involves the use of enzymes to catalyze the formation of covalent bonds between protein molecules. One enzyme commonly used for this purpose is transglutaminase (TGase). TGase catalyzes the formation of covalent bonds between the side chains of glutamine and lysine in proteins, leading to cross-links between protein molecules. Enzyme crosslinking is a gentle and environmentally friendly method of protein crosslinking that does not require the use of harsh chemicals or high temperatures. It also provides precise control over the degree of crosslinking, tunable by varying enzyme concentration and reaction time. Enzymatic crosslinking has been used to improve the mechanical and barrier properties of soy protein films, making them more suitable for applications such as food packaging. In one study, soybean protein isolate films were crosslinked using TGase, resulting in improved tensile strength and water resistance. The use of TGase also resulted in membranes with improved oxygen and carbon dioxide-blocking properties **[24]**.

Applications of Crosslinked Soy Protein-Based Films in Meat Packaging

Preservation of meat quality

Using cross-linked soy protein films in meat packaging can help preserve meat quality. The film provides a barrier to oxygen, moisture, and other gases that can cause meat to spoil or spoil. In addition, these films can prevent the loss of moisture in the meat, leading to the meat's shrinking and hardening. Films made from cross-linked soy protein can also help extend the shelf life of meat products. Food wrap can slow the growth of bacteria and other spoilage microorganisms by reducing the amount of oxygen that comes into contact with meat. This can help maintain meat freshness and quality for longer. In addition, using films made from cross-linked soy proteins can help reduce the packaging material required for meat products. Because these films are strong and durable, they can be used in thinner layers than traditional packaging materials, such as plastic or paper. This can help reduce waste and reduce the environmental impact of meat packaging **[16]**, **[18]**, **[20]**. Table 1 presents a comprehensive overview of the properties of crosslinked soy protein-based films developed explicitly for meat packaging. It provides essential information on characteristics such as tensile strength, water vapor permeability, oxygen permeability, barrier properties, mechanical properties, transparency, and shelf life. This valuable resource serves as a reference for researchers and industry professionals seeking to explore sustainable alternatives in meat packaging.

Table 1. Properties of Crosslinked Soy Protein-Based Films for Meat Packaging

Application	Description	References
Oxygen Barrier	Crosslinked soy protein-based films possess excellent oxygen barrier properties, reducing oxygen permeability. This minimizes oxidative reactions, such as lipid oxidation, which can lead to off-flavours and spoilage.	**[58]**, **[59]**, **[60]**
Moisture Retention	Crosslinked soy protein-based films can retain moisture within the meat, preventing excessive drying. This helps to maintain the meat's juiciness, tenderness, and overall quality.	**[33]**, **[58]**
Antimicrobial Activity	Crosslinked soy protein-based films possess antimicrobial properties, inhibiting the growth of spoilage and pathogenic microorganisms. This extends the shelf life of meat products and reduces the risk of foodborne illnesses.	**[14]**, **[60]**, **[61]**
Mechanical Strength	Crosslinked soy protein-based films exhibit good mechanical strength and flexibility, protecting against physical damage during handling and distribution. They help preserve the integrity and appearance of the meat.	**[14]**, **[33]**, **[58]**, **[60]**, **[61]**

Extension of shelf life

By acting as a barrier against gases like oxygen, moisture, and others that might hasten deterioration, films formed of soy protein can help increase the shelf life of meat products. These membranes' barrier qualities and their capacity to stop meat products from degrading can be further improved by using cross-linking chemicals like glutaraldehyde. Soy protein films can be created to release antimicrobial substances like bacteriocins or essential oils that may help stop the growth of germs and fungus on the surface of meat products and act as a physical barrier to the outside environment. This can increase the product's shelf life and lower the chance of contracting a foodborne disease. The moisture level of meat products may be maintained using soy protein-based films, which is crucial for their quality and safety. Consumers may find dry, chewy meat less appetizing as a result of moisture loss. Additionally, it may raise the possibility of microbial development and deterioration. By creating a barrier against moisture loss, soy protein films can help maintain the quality and safety of meat products for longer periods **[23]**, **[62]**.

Enhancement of food safety

Crosslinked soy protein-based films can decorate food protection in several approaches. Firstly, by improving the movie's barrier homes, the movies can save you the migration of harmful contaminants such as bacteria, fungi, and viruses into the packaged meat. This can help reduce the risk of foodborne ailments from contamination throughout dealing with, transportation, and storage. In addition, crosslinked soy protein-based films can also reduce the risk of oxidation and spoilage of packaged meat. Films can act as a barrier to oxygen and other reactive species, which can cause meat quality degradation and shorten its shelf life. This may be particularly important for meat products susceptible to oxidative rancidity, such as ground meat and meat products that contain high amounts of unsaturated fatty acids . Furthermore, soy protein-based films can be used as an alternative to synthetic polymer-based packaging materials, which are non-biodegradable and can accumulate in the environment, and cause contamination and harm to wildlife Soy films made from soy protein, on the other hand, are bio - they are biodegradable, can be disposed of safely, and reduce the environmental impact of meat packaging on the snow **[24]**, **[63]**, **[64]**.

Improving packaging efficiency and reducing waste

Soy protein-based films can improve the barrier and mechanical properties of packaging materials in the meat industry, leading to enhanced packaging efficiency and reduced waste. The use of such packaging materials with excellent barrier properties can reduce the need for additional layers or packing materials, resulting in cost savings and a reduced environmental impact. Additionally, improved packaging can extend the shelf life of meat products, reducing food waste. By retaining moisture and oxygen effectively, the growth of bacteria and other microorganisms that cause spoilage can be slowed down, resulting in a longer shelf life of meat products and a lower likelihood of product rejection due to spoilage. Furthermore, improved packaging can enhance food safety by reducing the risk of contamination during transportation and handling. The use of tear-resistant and perforation-resistant packaging materials can minimize the potential for contamination.

Moreover, using packaging materials that do not release harmful chemicals or compounds into foods can ensure the safety of meat products for consumption. Several studies have been conducted on the use of soy protein-based films for meat preservation, and the incorporation of antimicrobial agents such as cinnamaldehyde or tea polyphenols has been found to be effective in maintaining the quality and safety of meat products. Edible films made from natural compounds such as polysaccharides, proteins, and lipids have also been studied for their antioxidant and antibacterial activities in meat and meat products and have shown potential as eco-friendly alternatives to petrochemical-based plastic packaging. The incorporation of essential oils from oregano or thyme into soy-based edible films is effective in retarding oxidative changes in meats and can be used as an antioxidant-active packaging. Proteins are excellent materials used for obtaining edible or non-edible coatings and films, and different vegetable and animal protein sources have been studied for their mechanical properties, thickness, moisture content, water vapor permeability, sensorial properties, and suitability for the environment **[15]**, **[23]**, **[65]**.

Industrial Considerations for Soy Protein-Based Films in Meat Packaging

Manufacturing and processing challenges

Soy protein-based films for meat packaging applications can present several challenges. These challenges include achieving consistent film properties, ensuring the safety and quality of the final product, and logistical challenges associated with production and distribution. Factors such as film composition, treatment conditions, and curing methods must be carefully controlled during large-scale production to achieve consistent film properties. It is also important to ensure that the membrane meets regulatory requirements by performing rigorous testing. Residual crosslinkers or other additives in the film can potentially migrate into the meat product, causing

health and safety problems. Therefore, careful control of the manufacturing process is essential. In addition, logistical challenges may be associated with the production and distribution of soy protein films, such as transportation and storage requirements as well as cost and scalability considerations. Despite these challenges, soy protein-based films have become widely used for a variety of different products and different food categories such as meat products, vegetables, or dairy products. Proteins are excellent materials used for obtaining edible or non-edible coatings and films, and different vegetable and animal protein sources have been studied. Soy protein-based films have been used to preserve meat analogues, and the incorporation of antimicrobial agents such as cinnamaldehyde has been shown to be effective in retarding the growth of bacteria **[15]**, **[16]**, **[66]**.

Future Directions and Opportunities

The utilization of soy protein-derived films in meat packaging has garnered considerable interest in contemporary times, owing to their eco-friendliness, replenishability, and prospective capacity to enhance meat preservation, food security, and longevity. Notwithstanding, multiple obstacles and prospects exist pertaining to the subsequent investigation and advancement in this area. Possible academic rewrite: One promising avenue for enhancing the mechanical and barrier properties of soy protein-based films is the exploration of innovative crosslinking methods and utilising diverse additives like nanoparticles, biopolymers, and antimicrobial substances. The implementation of this technique has the potential to yield films displaying heightened robustness, suppleness, and antimicrobial properties, thus amplifying the efficacy of such materials as meat packaging. A promising avenue for further research lies in investigating the potential application of soy protein-based films in conjunction with other types of biodegradable packaging materials, such as cellulose and chitosan. The creation of multi-layered films through such intermixing has the potential to yield enhanced functional properties. Cost efficiency and market feasibility are key considerations in introducing and implementing soy protein-based films in meat packaging applications. The manufacturing cost of soy protein-based films can be higher than traditional petroleum-based packaging materials due to higher raw material and processing costs. However, the use of soy protein-based films has the potential to reduce overall packaging costs by improving packaging efficiency and reducing food waste. Marketability is also important as consumer acceptance and demand for sustainable and environmentally friendly packaging materials increase. The introduction of soy protein-based films could meet the growing consumer trend for environmentally friendly and sustainable packaging options and could provide a competitive advantage for companies in the meat industry.

Moreover, advancing sustainable and economically efficient manufacturing techniques for soy protein-derived films could augment their market viability. The use of biodegradable and sustainable packaging materials has gained considerable attention as a viable solution to address the mounting environmental concerns. The utilization of films derived from soy protein within the realm of meat packaging is consistent with the objectives mentioned above, subsequently presenting an avenue for waste reduction and augmenting the sustainability of the food sector.

CONCLUSION

In summary, soy protein-based films have great potential as sustainable and biodegradable alternatives to conventional meat packaging. Crosslinking techniques have been investigated to improve the mechanical barrier properties of soy protein-based films, making them more suitable for use in meat packaging. Various cross-linking agents have been studied, to improve performance and safety Promising results have been obtained. However, there are still challenges to overcome regarding product development and manufacturing, cost efficiency and market power. However, the increasing demand for sustainable and environmentally friendly packaging solutions will create opportunities for the production and commercialization of soy protein-based films in meat products packaging industry Further R&D efforts are needed to overcome these challenges and to better realize the potential of soy protein films in meat packaging.

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Low calorie diets in the prevention and treatment of human diseases

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ABSTRACT

Phytochemicals affect metabolic changes as well as organ changes. With their effects, they can prevent diseases or, in the case of established disease, affect speeding up conventional treatment. Low-calorie diets and other restrictive diets are challenging to follow for an extended period. As a result, they are less popular than non-restrictive programs that instead encourage good eating habits. In our experiment, we dealt with the health problems of 8 probands with the following health problems: high blood sugar level, overweight, high blood cholesterol level, high blood pressure. Before and after the end of the restrictive diet, we determined changes in the organism. Blood sugar levels, body weight, changes in blood pressure, subjective expression of pain, physiological values in the blood (cholesterol, hemoglobin, white blood cells, glucose, urea, cholesterol, thyroid function and hormones (T3 – triiodothyronine, T4 – thyroxine)) and urine (urine pH, urine proteins, glucose, ketones, urobilinogen, blood in urine and hemoglobin) were determined. As for blood biochemical parameters, positive changes were observed in almost all probands, except for the decreased creatinine level. After completing the restrictive diet, the participants' blood pressure moved towards normal values (120/80). In addition, the participants' body weight decreased by an average of 3-4.5 kg, which led to a change in their BMI (Body Mass Index). During the entire 7-day period of the diet, most of the participants initially reported negative subjective experiences, but at the end of the restrictive diet, they generally felt good.

Keywords: restrictive diet, health status, blood sugar level, physiological blood values, body weight

INTRODUCTION

Bioactive compounds have emerged as key food components related to healthy status and disease prevention. As population is getting older and less physically active, non-communicable diseases are increasing. In some cases, bioactive compounds are regarded as an interesting alternative for disease prevention and treatment. This is increasingly boosted by the increased need for natural products by the consumers, who require sustainable solutions for improving quality of life focused on personalized nutrition. The knowledge of the chemistry of natural products and their mechanistic approach are key elements for developing new solutions for this market. This chapter briefly addresses the importance of bioactive compounds and their role in non-communicable diseases **[1]**. There is no consensus in the literature to define the term “bioactive compound”. However, within the most widely accepted denominations, they are “compounds which have the capability and the ability to interact with one or more component(s) of living tissue by presenting a wide range of probable effects” **[2]**.

Unhealthy diets, malnutrition, and NCDs are closely linked. They are the logical consequences of, among other factors, today’s food systems, which have changed dramatically in the past 50 years. A focus on efficiency has seen an increase in the availability of inexpensive, high-calorie foods, often from staple cereal crops, reducing hunger for many. This has, however, often been at the expense of diversity and has displaced local, often healthier, diets. Access to diverse, micronutrient-rich foods – such as fresh fruits, vegetables, legumes, pulses, and nuts –

has not improved equally for everyone, and unhealthy foods with salt, sugars, saturated fats, and trans fats have become cheaper and more widely available.

Furthermore, global demand for and supply of meat, dairy products, sugar-sweetened drinks, and processed and ultra-processed foods has increased dramatically **[3]**. The increased consumption of sodium, used in food as a preservative and flavoring, contributes significantly to an unhealthy lifestyle. Table grapes belong to the most dietary balanced fruit **[4]**.

Under the term restrictive diet, we can imagine giving up certain eating habits. Such as fried steaks, mayonnaise salads, and alcoholic or non-alcoholic sugary drinks. We will include drinking vegetable and fruit juices, more fresh fruits and vegetables, various vegetable broths flavored with herbs and omit salting in the restrictive diet menu. Procyanidins are an important component of grapes and grape juices. These bioreducers have a broad-spectrum effect against cardiovascular diseases, inflammation, UV radiation, and trap free radicals. They are also a suitable nutritional supplement to the diet and can significantly ease the restrictions resulting from the diet **[5]**.

Most people think that if they should go on a diet, it will mean some suffering or restriction in their life. After going on a restrictive diet, they suddenly find that they are much better. They are more vital, full of energy, and have better blood count and systolic pressure. Several experimental studies that have been done on animals and humans have shown that diet is very important. It plays a huge role in the primary and secondary prevention of various diseases and is an important treatment element. In addition to eating, physical activity is also important, which reduces the risk of civilization diseases **[6]**.

Restrictive diets appear to improve eating behaviors, and the evidence reviewed argues against the notion that they may worsen the severity of binge eating. Moreover, they may lead to short-term changes in brain structure and improvements in cerebrovascular markers which, in turn, could impact eating behaviors. Non-restrictive interventions may have apositive effect on weight management and eating behaviors. However, evidence of their neural effects is scarce **[7]**. Obesity is a serious health problem that has spread worldwide and is thought to be a modifiable risk factor for several co-morbidities, including cancer, type 2 diabetes, hypertension, and sleep apnea **[8]**. There are many ways to manage and treat obesity, thus current tactics employed by healthcare experts shouldn't be viewed as a "one size fits all" strategy **[9]**, **[10]**, **[11]**. Current dietary intervention recommendations offer various methods to promote body weight loss and enhance other health aspects **[11]**.

Ailer et al. recommend the following energy intake for women and men of different ages (kJ/day) according to the physical load performed (Table 1) **[12]**.

Table 1 Energy intake for women and men of different ages (kJ/day).

	Woman		**Man**	
	Age			
Physical exercise	**19-30**	**31-50**	**19-30**	**31-50**
Low	8368	7531.2	10041.6	9204.8
Medium	9204.8	8368	10878.4	10460
High	10041.6	9204.8	12552	12133.6

Low-calorie diets and other restrictive approaches have historically been employed alone or in conjunction with behavioral and/or physical activity techniques to reduce body weight. However, these restrictive techniques have also been linked to many detrimental physiological or psychological outcomes, including weight regain over time, increased appetite, and more severe depressive symptoms **[13]**, **[14]**, **[15]**, **[16]**, **[17]**, **[18]**, **[19]**. These reactions to restrictive dietary practices may help to explain why it is difficult to maintain weight over the long term. However, current recommendations recommend interventions based on dietary patterns emphasising healthy eating (e.g., high consumption of fruits and vegetables, whole grains, nuts, low-fat dairy). Several non-restrictive strategies aim to improve body weight, appetite regulation, and other metabolic health markers. The management of eating disorders like binge eating disorder (BED) and disordered eating, such as emotional eating, restriction eating, or binge eating habits, may also be effectively accomplished using some of these non-restrictive measures **[20]**, **[21]**.

In the experiments, we investigated the effect of phytochemical, bioactive substances, contained in plant products consumed in food dishes as part of a modified diet on reducing the disease state of selected probands. We will evaluate the influence of the modified diet consumed based on the input values of the probands before completing the diet, which we compared with the output values (analysis of blood and urine), immediately after completing the modified diet.

Scientific Hypothesis

Bioactive plant metabolites have an impact on disease reduction and human treatment. Adjusting the diet is important for reducing the risk of diseases and speeding up the process of treating the disease.

MATERIAL AND METHODOLOGY

Samples

A total of eight probands were involved in the experiment (6 women and 2 men) at the age of 31 to 66 years.

Instruments

Glucometer BioLand G-423, BioLand Technology Ltd., China.
Arm Pressuremeter Beurer BM 65, Beurer GmbH, Germany.
Personal weight HBF-511B/T (Omron, Japan).

Laboratory Methods

Measurements were taken during the restrictive diet, 36 hours of food deprivation and immediately after the end of the experiment. G-423 glucometer was used to determine the change in blood sugar level. Blood pressure changes were determined with a Beurer BM 65 sphygmomanometer. Body weight changes were recorded by a personal digital scale (kg) and a height-length scale (cm). Changes in cholesterol levels were determined by a biochemical blood test in the laboratory. BMI (body mass index) was obtained by calculating – weight/height (in m^2). Changes in the subjective expression of pain were evaluated based on the probands' own feelings. Physiological values of blood (hemoglobin, white blood cells, glucose, urea, cholesterol, thyroid function, thyroid hormone (T3 – Triiodothyronine, T4 – Thyroxine)) and urine (urine pH, urine protein, glucose, ketones, urobilinogen, blood in urine and hemoglobin) were determined in certified medical laboratories.

Description of the Experiment

Sample preparation: For two days before arrival, the probands ate only light non-meat meals.

Design of the experiment: The experiment lasted 7 days. We were in constant contact with the probands, while we consulted with them about all the changes in the organism recorded during and after completing the 7-day adjusted diet. After the end of the experiment, there was subsequent longitudinal monitoring of their health status, especially those probands for whom the detoxification diet's duration was insufficient for a "complete" recovery, a return to optimal physiological values. The foods used and their preparation were:

- potatoes boiled in their skins, natural rice,
- vegetable juices: carrot (always about 2/3 of the juice volume), parsley, black radish, beetroot, cabbage, broccoli, celery, chicory (endive), herbal tincture (liquid extract from herbs),
- fruit juices from fruits: apples, pears, lemons, grapefruits,
- vegetable broth: a mixture of vegetables,
- herbs as ingredients: seasoning potatoes and rice with herbal spices – anise, true basil, kitchen garlic, garden marjoram, peppermint, nutmeg, oregano, rosemary, sage, saffron, thyme.

Regime actions during the 7-day restrictive diet are shown in Table 2-3.

Table 2 Guidelines for the 7-day restricted diet.

Day	Actions
1.-3.	reducing the energy intake of food in order to prepare the organism for not taking food
4.-5. until 12:30	without eating
5. from 12:30	increasing the energy intake of food in order to return to normal eating, such as on the 3rd day after lunch (vegetable salad)
6.	same as 2nd day (rice),
7. until 6:00 p.m.	same as 1st day (potatoes)

Table 3 Regime actions during the 7-day restrictive diet.

Time	Days			
	1.	**2.**	**3.**	
8:00	vegetable juice (2-3 dcl)	vegetable juice (2-3 dcl)	vegetable juice (2-3 dcl)	
8:30	1 ½ hour walk	1 ½ hour walk	1 ½ hour walk	
10:20	fruit juice (2 dcl)	fruit juice (2 dcl)	fruit juice (2 dcl)	
12:00	lunch (3 potatoes boiled in their skins individually flavoured with herbal spices, vegetable salad, vegetable broth)	lunch (rice individually seasoned with herbs, vegetable salad, vegetable broth)	lunch (vegetable salad individually seasoned with herbs, vegetable salad, vegetable broth)	
12:30	afternoon rest	afternoon rest	afternoon rest	
14:00	1 ½ hour walk	1 ½ hour walk	1 ½ hour walk	
15:45	vegetable juice (2 dcl)	vegetable juice (2 dcl)	vegetable juice (2 dcl)	
18:00	2 dcl of red wine	2 dcl of red wine	2 dcl of red wine	
21:00	bedtime, sleep	bedtime, sleep	bedtime, sleep	
	4.	**5.**	**6.**	**7.**
8:00	without eating (drinking water according to individual needs)	without eating (drinking water according to individual needs)	vegetable juice (2-3 dcl)	vegetable juice (2-3 dcl)
8:30	1 ½ hour walk	1 ½ hour walk	1 ½ hour walk	1 ½ hour walk
10:20	without eating (drinking water according to individual needs)	-	fruit juice (2 dcl)	fruit juice (2 dcl)
12:00		lunch (vegetable salad individually seasoned with herbs, vegetable salad, vegetable broth)	lunch (rice individually seasoned with herbs, vegetable salad, vegetable broth)	lunch (3 potatoes boiled in their skins individually flavoured with herbal spices, vegetable salad, vegetable broth)
12:30		-	afternoon rest	afternoon rest
14:00	1 ½ hour walk	1 ½ hour walk	1 ½ hour walk	1 ½ hour walk
15:45	without eating (drinking water according to individual needs)	vegetable juice (2 dcl)	vegetable juice (2 dcl)	vegetable juice (2 dcl)
18:00		2 dcl of red wine	2 dcl of red wine	-
21:00	bedtime, sleep	bedtime, sleep	bedtime, sleep	-

The mentioned fruits and vegetables were provided uniformly from the family-growing farm. The drinking regimen was applied individually by the probands as needed, drinking only fresh water or unsweetened green tea (except during the period of not taking food, when they only drank freshwater). During the entire experiment, we were in contact with the doctors to intervene in case of deterioration of the probands' health condition. Probands performed random physical exercises every day (1½ hours) even when not taking food and drank only pure water (36 hours in the middle of the diet). Table 4 shows basic information about the probands before the restrictive diet.

Probands recorded daily in writing all ongoing cognitive and somatic changes in their organism, which we subsequently evaluated and were part of the overall evaluation. After taking blood, urine, and stool (after completing a modified diet), we evaluated the changes in the given indicators. After the end of the experiment, we had constant contact with the probands and then longitudinally monitored their health status, especially those probands for whom the detoxification diet's duration was insufficient for a "complete" recovery, a return to optimal physiological values.

Table 4 Basic information about the probands before the restrictive diet.

Probands	Sex	Age	Body weight	High	Profession, activity	Health problems
1	W	43	69.5	172	teacher, trainer, regularly plays sports (running, weight training)	high cholesterol, problems with hydration
2	M	66	108	180	teacher, veterinarian, she played sports in her youth, now she bikes and hikes	swelling of the lower limbs (especially in the evening), migraine, type 2 diabetes mellitus
3	W	31	55	165	housewife swims regularly	low pressure, dizziness, digestive problems (bloating), zhaemorrhoids
4	W	52	65	169	saleswoman, exercises at home, goes to the gym, goes for long walks	lupus, joint pain
5	M	35	80.6	178	computer scientist played football, now works out, cycles and skis	tense body, trouble falling asleep, swollen abdomen in the stomach area
6	W	29	48	156	kindergarten teacher, seasonal sports	scattered hormones, problems with menstruation, acne on the back and face
7	M	42	98	180	fitness trainer, strengthens	joint and knee pain
8	W	56	76	168	teacher, veterinarian, hiking, cycling and seasonal sports	migraine, knee pain, high cholesterol

Statistical Analysis

The results were processed using Microsoft Excel software (Microsoft, USA).

RESULTS AND DISCUSSION

Table 5 shows the reference values of blood biochemical parameters and Table 6 shows the values of these parameters determined before and after the restrictive diet. In proband 1 there was a decrease in glucose, cholesterol (LDL and HDL cholesterol) and urea. On the contrary, there was a slight increase in triglycerides and creatinine. In proband 2 there was a decrease in glucose, cholesterol (LDL and HDL cholesterol) and urea. After the post-restrictive diet, creatine also decreased, but triglycerides increased (with which the subject has a long-term problem). In proband 3, there was a decrease in glucose, cholesterol (LDL and HDL cholesterol), triglycerides and urea, the value of creatinine did not change. The same trend was observed in proband 4, but the value for creatinine was slightly higher. In probands 5-8 there was a decrease in glucose, cholesterol (LDL and HDL cholesterol), triglycerides, urea and creatinine

Table 5 Selected reference values of blood biochemistry before starting the diet (mmol/l).

Parameter	Reference values
Glucose	4.10-5.80 mmol/l
Cholesterol	2.90-5.20 mmol/l
LDL cholesterol	1.00-3.10 mmol/l
HDL cholesterol	1.00-2.13 mmol/l
Triglycerides	0.45-1.70 mmol/l

Table 6 Biochemical parameters of blood of the probands.

Parameter	Blood analysis values of the probands before and after the diet (mmol/l)							
	1		2		3		4	
	before	after	before	after	before	after	before	after
Glucose	4.12	4.05	14.63	6.02	3.76	3.11	4.99	3.56
Cholesterol	7.95	6.56	5.33	5.10	5.65	5.32	6.87	6.20
LDL cholesterol	4.55	4.22	2.74	3.05	2.55	2.30	5.16	5.08
HDL cholesterol	2.19	1.78	1.52	1.20	2.19	2.05	1.67	1.52
Triglycerides	0.60	1.07	2.52	2.88	1.45	1.43	1.20	1.00
Urea	6.00	3.86	8.16	9.670	4.30	3.86	5.00	3.86
Creatinine	91.01	101.1	97.84	119.60	79.0	79.0	98	95
Leukocytes	3.81	4.20	8.34	9.02	5.97	6.11	7.13	7.82
Erytrocytes	4.91	5.03	5.03	5.03	4.72	4.75	4.09	5.13
Hemoglobín	151.00	152.00	128.00	130.00	144	144	128	132.00
Trombocytes	181.0	181.0	317.00	317.00	268	210	318	340
	5		**6**		**7**		**8**	
	before	**after**	**before**	**after**	**before**	**after**	**before**	**after**
Glucose	4.50	3.25	5.10	4.04	5.4	4.18	5.20	4.16
Cholesterol	6.02	4.64	4.68	4.03	8.7	6.5	6.45	4.80
LDL cholesterol	4.22	3.66	3.20	3.00	4.90	6.22	3.15	3.03
HDL cholesterol	1.68	1.47	1.52	1.12	2.50	2.01	1.92	1.28
Triglycerides	1.09	1.04	0.67	0.52	1.45	1.22	1.00	0.90
Urea	7.00	4.18	5.20	3.86	6.8	4.18	4.30	3.00
Creatinine	90.00	85	72	64.5	98	102	75	71
Leukocytes	4.67	5.13	3.81	5.30	4.20	5.13	5.20	6.80
Erytrocytes	4.56	5.03	4.91	5.03	4.93	5.00	4.50	5.70
Hemoglobín	153.00	153.00	124	126	155	157	123	125
Trombocytes	178	179	160	163	200	200	160	160

Table 7 lists the reference blood pressure values by categories. Table 8 shows the blood pressure values measured in the probands. A trend was noted for all probands when blood pressure was positively adjusted.

Table 7 Reference blood pressure values.

Extremely low pressure	<49	<34
Very low pressure	50-69	35-39
Low pressure	70-89	40-59
Lower normal	90-110	60-75
Normal	120	80
Prehypertension	120-139	80-89
High pressure - grade 1.	140-159	90-99
High pressure - grade 2.	160-179	100-109
High pressure - grade 3.	180-209	110-119
High pressure - grade 4.	>210	>120

Table 8 Blood pressure values of the probands.

Proband	Blood pressure before starting the diet (mm Hg)	Blood pressure after the diet (mm Hg)
1	135/91	130/86
2	110/70	117/80
3	77/58	100/60
4	122/84	120/80
5	121/80	117/80
6	90/70	100/75
7	125/90	120/80
8	80/120	80/100

Tables 9 and 10 show the reference values for BMI and the values that were measured in the probands before and after the restrictive diet. All probands experienced body weight loss in the range of 3-4.5 kg.

Table 9 BMI (body mass index).

Underweight	<18.5
Normal weight	18.5-24.9
Excess weight	25-29.9
First obesity zone – Moderate obesity	30-34.9
Second obesity zone	35.39.9
Morbid obesity	>40

Table 10 Body weight and BMI of probands.

Proband	Body weight before/after the diet (kg)	BMI before / after the diet
1	69.5/65	23.3/22.61. i.e. normal body weight
2	108/104	33.3 1st degree obesity/32.1 1st degree obesity
3	55/52	20.2 ideal weight/19.1 underweight
4	65/62.2	22.76 normal weight/21.78 normal body weight
5	80.6/76.5	25.44 overweight/24.14 normal body weight
6	48/45.5	19.72 underweight/18.7 underweight
7	98/93.5	30.25 1st degree obesity 1. (due to strengthening and the total % of muscle mass, data on the BMI result are distorted in this proband)/29.01 overweight
8	76/73	26.96 overweight and after restrictive diet 25.86 i.e. overweight.

Table 11 shows the recorded subjective feelings of all 8 probands that they reported during the 7-day restrictive diet.

Table 11 Recorded subjective feelings during a seven-day restrictive diet.

Probands	Subjective feelings during a restrictive diet						
	1	**2**	**3**	**4**	**5**	**6**	**7**
1	15.00 - headache and a cold, 22.30 – shivering, heat, cold, conditions for vomiting, sweating (headache pills)	limb stiffness during exercise, cold, difficult speech, mild headache, an increase in energy in the afternoon, good sleep with dreaming	heavy legs in the morning after waking up, inability to exercise (only walking), mild headache, sleepy, low energy, afternoon nap 20 min.	tired, nervous, sleepy, morning nap 30 min. with dreams, heavy legs, malaise	afternoon nap 1 h 20 min, malaise, heavy legs, dark urine at night, constatnt dreams at night	great sleep, doesn´t feel hungry, feels good, heavy legs, a burst of energy, dreams at night constantly	heavy legs in the morning, cold, rush of energy and a good feeling
2	feels good	hungry, mild headache	rush of energy, doesn´t feel hungry	rush of energy, doesn´t feel hungry	weakness, fatigue	malaise	fatigue

Table 11 Cont.

Probands	Subjective feelings during a restrictive diet						
	1	2	3	4	5	6	7
3	hunger, dizziness, fatigue	hunger, mild headache, restlessness	nervousness, sleepy, cold, low energy	tired, nervous, malaise	dizziness	energy boost, great sleep, doesn´t feel hungry	cold, rush of energy, good feeling
4	conditions for vomiting, hunger	mild headache, good sleep	sleepy, low energy, nerveless	energy boost, good feeling, doesn´t feel hungry	rush of energy, good feeling	great sleep, doesn´t feel hungry, good feeling	heavy legs in the morning, cold
5	bad sleep at night, headache until 11:00, sluggish, weak, after 11:00 euphoria, good mood, a rush of energy, evening depression feeling like before an illness, headache, watery diarrhea strange taste in the mouth and plaque on the teeth, blurred image of headache, hunger and trembling hands, tensed body, problem with falling asleep, bloated abdomen in the stomach area	a surprising day of the opposite nature, a good feeling calm, slightly tired, he managed the whole training well, good at work, headaches are not the only gentle pressure in the forehead area	tired	tired, heavy legs, malaise	malaise	tired, tired muscles, hard to walk, great sleep, doesn´t feel hungry, feeling good	heavy legs in the morning. cold, energy boost and godd feeling

Table 11 Cont.

Probands	Subjective feelings during a restrictive diet						
	1	2	3	4	5	6	7
6	headache, tingling and trembling of the limbs, urge to vomit after eating potatoes 2 hours apart, cold, conditions for vomiting, sweating	malaise, small headache, energy boost after lunch, good sleep	heavy legs in the morning, inability to exercise (only walking), in the evening depression, skin manifestation on the face and back in the form of red rashes, headache, sleepy, low energy	tired, at lunch, the crisis began, shaking of the hands, trembling of the whole body, malaise	malaise, tired	great sleep, doesn´t feel hungry, feels good, heavy legs, rush of energy	cold, rush of energy
7	sleeps after training, unfocused, headache, sweating (medication used)	Headache, sweating, limb stiffness during exercise, cold, difficult speech, good sleep	heavy legs in the morning, inability to exercise (only walking), mild headache, fatigue, low energy	weak, nervous, sleepy, heavy legs, malaise	heavy legs, malaise	dreams at night constantly, great sleep, doesn´t feel hungry	heavy legs in the morning, cold
8	feels good, hunger	feels good, hunger, mild headache	feels good, hunger	hunger, nervosity	cold, hunger, nervosity	nervosity	dizziness**,** weak, without energy

Proband 1 reported feeling good about managing the restrictive diet. After the restrictive diet, cramps, tremors and excessive stool appeared the next day. Proband 2's creatine decreased, but cholesterol triglyceride increased (proband has a long-term problem with this). After completing the restrictive diet, they discovered cramps, tremors and excessive stool the next day. After the restrictive diet, he feels great. After completing the restrictive diet, Proband 3 felt well. Digestive problems improved. Proband 4 reported that he felt great after the restrictive diet. Proband 5 feels hungry and in a good mood. After completing the restrictive diet, he feels great and is determined to repeat it. After the restrictive diet, proband number 6's acne improved, and she felt relieved and great. Proband 7 developed convulsions, tremors, and excessive stools the next day after completing the restrictive diet. He was already looking forward to finishing the diet, it was suffering for the proband. Because he does weight training, his glycogen was quickly depleted, and he had the above conditions (Table 10). Proband 8 developed convulsions, tremors and had excessive stools on the second day after completing the restrictive diet. After the restrictive diet, he feels great.

Several studies have evaluated the signifcance of the glycemic index of various foods and glycemic load in patients with acne, demonstrating individuals with acne who consume diets with a low glycemic load have reduced acne lesions compared with individuals on high glycemic load diets. Dairy has also been a focus of study regarding dietary infuences on acne; whey proteins responsible for the insulinotropic efects of milk may contribute more to acne development than the actual fat or dairy content. Other studies have examined the effects of omega-3 fatty acid and γ-linoleic acid consumption in individuals with acne, showing individuals with acne beneft from diets consisting of fsh and healthy oils, thereby increasing omega-3 and omega-6 fatty acid intake **[22]**.

Millions of people suffer from painful and swollen joints associated with arthritis. In thepast, many doctors told arthritis patients that dietary changes would not help them. However, this conclusion was based on older research with diets that included dairy products, oil, poultry, or meat **[23]**, **[24]**. New research shows that foods

may be a more frequent con-tributor to arthritis than is commonly recognized. Patient interest in the effect of diet on RA has been noted for decades. Among a number of small clinical trials of dietary manipulation in RA (Rheumatoid arthritis), modest benefit has been noted for high-dose omega-3 fatty acids, fasting, vegetarian diet, and Mediterranean-type diet **[23]**, **[25]**, **[26]**. Safiri et al. **[27]** examined the effects of dietary modification on arthritis pain and disease severity in 44 adults previously diagnosed with rheumatoid arthritis randomized to a diet (vegan diet for 4 weeks, elimination of other foods for 3 weeks, and then reintroduction of eliminated foods individually for 9 weeks) or an additional (placebo) phase for 16 weeks. As a result, the disease activity score decreased. The mean number of swollen joints decreased from 7.0 to 3.3 in the diet phase ($p = 0.03$) and increased from 4.7 to 5 in the classic diet phase.

Kjeldsen et al. **[28]** in a controlled, single-blind trial tested the effect of fasting for 7-10 d, then consuming an individually adjusted, gluten-free, vegan diet for 3.5 mo, and then consuming an individually adjusted lactovegetarian diet for 9 mo on patients with RA. For all clinical variables and most laboratory variables measured, the 27 patients in the fasting and vegetarian diet groups improved significantly compared with the 26 patients in the control group who followed their usual omnivorous diet throughout the study period. One year after the patients completed the trial, they were reexamined. Compared with baseline, the improvements measured were significantly greater in the vegetarians who previously benefited from the diet (diet responders) than in diet nonresponders and omnivores. The insufficient intake of fruit regularly 5 times a day has been evaluated in research work of Juríková et al. **[29]**.

In the study by Markovič et al. **[30]** energy restriction (d4) reduced fasting plasma glucose, independently associated with reduced carbohydrate intake. Both energy restriction and body weight loss have beneficial effects on insulin action and glycemic control in obesity. The effect of energy restriction is related to changes in individual macronutrients, while the effects of weight loss are related to changes in abdominal fat.

It is well-recognized that standard caloric restrictions (1500 kcal/day) are usually poorly effective in achieving body weight losses in overweight type 2 diabetic patients. For that reason very low-calorie diets (VLCDs) were developed as a mean for initiating or accelerating body weight reduction. Short-term studies indicate that VLCDs generally result in body weight losses that are three times greater than those obtained with standard low-calorie diets. Fasting blood glucose values are improving in parallel to body weight losses and in many patients the improvement in glycemic control is better than that expected from the magnitude of body weight losses **[31]**.

Vegetarian diet consumption associated with lower concentrations of TC, LDL-C and HDL-C. High-density lipoprotein cholesterol was also lower in the vegetarian groups than in the Western diet groups. Clinical studies also reflect the long-term effects of a vegetarian diet on plasma lipids. Those who follow a vegetarian diet for longer may have a healthier body composition, which may affect blood lipids. The effects of a plant-based diet on plasma lipids are likely to be largely the result of differences in saturated fatty acid intake and, to a lesser extent, cholesterol intake **[32]**. In the set of assayed in research Fatrncová-Šramková et al. **[33]** female was noticed overlapped intake of cholesterol (450 mg and more) among 60% offemale with negative impact on incidence of cardiovascular diseases. The better results in cholesterolintake achieved Ervin **[34]**, 72% of older adults met the guidelines for cholesterol.

A significant difference in a study **[35]** was reported for TC, LDL and TG levels between samples. Higher levels were reported by consumers of a Western diet versus reduced levels in vegetarians, with the lowest levels reported by vegans.

According to Bunner et al. **[36]** dietary interventions may offer a promising approach to migraine. A low-fat, plant-based diet reduces headaches' frequency, intensity, and duration while reducing medication use. The most commonly reported triggers in these and other analyzes include: chocolate, cheese, citrus, alcohol, and coffee. In this study, patients reported triggers retrospectively, but other studies have identified triggers through elimination diets.

Although many acute and preventive medications are now available to treat migraine headaches, many patients will not experience significant improvement in headache frequency and severity unless lifestyle modifications are made. Due to the myriad side effects of traditional prescription drugs, the demand for "natural" treatments such as vitamins and supplements for common ailments such as headaches is also increasing. Studies examine the evidence for supplements in the treatment of migraine **[37]**.

The beneficial effects of a vegetarian diet on blood pressure control have been confirmed in many studies. A vegetarian diet is associated with a significant reduction in blood pressure compared to a Western diet, suggesting that it may play a key role in the primary prevention and overall management of hypertension **[38]**.

In our study, we used a diet that we chose, and it was consistent with other studies. Bernard study examined a vegan diet that incorporated vegetables, fruits, grains, and legumes, including vitamin B12 supplementation. This diet was characterized by its high fiber content and low-fat content, and there were no limitations on the amount, energy, or carbohydrate intake **[39]**. The 2017 study by the same author also implemented a diet with the same

composition **[40]**. In a study conducted by Bloomer et al. in 2015, they utilized a vegan-based Daniel fast diet that eliminated processed foods and animal products, without limiting food portion sizes **[41]**. The GEICO studies conducted in South Korea by Ferdowsian et al. and Mishra et al. **[42]**, **[43]**, as well as the studies by Macknin et al. **[44]** and Nicholson et al. **[45]**, all adopted a similar low-fat vegan diet similar to the one practiced in Bernard's study. Hunt et al.'s study, on the other hand, employed a lacto-ovo-vegetarian diet that included legumes, whole-grain bread and cereal products, and higher quantities of fruits and vegetables. This diet had 25% less protein, 12% less fat, 16% more carbohydrates, 21% more ascorbic acid, slightly lower saturated fat, and less than 100 mg/d less cholesterol than the nonvegetarian diet **[46]**. Prescott et al.'s interventional diet was based on the dietary practices of Seventh Day Adventist vegetarians as studied in Rouse et al. **[47]**. Ramal et al.'s study **[48]** utilized a plant-based diet adapted from the 30-Day Diabetes Miracle Cookbook **[49]** to accommodate the ethnic groups involved.

CONCLUSION

Eight probands participated in the seven-day restrictive diet. During this diet, the probands could consume a specifically vegetarian diet. In the case of blood biochemical parameters, there were positive changes in all probands, except for two probands, in which there was a significant decrease in creatinine. The probands' blood pressure after the restrictive diet's end shifted towards the values for normal pressure of 120/80. The body weight of the probands after the end of the diet decreased in the range of 3-4.5 kg, and thus there was also a change in their BMI. The subjective feelings of most of the probands recorded during all 7 days of the diet were negative on the first days (they felt tired, hungry, had headache, the feeling of heavy legs), but on the seventh day they felt surges of energy, had good sleep, and felt better. Dietary changes are crucial for lowering illness risk and hastening the course of treatment after a disease has been identified. Therefore, we recommend increasing the consumption of fruits and vegetables – fresh vegetables are the most suitable, salt and season food only moderately, limit fatty foods, eat regularly 5-6 times a day and in smaller portions, ensure enough movement.

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Changes in consumer behaviour in the food market in a crisis

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ABSTRACT

This article deals with changes in consumer behavior in the food market during a crisis. A crisis can be described as a pandemic during the COVID-19 pandemic, war conflict in Ukraine and a high inflation rate that causes increasing prices of food and other items. All of this affected consumer behaviour in terms of purchasing behaviour and preferences. Consumers changed their behaviour, and we could notice rationality and irrationality in many cases. The research involved 565 respondents in a questionnaire survey conducted in the spring of 2022. The confidence interval at the level of max determined the sample size. +-5% at the significance level α=0.95. The paper presents and examines three hypotheses directly connected with the main aim of the paper. The questionnaire survey provided a solid base for our statistical evaluation, where we used the Mann-Whitney test, Kruskal-Wallis test, Friedman test, Principal component analysis (PCA) and Divisive hierarchical cluster analysis. Research results proved that food design and packaging were among the least important factors when buying food online during a crisis, and younger consumers least avoided the Internet when buying food. Just partially accepted was proven our hypothesis that demographic characteristics (age, gender, place of residence) significantly influenced the importance of factors when buying online food in times of crisis.

Keywords: consumer behaviour, food, crisis situation, rationality and irrationality, preferences

INTRODUCTION

Understanding the purchasing patterns of consumers emerging during a crisis plays an important role in achieving the success of any business organization **[1]**. So far, the professional literature on food systems has mainly focused on the problems of adaptation to climate change but needs to pay more attention to how to solve the response of food systems to crises **[2]**. The world has experienced several health crises, such as epidemics or pandemics like Ebola, SARS, MERS, swine flu and dengue fever **[3]**. With short incubation periods and high lethality rates, Ebola, MERS, and SARS mainly shocked the food systems in certain areas where they spread **[4]**. They devastated agricultural production by damaging agricultural labour forces **[5]** and hindering other input factors **[6]**. One of the most dramatic events in recent decades was the COVID-19 pandemic. The virus spread quickly worldwide, leading to massive government interventions not seen in Western countries since the end of World War II. Retail has entered a new "abnormal state" driven by the pandemic. Personal shopping in brick-and-mortar stores was limited to buying food, fuel and drugstore goods **[7]**. The contagious disease broke out in China, quickly spreading throughout the world. Medical facilities were overwhelmed, and the government institutions of most countries declared a state of emergency in the aftermath of WHO recommendations **[8]**. In February 2020, the World Health Organization (WHO) officially named this coronavirus disease; in March of the same year, it was declared a pandemic **[9]**. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) **[10]**. The pandemic has had an impact on agricultural production and poses a threat to long-term food supplies and food security **[11]**. It represents a threat, especially to countries dependent on food imports, because many food-exporting countries have limited food exports as a precaution, which has caused a shortage, especially of animal foods, in developing countries. For example, in India, supplies of rice, lentils, bananas and tomatoes fell by 25% between March 15 and May 31, 2020, compared to the same period in 2019 **[12]**. Global and labor and trade restrictions have significantly complicated mutual interactions between different members of the food

vertical and revealed its vulnerability to externalities **[13]**. The food processing sector has proven relatively stable during the pandemic, and food price increases have been minimal in most cases **[14]**. Subsequently, the world was also affected by another crisis - the war conflict in Ukraine (from February 2022) and high inflation, reflected in the high food prices. Consumers began to behave differently. The virtual online world is becoming more and more important.

Online shopping brings many advantages for every consumer; the only condition is a connection to the Internet. Today, online shopping is almost unlimited and saves time and money **[15]**. Online marketing is becoming more and more popular among marketers every year. Marketing departments are gradually changing classic marketing approaches to online marketing tools. It is understandable. Informational changes in global markets lead to declining consumer interest in traditional information channels. Many of the marketing specialists of the young generation have stopped working with classic marketing tools but have entirely switched to online marketing. The target audience is trying to reach and connect with the company's products on the Internet **[16]**. Due to the crisis, consumers have suddenly been forced to change their habits and prioritize online channels in their shopping. Also, in the grocery sector, the pandemic has driven sales strongly toward online channels **[17]**. During the crisis, grocery retail witnessed changes in collective patterns of consumer behaviour, even a radical change in demand for certain products, online shopping and home delivery services. Retail operations with essential products such as food and healthcare have faced challenges in inventory, logistics management and ensuring a clean environment due to increased demand and requirements. **[18]**. Approximately one-third of food businesses changed their marketing strategy and, due to the crisis, used the Internet store for face-to-face food delivery and online communication with customers **[19]**.

The unexpected outbreak of the crisis had a global impact on health, the economy and humanity worldwide. The closure of retail establishments had an impact on changing consumer behaviour. Many consumers who shopped offline due to the outbreak of the pandemic have started shopping online. Not only the way of shopping is changing, but also the way of thinking. Online shopping is focused on basic products, but offline shopping is still preferred when buying groceries **[20]**. Many government institutions worldwide have implemented quarantine measures to limit the spread of the virus, forcing people to stay home and leave the house only to buy medicine, food, or work if working from home was impossible. These restrictions led to changes in consumer behaviour when buying food. These include rediscovering the home kitchen, shopping from small and local vendors, and shopping for groceries online **[21]**. A few years ago, food delivery was an insignificant topic. This was only changed by the anti-pandemic measures in 2020 and 2021, which forced many companies to reconsider their previous attitude towards digitization and business.

Home delivery of food gave many people a sense of relative safety, as they could avoid long queues at shops and thus limit the risk of contracting the coronavirus **[22]**. Strict hygiene measures were applied in supermarkets, food stores, and grocery stores as a result of the COVID-19 pandemic situation **[23]**.

Various measures caused by the COVID-19 pandemic have caused changes in consumer behaviour. There was an increase in purchases of food and alcohol and, consequently, their increased consumption. Screen time has increased with working and studying from home **[24]**. Consumers' grocery shopping behaviour has undergone significant changes since the outbreak of the COVID-19 coronavirus at the beginning of 2020. The immediate threat of COVID-19 hitting the cities encouraged panic buying behaviour, leading to food stocks being sold out **[25]**. The measures introduced after the outbreak of the COVID-19 pandemic led to significant changes in consumer behaviour in the food market. These were primarily larger volumes of certain types of food, panic buying and the rise of online grocery shopping. They consider stockpiling as panic buying, which can be rational and irrational **[26]**.

Scientific hypothesis

The main goal of the research was to determine changes in consumer behaviour in the food market in a crisis situation. In the presented research, the results would answer various questions about the given issue. In order to reach the given aim, the following scientific hypothesis was stated.

1. Food design and packaging are among the least important factors when buying food online during a crisis.
2. Younger consumers least avoid the Internet when buying food.
3. Demographic characteristics (age, gender, place of residence) significantly influence the importance of factors when buying food via the Internet in times of crisis.

MATERIAL AND METHODOLOGY

Research Sample

The research population is represented by 449,230 residents of productive age (15-64 years) living in the Nitra Region. The research involved 565 respondents in a questionnaire survey conducted in the spring of 2022. The confidence interval at the level of max determined the sample size. +-5% at the significance level α=0.95. The majority of respondents were women (72%) compared to representatives of the male gender (28%). He considers the above a sufficient ratio, considering that in CEE countries, women mostly take care of household purchases **[27]**. In the first step, we excluded some respondents with no online shopping experience (6.73%). The age and gender structure of this part of the respondents are presented below (Figures 1-3).

Moreover, as is clear from the graphic display, there are no significant differences between the gender ($p=0.028$), but from the point of view of age categories, it is clear that the smallest proportion of respondents who do not shop online can be found in the youngest generation ($p<0.001$).

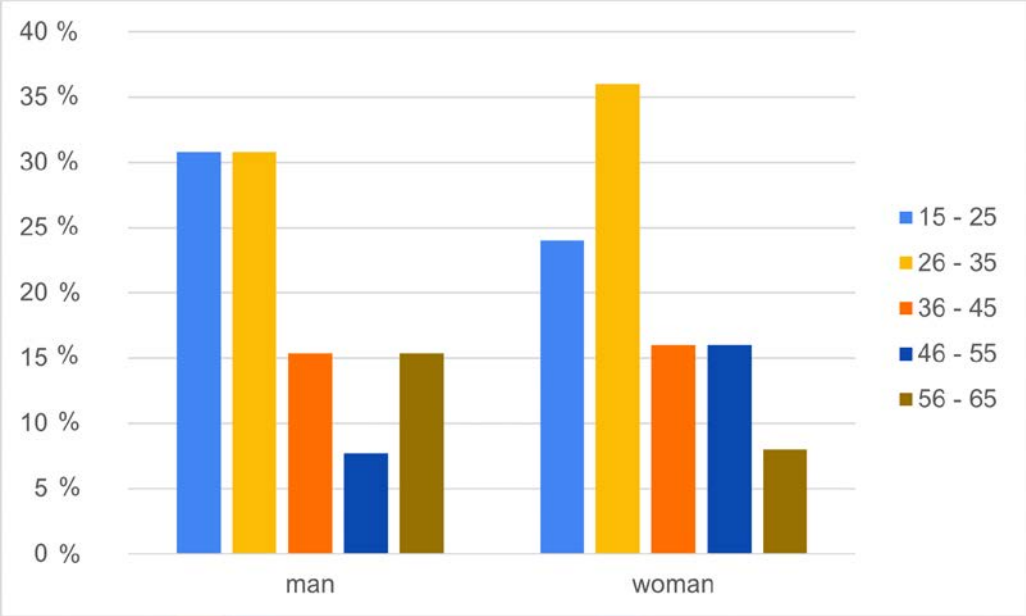

Figure 1 Age and gender structure of respondents.

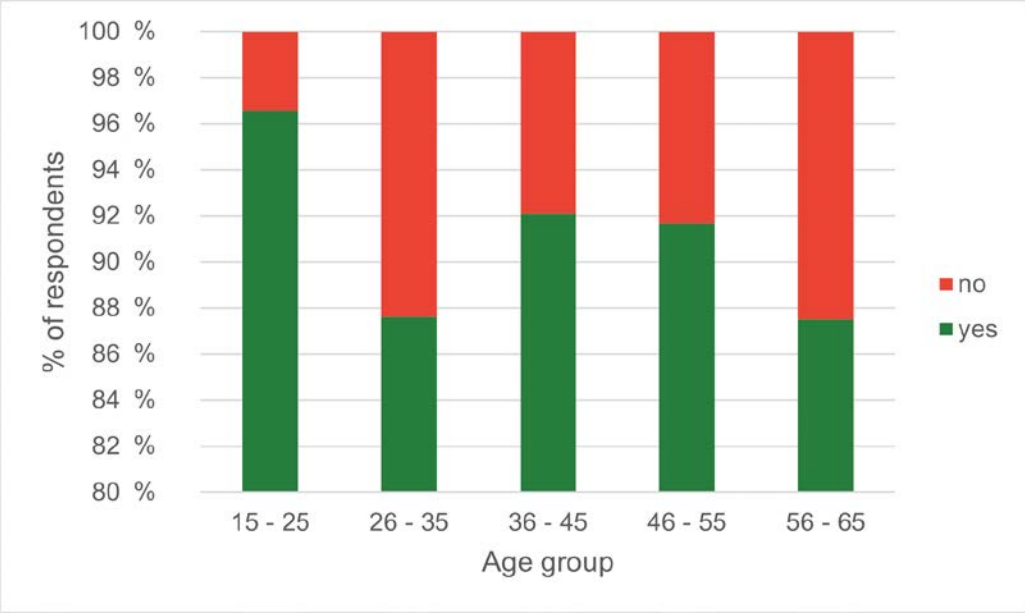

Figure 2 Respondents divided by age group.

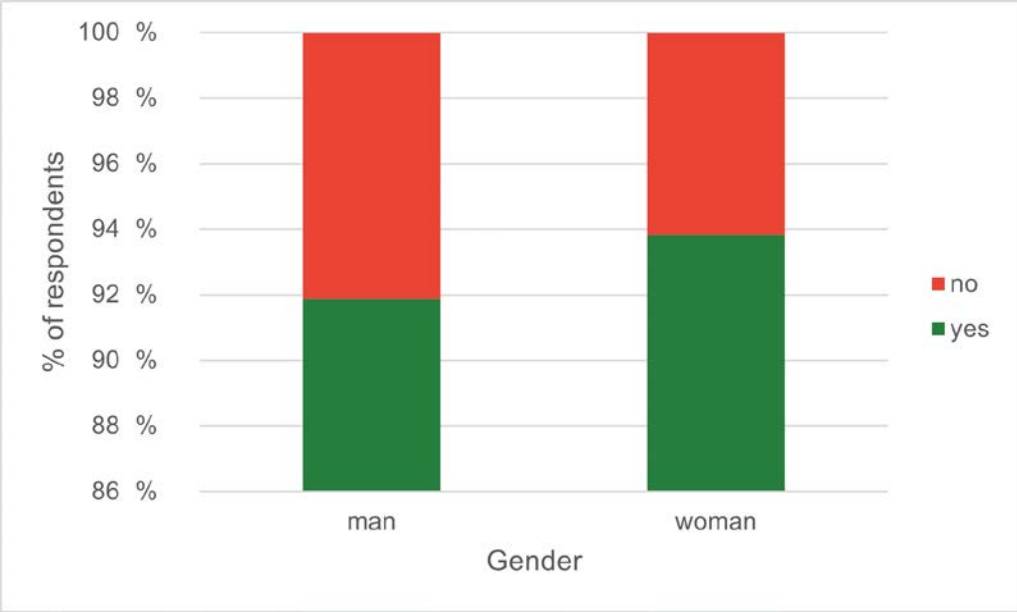

Figure 3 Respondents divided by gender.

Statistical Analysis

Based on the character of our research and above mention research sample, we decided to use the questionnaire survey to collect all relevant data, representing a base for our statistical evaluation. In order to achieve the appropriate results, the Mann-Whitney test, Kruskal-Wallis test, Friedman test, Principal component analysis (PCA) and Divisive hierarchical cluster analysis were used.

The Mann-Whitney test is a statistical method used to compare two independent groups of samples to see if there is a statistically significant difference between their distributions. This test is a non-parametric alternative to the t-test based on the normal data distribution. The Mann-Whitney test is also called the U test or the Wilcox test and is based on comparing the ranks of values.

The Kruskal-Wallis test is a statistical method used to compare three or more independent groups of samples unless the samples are normally distributed. The purpose of the test is to determine whether there is a statistically significant difference between the distributions of these groups. The Kruskal-Wallis test is a non-parametric alternative to the one-factor ANOVA test, which assumes a normal data distribution. The test is based on comparing the order of values of individual groups and calculating a statistical measure called the H-standardized statistical test.

The Friedman test is a statistical method used to compare multiple continuous groups of samples. This test is used when the data are not normally distributed, and the samples are measured in the same units. The purpose of the test is to determine whether there is a statistically significant difference between the distributions of these groups. The Nemenyi method is a post-hoc analysis method used to compare pairs of groups in the Friedman test. This method identifies statistically significant differences between groups and allows you to determine which group pairs differ.

Principal component analysis (PCA) is a statistical method that reduces the dimensions and obtains new independent variables (principal components) from the original variables. This method is often used in data analysis and visualization.

Divisive hierarchical cluster analysis is a method of data analysis that starts with one large cluster containing all observations and gradually divides it into smaller clusters based on the similarity between them. In this methodology, we will use the Euclidean distance to measure the similarity between observations.

RESULTS AND DISCUSSION

In order to aim at purchase factors that shape consumer decision-making, respondents were asked to rate the importance of the following factors on a scale from 1 to 5: food price, food quality, food freshness, food packaging, food design, country of origin of food, brand food and food delivery area. These factors were selected based on previous research and their potential impact on food purchasing decisions in times of crisis. We used principal component analysis (PCA) to process the obtained data (Figure 4).

Our analysis showed that the first two principal components explain more than 70% of the variability in the factors important in food purchasing in times of crisis. The first principal component includes all factors except food

design and packaging, which were included in the second principal component. This indicates that these two factors have a different influence on the respondents' decision-making when purchasing food in times of crisis.

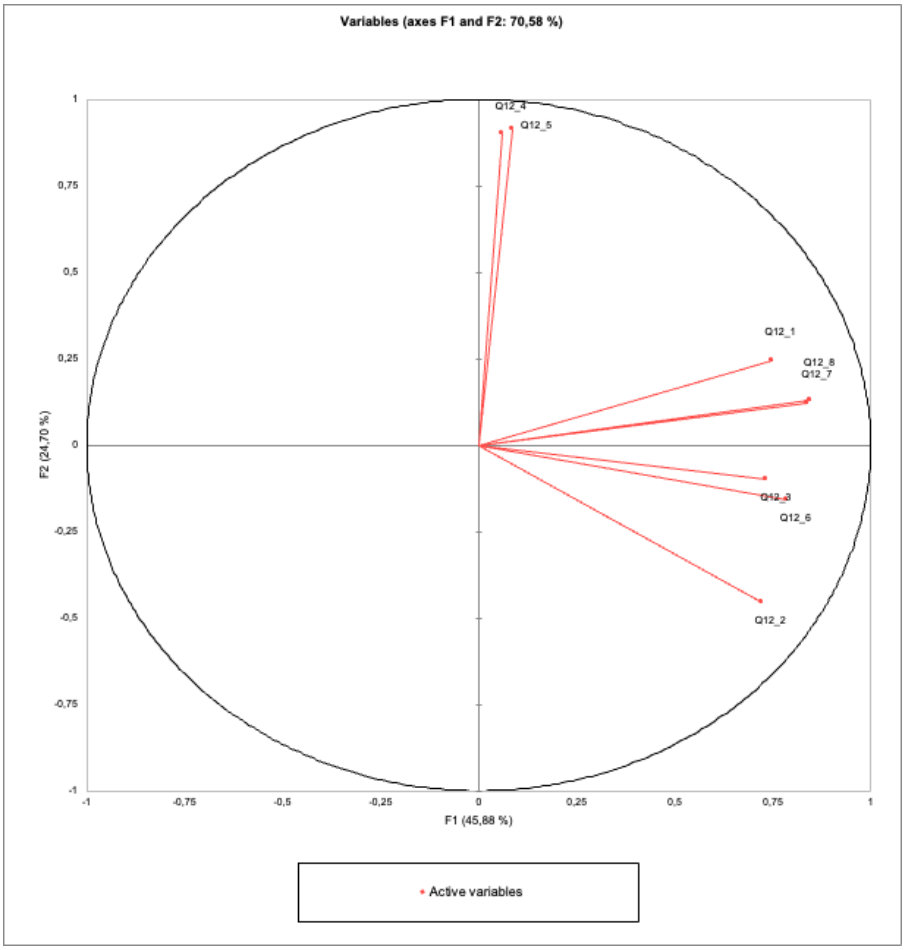

Figure 4 Principal component analysis (PCA).

Further analysis confirmed that the factors included in the second component, i.e., food design and food packaging, are the least important for the respondents. The Friedman test confirmed this result, in which we achieved a statistically significant result ($p < 0.001$). This finding suggests that respondents in our study considered food design and food packaging to be less important factors in food purchasing decisions during a crisis.

Table 1 Hierarchical cluster analysis of respondents.

Sample	Frequency	Sum of ranks	Mean of ranks	Groups		
Q12_5	527	1,169.000	2.218	A		
Q12_4	527	1,227.000	2.328	A		
Q12_7	527	2,617.000	4.966		B	
Q12_6	527	2,634.500	4.999		B	
Q12_2	527	2,712.000	5.146		B	C
Q12_8	527	2,757.500	5.232		B	C
Q12_3	527	2,923.500	5.547			C
Q12_1	527	2,931.500	5.563			C

Note: Statistical significance of differences between factors ranking (A, B, C). Q12_1 price of food, Q12_2 food quality, Q12_3 food freshness, Q12_4 food packaging, Q12_5 food design, Q12_6 food country of origin, Q12_7 food brand, Q12_8 food delivery area.

Subsequently, using hierarchical cluster analysis (Table 1), we classified the respondents into three categories (clusters), which can be characterized as follows:

Cluster 1 (least numerous cluster, n = 42):

This cluster consists of respondents who rate both factors, i.e., food design and packaging, as less important than others. This cluster represents a minority of respondents and indicates that for them, the price and quality of food are significantly more important when deciding to buy food in times of crisis.

Cluster 2 (n = 233):

This cluster consists of respondents who rate factors related to food design as more important and, conversely, the second group of factors, i.e., factors related to food packaging, as less important than other respondents. This cluster represents a substantial part of the respondents and indicates that food design has a greater,more significant influence on food purchasing decisions in times of crisis.

Cluster 3 (the most numerous cluster, n = 252):

This cluster consists of respondents who rate factors related to food design as less important as the first group of factors, i.e., factors related to price and food delivery area, are for them more important than for other respondents.

Table 2 presents the difference in assessing individual partial purchase factors based on basic demographic indicators, i.e., gender, age and place of residence of the respondents.

When we focus on the difference in the assessment of individual partial purchase factors based on basic demographic indicators, i.e., gender, age and place of residence of the respondents, we reach the following conclusions (Table 2) - differences can be seen in the factor price of food (Q12_1), which is evaluated differently among respondents in terms of their gender and place of residence. Food packaging (Q12_4) and food design (Q12_5) are evaluated differently among respondents according to age and place of residence.

Table 2 Differences in evaluating individual partial purchase factors based on gender, age and respondents' place of residence.

Variables	Countryside/Town	Age Older/younger	Gender Women/Men
Q12_1	C>T**	O=Y	W>M***
Q12_2	C=T	O=Y	W=M
Q12_3	C=T	O=Y	W=M
Q12_4	C>T*	Y>O**	W=M
Q12_5	C>T**	Y>O*	W=M
Q12_6	C=T	O=Y	W=M
Q12_7	C=T	O=Y	W=M
Q12_8	C=T	O=Y	W=M

Note: Q12_1 price of food, Q12_2 food quality, Q12_3 food freshness, Q12_4 food packaging, Q12_5 food design, Q12_6 food country of origin, Q12_7 food brand, Q12_8 food delivery area.

Next, we focused on the differences between individual food commodities from the point of view of their frequency of purchase. In our research, we examined 12 different food commodities. Using principal component analysis (Table 3), we successfully identified 4 foods with similar characteristics and purchase frequency. The first group is fast-moving foods, which include quickly perishable foods. This group includes meat, bakery products, vegetables and fruits, and fish and fish. These foods require more frequent purchases to prevent spoilage. The second group is products with a long shelf life, such as confectionery, frozen food and drinks. These foods have a longer shelf life and are less prone to rapid spoilage.

For this reason, the frequency of their purchase is lower. The third group is made up of vegetable oils. These foods have a specific character and are often used to prepare meals. Their purchase frequency depends on the individual required doses and use in the kitchen. The fourth group is specific foods, such as organic foods, healthy foods and tobacco products. These foods have their specific place in the market and are sought after for various reasons. Their purchase frequency differs from the remaining groups and depends on individual consumers' preferences and lifestyles.

Table 3 Analysis of main components - 4 food groups with similar characteristics and purchase frequency.

Variables	F1	F2	F3	F4
Q16_1	**0.800**	0.026	0.003	0.009
Q16_2	**0.805**	0.031	0.025	0.002
Q16_3	**0.790**	0.027	0.005	0.007
Q16_4	**0.781**	0.034	0.027	0.001
Q16_5	**0.573**	0.008	0.007	0.039
Q16_6	0.017	0.163	**0.537**	0.244
Q16_7	0.076	0.130	**0.610**	0.048
Q16_8	0.074	0.243	**0.464**	0.087
Q16_9	0.153	0.023	0.104	**0.601**
Q16_10	0.035	**0.513**	0.021	0.061
Q16_11	0.018	**0.534**	0.276	0.007
Q16_12	0.030	**0.554**	0.251	0.013

Note: Q16_1 milk and dairy products, Q16_2 bakery products, Q16_3 vegetables and fruits, Q16_4 meat and meat products, Q16_5 fish and fish products, Q16_6 confectionery, Q16_7 frozen products, Q16_8 alcoholic and non-alcoholic products, Q16_9 vegetable oils, Q16_10 tobacco products, Q16_11 nutritionally balanced foods, Q16_12 BIO foods.

Next, we focused on comparing the purchase frequency of these food commodities in online shopping. We found that fast-moving foods were the least frequently purchased online, while specific foods, especially tobacco products, were the most frequently purchased online. This difference was statistically significant, as we confirmed using the Friedman test ($p < 0.001$).

Table 4 presents the differences in the purchase frequency of individual commodities from the point of view of place of residence, age and gender. The table below presents the differences in the purchase frequency of individual commodities from the point of view of place of residence, age and gender. The research results show that the differences in the frequency of purchase of individual commodities, namely bakery products (Q16_2), vegetables and fruits (Q16_3), meat and meat products (Q16_4), confectionery (Q16_6) and vegetable oils (Q16_9) are striking depending on the age of the respondent; in the case of the commodity fish and fish products (Q16_5) they are striking in terms of gender and place of residence, and in the case of milk and dairy products (Q16_1) in terms of all three investigated demographic indicators, i.e., gender, age and place of residence.

Table 4 Differences in the purchase frequency of individual commodities from the point of view of the place of residence, age and gender.

Variables	Countryside/Town	Age	Gender
Q16_1	C>T**	O>Y*	W>M**
Q16_2	C=T	O>Y***	W=M
Q16_3	C=T	O>Y***	W=M
Q16_4	C=T	O>Y***	W=M
Q16_5	C>T*	O=Y	W>M**
Q16_6	C=T	Y<O*	W=M
Q16_7	C=T	O=Y	W=M
Q16_8	C=T	O=Y	W=M
Q16_9	C=T	O>Y***	W=M
Q16_10	C=T	O=Y	W=M
Q16_11	C=T	O=Y	W=M
Q16_12	C=T	O=Y	W=M

Note: Q16_1 milk and dairy products, Q16_2 bakery products, Q16_3 vegetables and fruits, Q16_4 meat and meat products, Q16_5 fish and fish products, Q16_6 confectionery, Q16_7 frozen products, Q16_8 alcoholic and non-alcoholic products, Q16_9 vegetable oils, Q16_10 tobacco products, Q16_11 nutritionally balanced foods, Q16_12 BIO foods.

Based on the presented results, we can conclude that our Hypothesis 1: Food design and packaging, among the least important factors when buying food online during a crisis, was accepted, and Hypothesis 2: Younger consumers least avoid the Internet when buying food. Just partially accepted was Hypothesis 3: Demographic characteristics (age, gender, place of residence) significantly influence the importance of factors when buying food via the Internet in times of crisis.

In the USA examined the behaviour of households when purchasing food during the COVID-19 pandemic on a sample of 1,370 respondents. They found three fundamental changes. The first was that the structure of spending on food changed. Expenditure on eating out was significantly reduced, caused by restrictions on restaurants, bars, etc. Secondly, the number of households that bought food online grew significantly. The third finding was that the price is not the decisive criterion for purchasing food, but its taste is **[28]**.
Similarly, a study in Germany showed that since the start of the crisis, there has been a strong boom in grocery shopping and an unprecedented increase in online grocery shopping. Existing retail chains primarily ensured this increase. There was no significant increase in new online grocers. Food retail remained unchanged regarding dominant business models and distribution mechanisms **[29]**.

During the pandemic, many people preferred a plant-based diet over an animal-based one. There was an increase in sales of plant foods and a decrease in animal meat. By increasing the consumption of plant-based food, consumers wanted to gain immunity or other health benefits; this food is also cheaper **[30]**. During the pandemic, many consumers experienced an interesting shift in eating habits. The consumption of plant-based foods, including meat alternatives, has increased significantly, which has also been reflected in the retail sales of plant-based foods in the USA **[31]**. Another survey conducted in the USA in May 2020 points to a 3% threat to food security, especially among households with children **[32]**. Due to COVID-19, consumers have suddenly been forced to change their habits and prioritize online channels in their shopping. Also, in the grocery sector, the pandemic has driven sales strongly toward online channels **[33]**. Given the increased concerns about human health, it seemed logical that consumers would buy more fresh food, but frozen and canned food products saw a significant increase in sales **[34]**. According to the survey agency, 2 must **[35]** among the most common barriers to buying food online is that people like to check food visually and sensorial, especially quality and freshness. Furthermore, it is the good availability of brick-and-mortar stores, the preference for personal purchases, or the impossibility of paying with food stamps. Moreover, last but not least, they are hindered by the number of fees for the delivery of purchases and the insufficient selection of food.

The crisis connected with the pandemic, the war conflict in Ukraine and inflation brought changes in consumer behaviour when purchasing food. The main findings indicate decreased food purchases in brick stores and an increasing preference for online shopping. The time spent in the store is shorter, but the average expenditure per purchase is higher. Despite this, the average monthly expenditure is lower **[36]**. People did not use these purchases because they perceived them as useful but because they were forced to use these services as part of the anti-pandemic measures. The most important purchase factors are the perceived usefulness of the product, ease of use of the seller's website, subjective standards and the buyer's trust in the seller. Only behind these factors is the product's price **[37]** in terms of importance.

Regarding online grocery shopping, we saw a 255% increase in households using grocery pickup and a 158% in households using grocery delivery services. These increases can be explained primarily by consumers worrying about the pandemic and feeling safer with these food shopping methods **[38]**. The pandemic has forced people to limit physical interactions, leading to a surge in online grocery shopping. Before the pandemic, up to 81% of U.S. consumers had never shopped for groceries online. This has changed significantly during the pandemic. Up to 79% of consumers bought food online **[39]**. Another study conducted in the USA points out that in 2020, compared to 2019, the purchase of food in retail stores increased by 4.8%.

On the contrary, food purchased in restaurants, buffets and other catering establishments decreased 19.5%. These changes result from anti-pandemic measures related to crises **[40]**. According to that **[41]**, only around 15% of consumers in Italy, Germany, and France are satisfied with their online grocery service. This supports the assumption that although the pandemic has forced consumers to use online grocery services, there is still an extensive need to improve these services. The results of a survey conducted in Russia on a sample of 1,297 respondents found these main changes in food consumption. Consumers reduced the number of purchases and bought larger quantities of food per purchase. They stocked up on non-perishable foods, bought healthier foods, cooked more at home and tried to reduce food waste **[42]**.

Similarly, a survey carried out in Finland on a sample of 2,568 respondents states that people are increasingly buying food online due to the impact of the crisis. The results indicate that the typical online food shopper is a consumer under 45 years of age with concerns about their health or the health of their loved ones. He lives in a larger household and the centres of larger cities **[43]**. Similarly, a survey carried out in Northern Ireland before the pandemic in March and April 2019 and the same period of 2020 during the pandemic recorded a significant

decrease in purchases reflected in the reduced number of completed transactions and a significant increase in the volume of the size of the shopping basket. This increase was due to a sharp increase in home food deliveries **[44]**.

In Slovakia, the authors found the following findings: Unfavorable price developments in 2022 changed the purchasing behaviour of Slovak consumers, who reduced the number of purchased goods and shopped more often. At the same time, the demand for products with lower added value and private label products increased **[45]**.

The crisis brought enormous economic uncertainty, which affected the consumers' shopping behavior and the choice of communication by companies **[46]**. It is important to add that not even during times of crisis, the behaviour of consumers is also strongly influenced by cultural, social, psychological and personality factors that influence the final consumer decisions **[47]**, however many of these decisions are irracional or based on emotions **[48]**, **[49]**.

Research **[50]** demonstrated that a high-risk perception, in the case of COVID-19 pandemic or other civil unrests, would cause the intention to buy goods that no longer follow common sense. The changes that emerged during the lockdown are persisting **[51]**.

The world has changed and never will be the same as before. Nowadays, we live in uncertain times. This is a simple way to summarize a situation that affects not only the global situation in the world but also the lives of each of us. We are facing the consequences of the ongoing war in Ukraine, migration, and the COVID-19 pandemic. Of course, there exist many issues that mark the current situation in the world, but from the point of view of marketing as well as how was changed our see of the world and the way of our think and conduct our lives, the most significant ones are those previously mentioned problems that influence our everyday lives and we will have to deal with them also in the future days. When having a closer look, we can conclude they have a huge impact not only on the economy and society but also on marketing and marketing decisions worldwide **[52]**.

CONCLUSION

The submitted paper aimed at changes in consumer behaviour in the food market in a crisis situation. The crisis can be described mostly as the COVID-19 pandemic, the war conflict in Ukraine and high inflation that causes increasing prices of food and other items. In the paper were presented and examined three hypotheses connected with the paper's main aim.

We can conclude that respondents in our study considered food design and food packaging less important factors in food purchasing decisions during a crisis and that younger consumers least avoid the Internet when buying food. We found that fast-moving foods were the least frequently purchased online, while specific foods, especially tobacco products, were the most frequently purchased online. Our research just partially proved the hypothesis that demographic characteristics (age, gender, place of residence) have a significant influence on the importance of factors when buying food via the Internet in times of crisis, as the results proved differences in the frequency of purchase of individual commodities in the crisis, namely bakery products, vegetables and fruits, meat and meat products, confectionery and vegetable oils which were depending on the age of the respondents. In the case of the commodity fish and fish products, these were striking in terms of gender and place of residence, and in the case of milk and dairy products, in terms of all three investigated demographic indicators, i.e., gender, age and place of residence.

However, our research has also some limitations. We focused just on the area of the Slovak Republic. The next limitation of our research is the fact that our research was focused only on consumer behaviour in the food market during the crisis. The solved issue might also be examined from the post-crisis period's perspective or in other areas besides the food market. We are convinced that the submitted paper creates a solid basis for further research and practical application in the food markets and consumer behavior field.

Consumer behaviour in a crisis situation shows rationality and irrationality when creating shopping behaviour preferences. The crisis taught consumers of all generations to purchase more online and behave more responsibly in many ways, as well as that life and health are the most important values.

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Characteristics of mucous-forming polysaccharides extracted from flax seeds

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ABSTRACT

The research used the seeds of long flax of the "Vruchy" variety and oil-curly flax of the "Original" variety. To extract mucus, whole flax seeds were hydrated for 3 hours. in tap water, at a ratio of 1:20 and a temperature of 18 – 20 °C with constant stirring with a magnetic stirrer. This study aimed to evaluate the effect of temperature and duration of extraction on the yield of mucilaginous polysaccharides in aqueous solution from flaxseed. Change range: the temperature is selected in the range from 0 °C to 100 °C with a step of 20 °C; with a duration, ranging from 10 min to 140 min in 10 min increments. The yield of polysaccharides from flax seeds was determined for each combination of controlled factors. It was established that in the first 10 – 20 min. there is an increase in the yield of polysaccharides and the rate slows over time. For 90 min. equilibrium occurs at a temperature of 80 °C. This period of the process is optimal for the extraction of mucilage-forming polysaccharides from flaxseed. The mass of the extracted polysaccharides, from the mass of the seeds after a time of 95 min was 5.74%, and 6.00% at a temperature of 80 °C. A package of applied statistical programs was employed during the research to process the experimental data. A mathematical model of the process of extracting mucus-forming polysaccharides in an aqueous solution of flax seeds was built using regression analysis methods. The obtained regression equations determined the optimal regimes of the sought values in terms of temperature (80 – 85 °C), time (85 – 90 min) and conducted in compliance with the prescribed amount of water of 200 cm^3. Within 10 – 20 min the formation of a transparent gel capsule around the flax with a phase separation boundary under seed contact with water, which does not change further. This indicates the completion of the hydration process.

Keywords: flax seeds, polysaccharides, mucus, extraction, additive

INTRODUCTION

Today there is a great challenge to the problems that often arise and human faces. The main ones are the high-quality provision of food to the population, energy provision, raw materials, including water, ecological and radiation safety of inhabitants, and protection of the person against the results of negative activity **[1]**. To a large extent, nutrition determines a person's health and life expectancy. The current rather complex ecological situation **[2]** dictates new approaches to processing natural raw materials: the ways of their fullest use are necessary.

Provision of the population with quality food is one of the most important tasks for Ukraine and any other country. Food affects the human body from birth and determines the development of the body. After all, getting into a human body, **[3]**, **[4]** at the expense of difficult biochemical transformations, during a metabolism, creates structural elements of cells. The body is provided with energy that is necessary for physiological and mental

activity. In addition, nutrition determines the health, activity, and protected life expectancy of a person with the ability to reproduce. Thus, the state of nutrition is and remains one of the most important factors determining the nation's health **[5]**.

Food should provide the body not only with nutrients but also contribute to the prevention and treatment of diseases. Bakery and confectionery products with the use of raw flour are a component of the daily human diet. Therefore, giving them the properties of a health product is an important problem today, because the chemical composition they are not sufficiently balanced with important ingredients **[6]**.

Flax is one of the most universal and valuable industrial crops and one of the most promising in dynamic development. Demand for flax and flax products in Ukraine and other countries is characterized by high interest in its use in medicine, cooking, and cosmetology **[7]**, **[8]**. Ukrainian food producers do not often use this type of raw material, although it has a unique biochemical composition and pharmacological properties. They are due to the high content of substances in the provision of preventive measures and treatment of cardiovascular, gastrointestinal, cancer, and many other diseases. Therefore, food manufacturers have begun to pay special attention to the extraordinary benefits of flax seeds for health prevention and treatment.

One of the ways to ensure healthy nutrition is to enrich basic products with non-traditional types of raw materials, particularly flax seeds. Flax seeds are a promising raw material for normalizing the fatty acid composition of food products, enriching them with water-soluble polysaccharides and flax lignans.

Despite the rapid development of scientific and technological progress and a mass of artificial fabrics, resins, and oils, economic interest in flax as an oil and yarn crop is not decreasing, but increasing sharply **[9]**. In recent years, the cultivation of oilseed flax in Ukraine has grown dynamically. Flax has several advantages over other crops due to stable yields (14 – 24 kg/ha) and early maturity (the end of July). Flaxseed's main components are oil (41%) and protein (21%). However, depending on the variety, growing environment, and processing methods, the ratio of these components in flaxseed can vary significantly **[10]**. In addition, flax is an important medicinal plant. Flaxseed oil is used in the diet of patients with disorders of fat metabolism, atherosclerosis, coronary heart disease, brain, hypertension, diabetes, liver cirrhosis, hepatitis, fatty liver disease, skin diseases, and various inflammatory processes.

Nowadays, only oil is produced from flax seeds on an industrial scale, using one of the traditional mechanical methods. In terms of biological value, flaxseed oil ranks first among other edible vegetable oils. It contains a lot of useful substances for the body (polyunsaturated acids, vitamins F, A, E, and K, and saturated fatty acids (10% of the composition), which is shown in Figure 1.

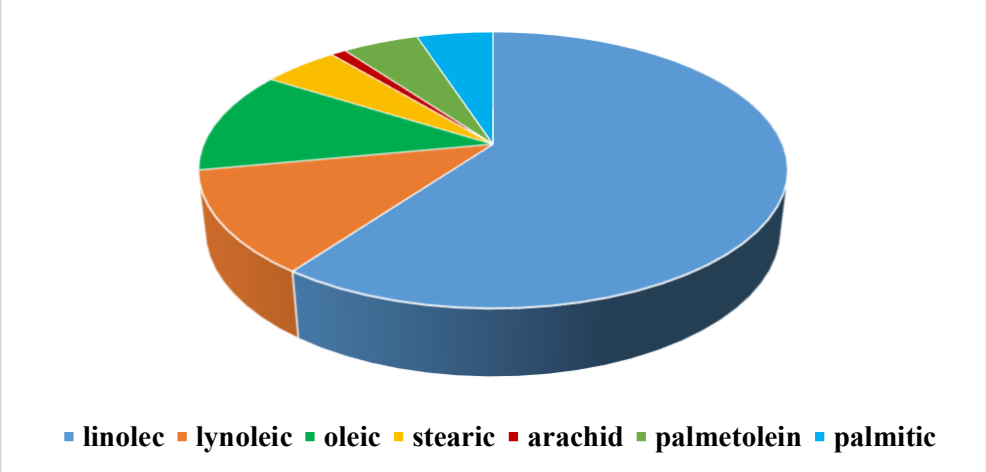

Figure 1 Fatty acid composition of linseed oil.

In scientific works **[11]**, **[12]** it was proved that linseed oil contains a large amount of unsaturated fatty acids, the consumption of which with food lowers the level of cholesterol. Based on the results of research presented in the manuscripts **[13]**, **[14]**, it was established that substances in flax seeds are effective in treating various types of tumors. The following scientific works **[15]**, **[16]** were devoted to the study of the properties of linseed oil, in which the author teams studied the content of polyunsaturated α-linolenic acid, which is part of almost all cell membranes, is an indispensable acid in the human diet and contains a large amount of vitamin E. The use of domestic raw materials of plant origin, which has a high potential for biologically active substances, allows you to purposefully create products with functional properties, as well as allows you to expand the range of products, and increase their nutritional and biological value. Flax seeds contain a significant amount of protein (about 25%),

fat (30 – 48%), which contains 35 – 45% glycerides of linolenic acid, 25 – 35% linoleic acid, 15 – 20% oleic acid and a small number of glycerides of palmitic and stearic (8-9%) acids.

Flax seeds are an excellent source of valuable polyunsaturated fatty acids ω-3 and ω-6. These are vital acids the human body cannot produce on its own, and should only be obtained from the foods we consume. The content of w 75.00% (of the total mucus content) of the most viscous neutral polysaccharide with a molar mass of 1.2C3.75% of acid polysaccharide AF1 with a molar mass of 6.5.10 5 g/mol; 21.55% acid polysaccharide AF2 with a molar mass of 1.7.10 4 g/mol. Flax seeds contain vitamins D, B2, B3, B4, B6, and B9, tocopherols, β-carotene, macro- and microelements: potassium, calcium, magnesium, iron, manganese, copper, chromium, selenium, aluminum, iodine, zinc **[17]**.

The authors **[23]**, **[24]**, present the characteristics of the insoluble fiber fraction. It consists of cellulose and complex polymeric compounds such as lignin. These fiber forms are valuable food components due to their physiological action. They promote bowel function, prevent atherosclerosis and improve lipid metabolism. Fiber is about 28% of the dry weight of non-fat flaxseed.

Note that a distinctive feature of carbohydrates is the content of water-soluble polysaccharides. When wet, they can form mucus on the surface of the seeds. Their number is 2-7% of the total mass. All other biologically active seed ingredients, such as mucus, are not so widely used due to the lack of rational and efficient technologies for their production.

It should be noted that the mucus of flax seeds is rich in macro-and micronutrients such as potassium, calcium, magnesium, iron, manganese, copper, zinc, chromium, aluminum, selenium, nickel, iodine, boron. According to **[18]** the mucous substances of flax seeds contain fibrous materials diameter of 18-45 nm **[19]** which are stretched in the presence of water, and are connected with the same fibers of seeds nearby (Figure 3). Hydrophilic groups of mucous molecules retain water in the middle of the cells of this grid, thus creating the effect of "freezing". According to various information data **[20]**, **[21]**, flax seeds can retain water in a multiplicity of 7 to 27 units, about its mass. This is a prerequisite for the use of flax seeds not only as enrichment but also to regulate the technological characteristics of food products.

Anatomomorphological studies of cross-sections of flax seeds **[22]** showed that the shiny, dry state, surface of the shell is determined by a vitreous layer of dehydrated mucus-forming polysaccharides

The study aimed to analyze the process of extracting mucilage-forming polysaccharides from flax seeds. To evidence their composition and properties and to develop a method of dehydration to form a dry powdered additive with structure-forming properties.

Scientific Hypothesis

This hypothesis is aimed at determining the technological influence of the main factors of moisture absorption and moisture release in the germination process of "Vruchiy" and "Original" flax seeds with the disclosure of the extracted characteristics of the complex of mucilaginous polysaccharides with further use in the bakery industry. Taking into account the complex process accompanied by mechanical, physical, and chemical phenomena, the influence of factors on the germination of flax seeds is mathematically described using the methods of regression and correlation analysis. This analysis helps to establish optimal modes of germination of flax seeds.

MATERIAL AND METHODOLOGY

Samples

Long-term flax seeds of the "Vruchiy" variety and oil-curly flax of the "Original" variety were used for research. Flaxseeds were cleaned thoroughly to free them from dust, dirt, and other foreign matter.

Damaged seeds were discarded. Previous studies **[23]** found that these varieties have the best biochemical composition and are promising raw materials in the production of dietary supplements and healthy and functional foods. Flax seed carbohydrates are of particular interest. Fibre and mucus-forming polysaccharides stand out among them. Figure 2 clearly shows that flax seed fiber consists of insoluble and water-soluble fractions. The insoluble fiber fraction is characterized by cellulose and complex polymer compounds such as lignins. These forms of fiber are valuable components of food products due to their physiological benefits.

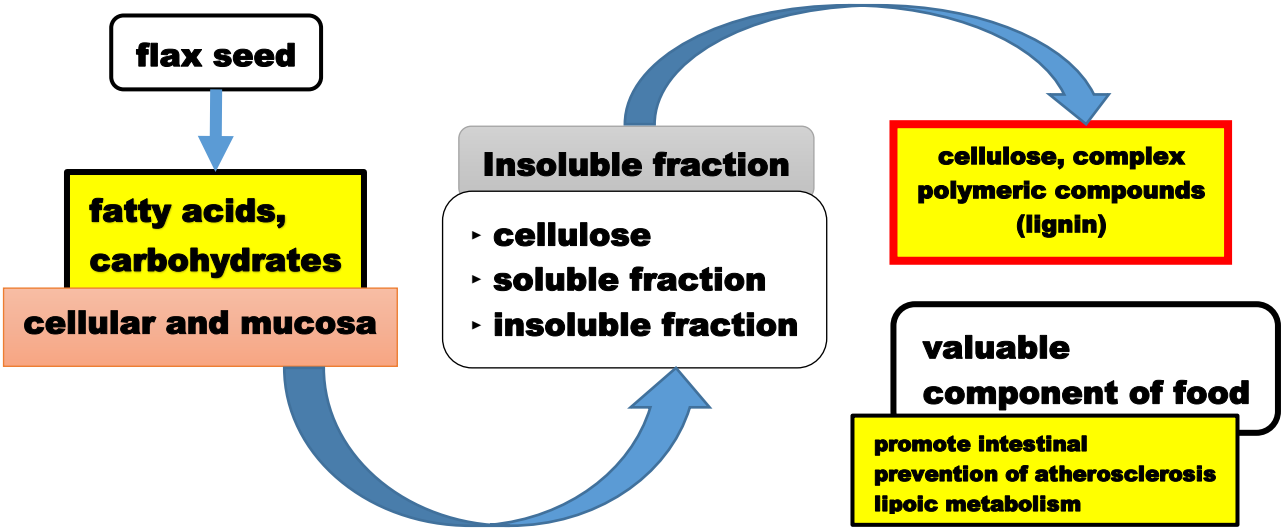

Figure 2 Scheme of the physiological action of carbohydrates of flax seeds.

The main physical and mechanical properties and physicochemical parameters of seed quality are presented in Table 1.

Table 1 Quality indicators of different varieties of flax seeds.

Grade	Humidity, %	Oil content, %	Bulk density, kg/m^3	Weight of 1000 seeds, gr
Vruchiy	8.6	33.82	712	6.62
Original	8.7	38.75	752	4.73

All methods of isolating polysaccharides from flax seeds are based on extraction processes with water or salt solutions. The extraction is carried out either from intact flax seeds or directly from the shell separated from the core. The polysaccharide product is isolated from the extracts, mainly by precipitation by alcohol (ethyl or isopropyl) **[24]**, then it is dried using a lyophilic or spray dryer.

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 extracted polysaccharides from flax seeds (1% NaCl solution).

Animals, Plants, and Biological Materials

Flax seeds of the following varieties were used for experimental research: Gladiator (producer: Sofia farm, Vinnytsia region, Ukraine); Hetman (producer Sofia farm, Vinnytsia region, Ukraine); Monk springs (supplier: Svitanok Farming, Rivne Region, Ukraine), which are recommended for cultivation in the Polissia and Forest Steppe zones.

Instruments

Drying of flax is performed on the drying cabinet SECH-3MK. Flax drying was carried out at a temperature of no more than +65 °C and heating of seeds 35 – 45 °C. To control the temperature of the seed during its drying period, samples are taken every 20 – 40 minutes for 10 hours. Photographs of samples using a microscope Bresser Biolux LCD 50x-2000x at a magnification of × 300 times. The temperature was measured with a thermometer TLS Ukrainian manufacturer "Glass Device". The obtained results were statistically processed using the standard Microsoft Office software package.

Laboratory Methods

Sampling was performed according to DSTU ISO 4803:2013 GUEST 4803:2013 **[26]**, Organoleptic evaluation "Descriptive (qualitative) method of profile analysis" DSTU ISO 4910:2008 **[27]**. The extraction efficiency is influenced by the following factors: the ratio of raw material and solvent (hydromodule), temperature, and time of the extraction process. When selecting a rational hydro module, it was taken into account that an increase in the mass fraction of the extractant leads, on the one hand, to an increase in the driving force and, on the other hand, to a decrease in the concentration of extracted substances. Irrational selection of the hydraulic module increases the cost of the target product, as it will require a larger volume of extractant or a longer process of their concentration. In this case, a decrease in the mass of the extractant leads to an increase in

the viscosity of the solution, which also leads to an increase in power consumption. The optimal and economical hydraulic module for the process on an industrial scale, as defined by the authors **[28]**, is a hydraulic module in the range of 18-20, which we used in research.

To remove mucus, whole flax seeds were hydrated for 3 h in tap water, at a hydromodule of 1:20 and a temperature of 18 – 20 °C with constant stirring with a magnetic stirrer. Water as an extractant is associated with its food and pharmaceutical applicability. Before extraction, the raw material was not subjected to any pre-treatment. The resulting mass was poured into boxes and dried at a temperature of 50 °C for 10 h in SECH-3MK. According to the method **[29]**, the dried mucus was separated from the seeds by rubbing through a sieve No. 40 with a through mesh of 0.42 mm. Using an Oswald viscometer, the viscosity of the resulting solution was measured, and the dry residue was determined.

The resulting dry residue was weighed and redissolved in a volume of water equal to the original (taken for extraction). The viscosity of the solution's polysaccharides is reduced, which is important in using dry polysaccharides. In fig. 3 shows the localization of mucus-forming polysaccharides in flax seeds based on an enlarged fragment of the cross-section of the seed, × 200. The arrows show a vitreous layer of dehydrated mucus-forming polysaccharides.

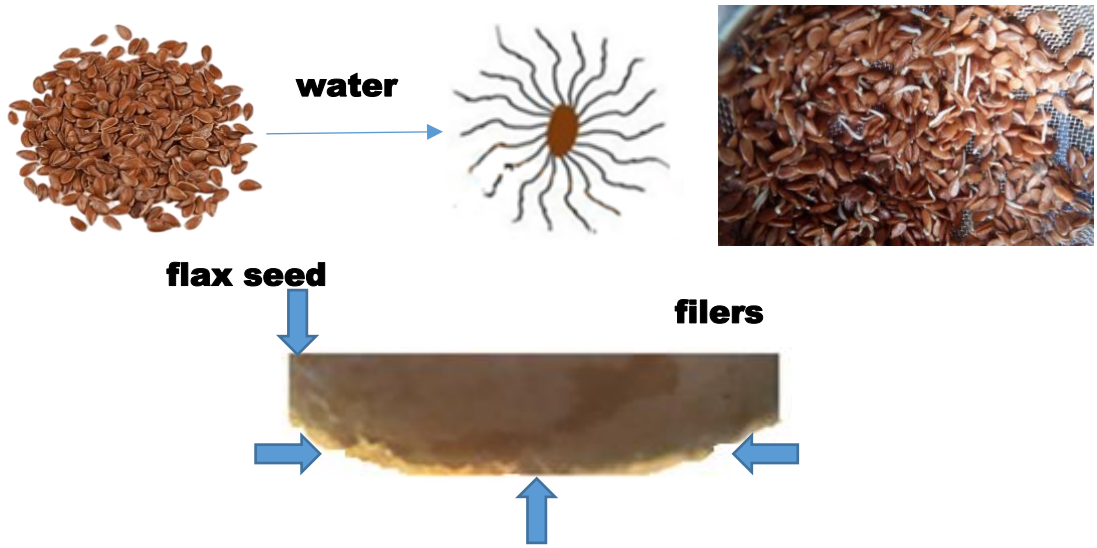

Figure 3 Modern ideas about the mechanism of gelation of mucous membranes substances of flax seeds.

Using salt solutions to extract polysaccharides from flax seeds (1% NaCl solution) facilitates the process. Thus, the viscosity of the extracts is reduced by almost three times, and the yield of the final product is increased **[30]**. However, in the product the carbohydrate part decreases and the content of protein and ash elements increases. Therefore, we used water in our research.

Methods for determining the study data: Sampling of flax seeds was performed by DSTU ISO 8837:2019 **[31]**. The mass fraction of moisture was determined by the method of accelerated drying in an oven SECH-3MK **[32]**.

Flax seeds' swelling dynamics were evaluated on a microscope Bresser Biolux LCD 50x-2000x at a magnification of × 300 times. The ability of flax seeds to retain water was determined by the amount retained in the sample after infusion and centrifugation of the appropriate suspension. The ratio of flax seeds: to water in suspension was 1:10. The indicator's value was determined as a percentage by the ratio of the difference between the amount of water used and the weight of the obtained supernatant to the weight of the sample **[33]**.

Description of the Experiment

Sample preparation: The dependence of the extraction of mucus from flax seeds by extraction over time and in tap water at a temperature of 18 – 20 °C sets the study's parameters. The influence of parameters on extraction was constructed by the method of the planned experiment. The zones of rational extraction parameters according to the direction of the process are established by the method of a planned experiment.

Number of samples analyzed: 5 samples of each of 3 hundredths of flax, weighing 0.1 kg, were selected for research.

Number of repeated analyses: All measurements of instrument readings were performed 5 times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was also 5 times.

Design of the experiment: Variable factors, optimization criteria, and the area of definition of factors are found. The choice of factors influencing the water extraction process of polysaccharides from flax seeds was carried out according to the parametric scheme (Figure 4).

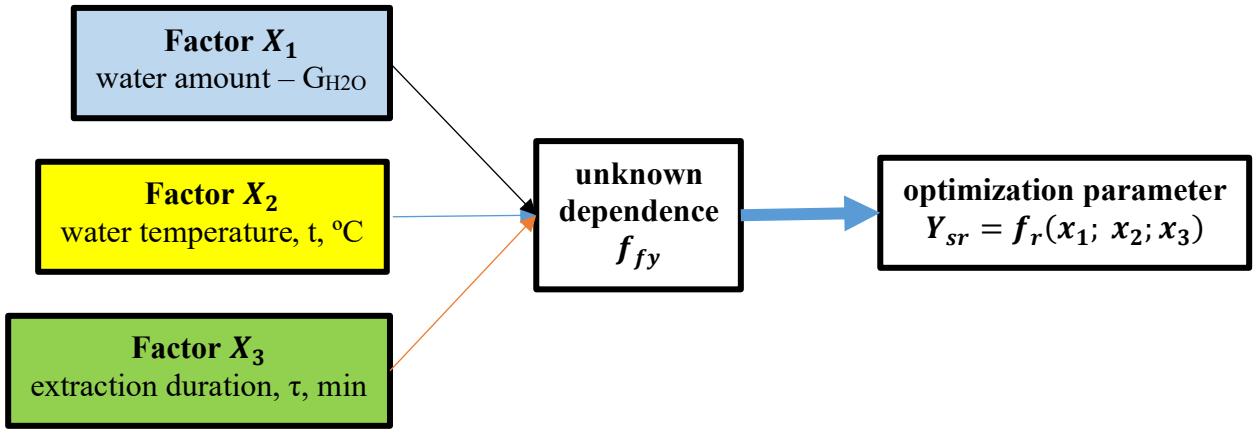

Figure 4 Scheme of the model of the planned experiment of PFE 3^3.

The main factors influencing the process are: water quantity - G_{H2O}, cm^3; water temperature – t, °C; extraction duration – τ, min The extraction efficiency was evaluated by the amount of dry matter transferred to the extract from 100 g of flax in terms of dry matter (Y_{sr},%). Independent variables were taken: the amount of water – G_{H2O}, cm^3, which was encoded by the index; water temperature – t, °C, which was coded by the index; extraction duration – *τ*, min, which was encoded by the index.

The reliability of the evaluation of the results of experimental studies with effective extraction by the amount of dry matter was ensured by the minimum number of measurements of the above indicators, the method of which is described in **[34]**.

After coding the factors, a planning matrix of the PFE 3^3 experiment was compiled for the total number of experiments $N = 3^3$. Thus, an approximate mathematical model in the form of a functional dependence $Y_{sr} = f_Y (x_1; x_2; x_3)$ was chosen to study dry matter Y_{sr}. The response function, namely the optimization parameter, was taken as a complete square polynomial, which describes the real experimental process:

$$T = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2$$

Where:

Y_{sr}, is the experimental value of the dry matter; b_0, b_1, b_2, b_3, b_{12}, b_{13}, b_{23}, b_{11}, b_{22}, b_{33} are regression coefficients that correspond to the corresponding values of the input factors; – input coded factors.

The coefficients of the approximating polynomial, represented as a complete quadratic equation under orthogonality and symmetry, were determined by known formulas **[34]**. According to the method **[34]**, the reproducibility of the obtained values from the experimental array was checked at the same number of repeats for each experiment performed according to Cochren's criterion. At the same time, after checking the adequacy of the distribution of random variables to the real process, the statistical significance of the regression coefficients was assessed using the Student's t-test. We used the statistical software package for the PC "Statistics 6.0" to build and analyze the obtained dependencies.

Statistical Analysis

Statistical processing of the results of experimental studies was carried out using the program (STATISTICA 12) from the company StatSoft for a series of parallel measurements (n = 4-5, p <0.05). During the optimization of technological parameters, the method of an incomplete factorial experiment with the formulation of a regression equation and optimization by the method of "least squares" using the Mathcad package of applied mathematical calculations is used. Approximation of the results, presented in the form of three-dimensional diagrams, was carried out using polynomial regressions. The importance of influencing factors on the dynamics of hydration of mucus-forming polysaccharides is the creation of objective control. It is aimed at economical use of material and energy resources. At the level of probability p = 0.95 and the value of the t-alpha criterion equal to 2.365, the

following statistics were obtained (Figure 5): coefficient of multiple determination D = 0.926; coefficient of multiple correlation R = 0.962; standard deviation of the estimate s = 0.084; Fisher's F-test is 10.776. The coefficient D is significant with a probability level of P = 0.99961.

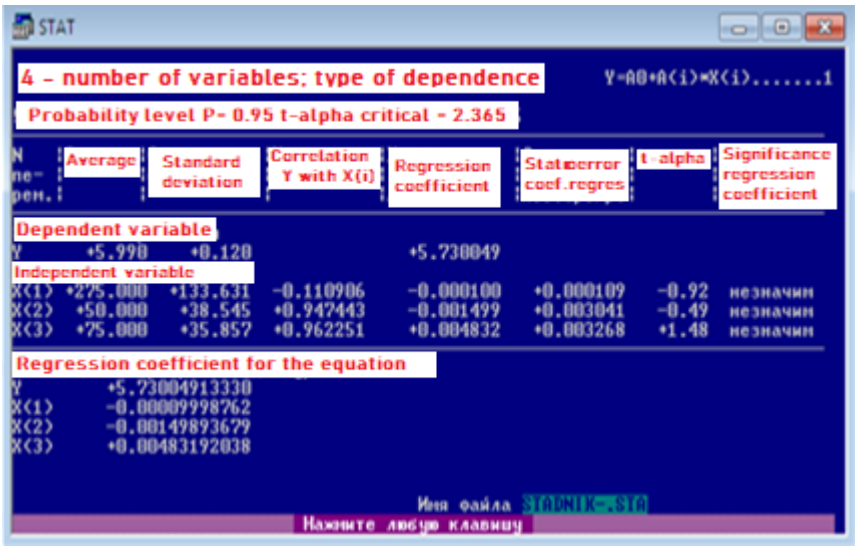

Figure 5 Statistical evaluation.

RESULTS AND DISCUSSION

Based on the results of the obtained calculations, which were performed using a package of applied statistical processing programs, as well as analysis of experimental research results, the obtained regression equations were recorded for a personal computer, and the spatial dependences of the response surfaces of the required dry matter values (Y_{sr}, %). They were determined from the obtained values of the amount of water – G_{H2O}, cm^3, water temperature – t, °C, the duration of extraction – τ, min.

The obtained regression equations characterizing the functional change of the required values of dry matter (Y_{sr}) in natural values for flax "Vruchiy" and "Original":

by temperature:

$$Y_{1tsr} = 5{,}73 - 0{,}99\ 10^{-4}\ G_{H2O} - 1{,}5\ 10^{-3}\ t + 4{,}83\ 10^{-3}\ \tau$$
$$Y_{2tsr} = 5{,}7 - 0{,}42\ 10^{-3}\ G_{H2O} - 0{,}33\ 10^{-2}\ t + 6{,}83\ 10^{-2}\ \tau$$

by time:

$$Y_{1\tau\,sr} = 4{,}55 - 0{,}99\ 10^{-3}\ G_{H2O} + 0{,}036\ t - 0{,}013\ \tau$$
$$Y_{2\tau\,sr} = 3{,}99 - 1{,}47\ 10^{-3}\ G_{H2O} + 0{,}038\ t - 0{,}86\ 10^{-2}\ \tau$$

When constructing the response surfaces of the influence of two independent values of factors on the change of dry matter (Y_{sr}, %), the third was assumed to be constant, giving it an average value from the corresponding range of the lower and upper limits. These regression equations characterize the change in dry matter (Y_{sr}, %) depending on the parameters within the following limits of change of input factors:

X_1, – $G_{H2O,}$ $см^3$(400-150); X_2 – t, °C (20-110); X_3 – τ, min (10-120).

According to the equations, the response surfaces of (Figure 6) are constructed.

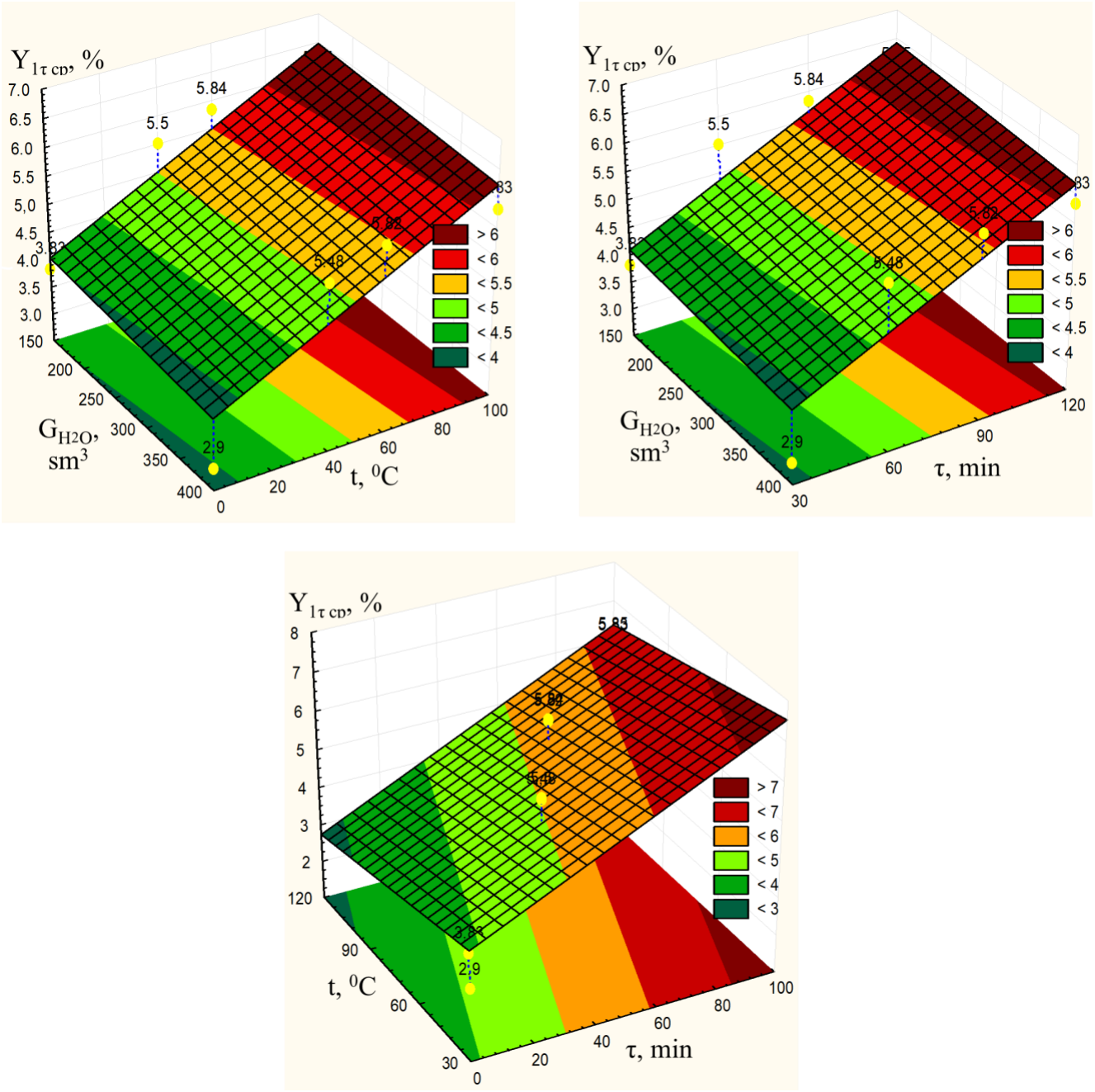

Figure 6 Surface response change of dry matter depending on water and temperature; water and time; temperature and time.

Figure 6 clearly shows the effect of parameters on the quantitative yield of mucus-forming polysaccharides. Determination of the effect of duration and temperature on extraction found that during the first minutes, there is a sharp increase in the yield of polysaccharides from flax seeds. This process period slows down over time, and equilibrium occurs after 70 minutes of water contact with flax seeds. Equilibrium occurs when the set amount of water is 200 cm^3, significantly playing during extraction. Therefore, these parameters can be optimal for extracting mucus-forming polysaccharides from flax seeds.

The interaction of water, duration, and temperature indicates a positive effect of hydration on the intensity of its dispersion. So, the response surface (Figure 6) shows that the influence parameters are important for establishing and determining the area of their influence. The interaction of the parameters depends on the percentage formation of polysaccharides. Thus, achieving the optimum mucilage-forming polysaccharides in flax seeds begins with a hydration time of 60 minutes. at a temperature of 75 – 85 ºC while maintaining the amount of H_2O 200 cm^3. their uniform connection. We can note that the beginning of mucus formation is already at 50 min showing 5.19% polysaccharides. Polysaccharides reach their maximum at 5.9% at parameters: t = 90 0C, G_{H2O} =

200 sm^2, and time τ = 80 min. Thus, without increasing the consumption, the effectiveness of the hydration dynamics of mucus-forming polysaccharides in flax seeds was achieved.

In scientific manuscripts **[35]**, **[36]**, **[37],** the authors conducted a study of the amino acid composition and polysaccharide complexes of the above-ground and underground parts of the spring primrose. Fractions of water-soluble polysaccharides and pectin substances were isolated from the studied raw materials, and their quantitative content was determined. It should be used (gas chromatography method) to determine free sugars' qualitative composition and quantitative content.

Indeed, the authors presented and disclosed in the paper **[38]**, **[39]** that molecules simultaneously interact with proteins and non-starch polysaccharides of seeds. The insoluble fraction of non-starch polysaccharides of flax seeds is 20-22% of its mass and consists mainly of cellulose, a small amount of lignin, and hemicelluloses **[40]**, **[41]**. Scientific works **[42]**, **[43]** noted that many hydroxyl groups and a developed system of thin submicroscopic capillaries characterize cellulose. Therefore, it gives high retention properties to the liquid.

Research devoted to the application of ultrasonic vibrations in chemical technology is quite promising: in many cases, they provide the exceptionally high intensity of the technological process, which cannot be achieved with the help of such widespread methods as mechanical mixing, application of high temperatures and pressures, etc. **[44]**, **[45]**. Therefore, the problem of using ultrasound in the processes of chemical technology deserves serious attention. In our opinion, it can be used to determine the qualitative composition and amount of free sugars.

Soluble non-starch flaxseed polysaccharides are represented by mucous substances (4-6% by weight of seeds) **[46]**, **[47]**, which are well hydrated in cold water with the formation of a mobile gel. In the cellular structures of the first three layers of the seed coat, after 1 min of hydration, the gel passes through the microscopic holes in the seed coat and becomes visible, forming a transparent capsule with a fairly clear phase boundary (Figure 7).

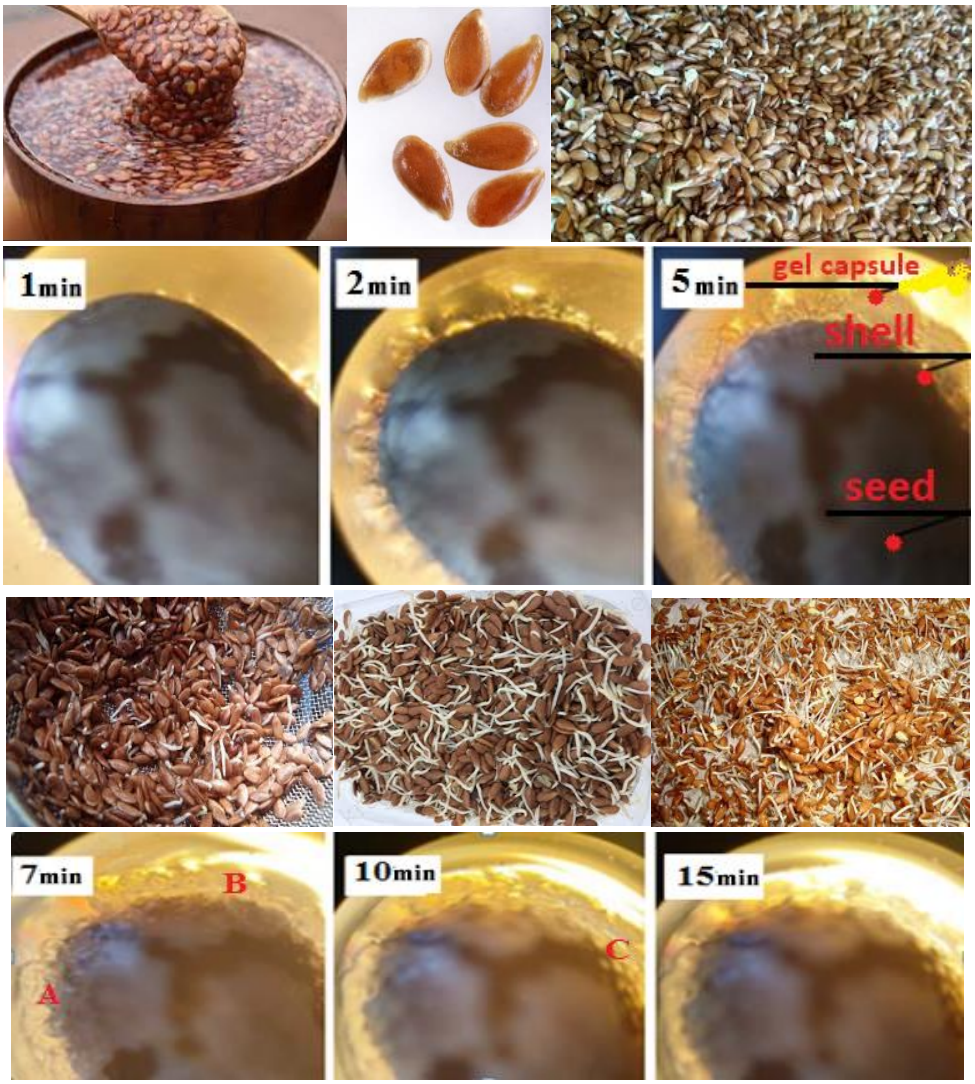

Figure 7 Features of the initial dynamics of hydration of mucus-forming polysaccharides in flax seeds with different duration of contact with water (x300).

The thickness of the gel capsule increases when the seeds come into contact with water for up to 10 minutes, and if the process of soaking the seeds is continued for up to 25 minutes, the size of the capsule does not change.

Figure 7 shows the volume A occupied by low molecular weight mucus formed above the surface of the shell at 7 minutes and 10 minutes of the process. Accordingly, B is a light loop-like strand of more high molecular weight mucus in a low molecular weight mucus solution. The formation of C – spheroidal formation of the highest molecular weight mucus.

Modeling allowed us to confirm our idea about the state of influence of the main factors on the dynamics of hydration of mucus-forming polysaccharides. In general, the hydration of flax seeds allowed us to isolate several fractions of mucus-forming components of polysaccharide nature with different physicochemical properties, in particular, with different hydration rates. At the same time, it is quite obvious that the biggest technological problems in various production operations with flax seeds are created by the low-molecular slime-forming component, which quickly hydrates even with a slight increase in the moisture content of the seeds.

The mucus fraction is presented as translucent (in micrographs – light) loop-shaped strands. These strands gradually increase in size and occupy the entire primary mucus area. The zone was previously formed around the flaxseed shell by a fraction of rapidly hydrating polysaccharides. Therefore, the polysaccharide nature of mucus formation is confirmed by a qualitative reaction to the influx of a weak aqueous solution of methylene blue. Blue gives a blue-violet color in the presence of polysaccharides (in Figure 6 – dark loose formations in the area of mucus).

Similar series of experimental studies are described in scientific works **[48]**, **[49]**, **[50]. The** author's teams investigated the properties of polysaccharides, which were obtained by extracting different types of oilseeds (sunflower, soybean, and rapeseed). But in the works mentioned above, there is no comparative analysis of the chemical composition of the obtained polysaccharides.

In the conducted studies and the given features of the initial dynamics of hydration of mucilage-forming polysaccharides in flax seeds (Figure 7), changes in the location, intensity, and shape of bands of the main functional groups that characterize protein, carbohydrate, and lipid complexes are visible. The periodicity of the processes occurring on the change in the intensity of the functional groups' main bands depends on the hydration and temperature duration. In our research, the intensity of breaking down protein and polysaccharide substances, which are few in the literature in this aspect, is reflected.

In the scientific literature, there is a large amount of information devoted to the study of the properties of polysaccharides, which were obtained by various methods:

Extraction from natural sources. This method involves harvesting the plant material, processing it, and extracting the polysaccharide using solvents or physical methods **[51]**.

Biosynthesis by bacteria or fungi. Some bacteria and fungi can be used for the biosynthesis of polysaccharides. For example, xanthan gum, used in the food industry, can be produced using the bacterium *Xanthomonas campestris* **[52]**.

Fermentation. In some cases, polysaccharides can be obtained by fermentation. This process uses living microorganisms, such as fungi or bacteria, to break down carbohydrates in substrates and form polysaccharides **[53]**.

Chemical synthesis. Some polysaccharides can be synthesized chemically using organic chemistry. This method involves stepwise reactions leading to the formation of the desired polysaccharide **[54]**, **[55]**.

Biotechnological methods. With the help of biotechnological methods, it is possible to use genetically modified organisms for the production of polysaccharides. This involves genetically engineering bacteria or fungi to produce the desired polysaccharide **[56]**.

The choice of method for obtaining polysaccharides depends on their natural source, properties, and application needs. Each method has its advantages and limitations, and the specific situation and research or production need to determine its use.

In general, the hydration of flax seeds allowed us to identify several fractions of mucus-forming components of polysaccharide nature with different physical and chemical properties, in particular, with different rates of hydration. It is quite obvious that the biggest technological problems in various production operations with flax seeds are created by the lowest molecular weight mucus-forming component, which is quickly hydrated even with a slight increase in seed moisture.

Flaxseed mucus is a mixture of water-soluble polysaccharides **[57]**, **[58]**, which include mainly L-lactose, D-xylose, L-rhamnose, and D-galacturonic acid. Mucus polysaccharides form two main fractions: neutral and acidic. The neutral fraction contains almost no galacturonic acid, xylose is the basis of this fraction. The acidic fraction is dominated by galacturonic acid and xylose residues are detected. According to the authors, the relative content of the neutral fraction in the composition of mucus polysaccharides **[59]**, **[60]**, is 75%. The ratio of these fractions

depends on the genotype of flax and largely determines the properties of polysaccharides of flax mucus, including rheological.

Chemically, the mucus is dominated by pentosans (up to 90%). Its complete solubility in water characterizes the physical properties of mucus. The mucous substances of flaxseed are complex chemical compounds of monosaccharides.

We see that the latter hydrate the most high molecular weight polysaccharides. We believe this is because they are localized in the inner layers of the seed coat and the endosperm. At the initial stage of hydration, these mucus are visually identified as small spheroidal translucent structures. Macromolecular mucus takes the form of transparent annular formations against the background of dark cell walls.

We obtained this result by compressing the flooded seed coat between the slides and the cover glasses. The inner surface of the flax shell with translucent annular structures of macromolecular mucus is reflected against the background of dark cell walls for 10 minutes. after the influx of water.

The effect of the duration of extraction on the quantitative yield of mucus-forming polysaccharides found that during the first 10 – 20 minutes there is a sharp increase in the yield of polysaccharides from flax seeds. In the future, the speed of the process slows down, and after 90 minutes of water contact with flax seeds equilibrium occurs. Therefore, this time can be considered optimal for removing mucus-forming polysaccharides from flaxseed. The research results are given in Table 2.

Table 2 Dependence of quantitative yield of mucus-forming polysaccharides from flax seeds on the duration of extraction.

Duration, min	Weight of extracted polysaccharides,% by weight of seeds	
	Vruchiy	Original
10	2.09	1.95
20	3.83	3.45
40	4.86	4.62
60	5.48	5.21
80	5.76	5.53
100	5.82	5.74
120	5.82	5.74
140	5.82	5.74

The Table 3 presents the results of the effect of temperature on the yield of mucus-forming polysaccharides from flax seeds. The extraction time is 100 minutes.

Table 3 Dependence of quantitative yield of mucus-forming polysaccharides on temperature.

Temperature, °C	Weight of extracted polysaccharides,% by weight of seeds	
	Vruchiy	Original
20	5.82	5.74
40	5.91	5.86
60	6.08	5.93
80	6.10	6.00
90	6.12	6.00
100	6.12	6.00

The results indicate that the increase in the amount of extracted polysaccharides depends more on the duration of the extraction process than on temperature. Because the increase in temperature requires energy consumption and also causes denaturation of flaxseed protein. We consider it inexpedient to increase it during the process of extraction of mucus-forming polysaccharides from flax seeds.

It can be noted that even simple visual observations of the dynamics of mucus formation in the case of hydration of flax seeds allow us to distinguish several fractions of mucus-forming components of polysaccharide nature. These fractions have different physicochemical properties, particularly with different hydration rates. The lowest molecular weight mucus-forming component creates the biggest technological problems in various production

operations with flax seeds. It quickly hydrates even with a slight increase in seed moisture. Therefore, all the mucus-forming components when moistened flax seeds are successively hydrated and go into solution, which indicates a gradual increase in its concentration.

We offer the following method of processing flax seeds to obtain a food additive with gel-forming properties. The raw material is brought into contact with water at room temperature and a hydraulic module of 1:3.

CONCLUSION

The hydration parameters and mechanism were studied to substantiate the technology for releasing mucilage-forming polysaccharides in an aqueous solution from flax seeds. The effect of temperature and duration of extraction on the yield of mucus-forming polysaccharides in an aqueous solution from flax seeds was evaluated. Based on all the investigated parameters in this study, the best for hydration will be recommended to be carried out with water at room temperature (18 – 20 °C) and ratio 1 : 3 for 90 min. It was determined that the maximum amount of polysaccharides released in the aqueous solution is observed at 10-20 min, it remains unchanged after that. From 85 min, equilibrium occurs at a temperature of 80 °C, which indicates the optimality of the process for extracting mucilage-forming polysaccharides from flax seed. With the proposed ranges of temperatures and time, the best result was observed in the mass of extracted polysaccharides from the mass of seeds: at a temperature of 80 ºC – 6.00% and at a time of 95 minutes – 5.74%. Microscopic studies have established that low-molecular fractions of polysaccharides are hydrated on the surface of the shell. When flax seeds come into contact with water for a few minutes, high-molecular polysaccharides enter the hydrated state.

It is also necessary to mention that flax mucilage polysaccharides can be used as a thickener, stabilizer, and moisture-retaining agent while providing a protective effect to the digestive system. The obtained results make it possible to use the soaking of flax seeds, under the established parameters, in the food industry, particularly in the technologies of bakery and flour confectionery products. Flax seed polysaccharides and lipids and proteins included in its composition have practical significance and can be used in pharmacy as:

- structure formers;
- water-retaining agents;
- stabilizers.

Nevertheless, further studies are needed to evaluate the yield of mucilaginous polysaccharides.

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The macroeconomic indicators influence the consumption of selected organic food under the conditions of global climate change – a case study from the Czech Republic

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ABSTRACT

Since the beginning of the 21st century, within the framework of food consumption in the Czech Republic, organic food consumption has also begun to be statistically monitored. This consumption is influenced by several factors, such as consumer demand, their changing attitudes, and beliefs about the correctness of their consumption, but also the owners and managers of companies producing organic food and their willingness and decision to offer organic food to consumers. The content of this paper is to search for the connections between selected macroeconomic indicators and their influence on total household consumption and, within it, on the consumption of certain groups of food and organic food. More than twenty years of statistical monitoring shows how selected macroeconomic indicators and food consumption, including organic foods and their main groups, were developed. During approximately twenty years of development, it is possible to identify several fluctuations with varying intensity in growth, stagnation and decrease. An example is the current economic situation manifested by significant movements in the leading macroeconomic indicators to varying extents in the Czech Republic and several other countries, not only in Europe. The deterioration of the macroeconomic indicators results understandably raises concerns about the future development of consumption and the applicability of the generally produced more expensive organic food on the market. Therefore, The author team investigated the correlation between selected macroeconomic indicators, total food consumption and, in particular, the consumption of selected organic foods and evaluated the course of changes over time between 1993-2021. The influence of selected macro indicators on changes in the consumption of organic foods in the Czech Republic was assessed. The previously published papers deal with food and organic food consumption from different perspectives but not from the perspective of examining the correlation between consumption and three chosen macroeconomic indicators. The present contribution thus aims to fill this existing gap.

Keywords: demand, consumption, gross domestic product, price indices, green marketing, organic food

INTRODUCTION

The theoretical starting point for examining the relationship between selected macroeconomic indicators and the total consumption of food and selected groups of organic foods in the Czech Republic explains their nature and interrelationships.

Macroeconomic indicators, also known as fundamental data, according to **[1]**, are statistics or data that reflect the production or output of an economy, government or sector and vary in frequency, impact, and significance (Nominal GDP is an indicator of current (real) prices, real GDP is an indicator of comparable (constant) prices.).

These include the gross domestic product (GDP), average inflation rate, household consumption, employment indicators, retail sales, public debt, monetary policy, and interest rate announcements. They are related to the economy, population, geography, etc. They are collected by agencies and offices of various government statistical organizations and sometimes by private organizations using similar techniques. Macroeconomic indicators are used to assess the state of a country's economy and measure a country's overall economic performance. These are different quantities that focus on certain countries or sectors. Their results can be continuously monitored, examined, evaluated and compared. By comparison, it is possible to determine the position of the national economy within other countries. Not all known macroeconomic indicators have a direct link to the consumption of food and organic food.

The most important indicator of the performance of the economy of the given country as a whole, which is related to supply and demand and consumption, is GDP. It is defined as the sum of monetary values of (final) products and services produced during one year by production factors allocated in a given country, regardless of the ownership of these factors **[2]**. GDP represents the total volume of products and services created for a certain period (final production) in monetary units **[3]**. According to its valuation method, two categories are distinguished, i.e., nominal GDP and real GDP. 1Nominal GDP is an indicator of current (real) prices. The real GDP is an indicator of comparable (constant) prices **[4]**. GDP captures the output created by production factors on a given state's territory. The number of resources that are available for (re)distribution in a given economy is also expressed by the gross national income (GNI), Gross National Product (GNP). In this concept, primary incomes from non-residents (wages, profits, annuities, etc.) are added to GDP, and conversely, incomes paid to non-residents are deducted. The difference between GNI and GDP is net income relative to the rest of the world. If this difference is positive, it means that more pensions flow into the economy from non-residents than are paid to them (the net income of non-residents is, therefore, negative from the point of view of the national economist). Gross disposable national income and gross national savings are important aggregate economic indicators related to consumption **[5]**.

The unemployment rate is among the predicted macroeconomic indicators that can influence consumption. The unemployment rate is calculated as the percentage of unemployed in the labour force of a given territory.

The labour force includes both employed and unemployed populations. According to the **[6]**, all persons aged 15 and over, usually living in the monitored territory, who meet three conditions during the reference week are considered unemployed: 1) they are not employed, 2) they are actively looking for work, and 3) they are ready to start to work no later than 14 days.

Frictional unemployment arises as a result of worker mobility or as a result of the constant movement of people between places or job opportunities. Workers have different abilities and preferences, which make them look for better employment. This type of unemployment is normal in the labour market because it is temporary. Changes in frictional unemployment can be caused by voluntary changes in the time spent searching for a job that the unemployed are willing to accept. Frictional unemployment is distinguished from other types of unemployment by three factors. The first factor is the existence of enough jobs for those who are frictionally unemployed. The second factor is that the frictionally unemployed have sufficient qualifications for available jobs where they are required. The third factor is the short time of searching for a job. A specific part of frictional unemployment is considered to be seasonal unemployment, which occurs in sectors whose production fluctuates depending on the season, for example, in construction or in agriculture for crop production **[7]**.

Structural unemployment arises when some sectors are depressed and others, on the contrary, are expanding. Employers affected by a shrinking industry are laying off workers, and retraining is needed to move these workers to another (expanding) industry. Structural unemployment usually lasts longer than frictional unemployment. For many people, the search for work can drag on for months or years because they do not have the qualifications that companies need. In the economy, there is constant technological development, which is manifested in the gradual decline of some fields and the development of other fields. Thus, structural unemployment can be considered a natural and inevitable part of every economy, but at the same time, it represents a more serious problem than frictional unemployment and, as such is not compatible with the idea of full employment **[8]**.

Cyclical unemployment is related to cyclical fluctuations in the performance of the economy. Unemployment of this kind is dependent on the performance of the economy, when unemployment will decrease with its growth and vice versa. If the economy is in recession, employers lay off workers **[9]**.

Among the mentioned indicators, the unemployment rate indicator has an effect on consumption, which could also be analyzed as the correlation between this indicator and total consumption, consumption of organic food and organic dairy food.

Inflation is a macroeconomic phenomenon that reduces the purchasing power of money over a defined period of time and increases the price level of all goods and services. It, therefore, contributes to economic instability and uncertainty about the development of macroeconomic variables. Inflation is an average quantity that expresses

the change in the price level in a given economy. "Inflation means a general increase in the price level over time **[10]**". An increase in the price of an individual product or a group of products does not necessarily mean inflation (In this case, it is a so-called short-term price shock). A rise in the price level is associated with a decline in the purchasing power of money. Deflation is the opposite macroeconomic phenomenon when the price level falls or disinflation when the inflation rate decreases. The price level is measured using price indexes. The price index is the ratio of the costs of acquiring a certain set of goods and services in the current period and in the base period. The price index can then be used to calculate the growth rate of the price level or the rate of inflation **[12]**.

The inflation rate is expressed as a percentage change in the average price level for the last twelve months compared to the average price level for the previous twelve months. **[2]**, i.e., the inflation rate is the percentage increase in consumer price indices. For the correct interpretation of each price index, it is always necessary to know the period for which it is calculated. When expressing the rate of the consumer price index, precise factual, spatial, and temporal delimitation is important. This means it is necessary to clearly state the period for which the inflation rate is calculated and determine the basis on which it is compared. Price indices are used to calculate inflation, such as the GDP or implicit price deflator, producer or consumer price index, and cost of living index **[11]**. Three price indices are commonly distinguished: consumer price index (CPI – Consumer's Price Index), GDP deflator (IPD – Implicit Price Deflator), and producer price index (PPI – Producer's Price Index). Inflation rates are expressed by the increase in the average annual consumer price index, expressed by the increase in the consumer price index to the same month of the previous year, or the inflation rate expressed by the increase in the consumer price index to the previous month or the increase in the consumer price index to the base period (e.g., year 2015 = 100) are most often used) **[12]**.

In the Czech Republic, the Czech Statistical Office monitors the movement of inflation based on the measurement of net price changes using consumer price indices **[10]**. The consumer basket represents a set of products and services purchased by households over a certain period of time. "Price indices measure the price level of a selected basket of representative products and services (approx. 450) in two compared periods, while the weight (or importance) assigned to individual price representatives in the consumption basket corresponds to the share of the given type of consumption they represent in the total household consumption **[13]**". The selection of representatives for the consumption basket depends on the share of household consumption **[14]**. The consumer basket is composed of food goods (food, beverages, tobacco), non-food goods (clothing, furniture, household goods, drugstore and small goods, transport and leisure goods, personal care goods, etc.) and services (repair from the areas of housing, household operations, healthcare, social care, transport, leisure, education, catering and accommodation, personal care and financial services).

A demand is a phenomenon that expresses the relationship between the number of goods buyers are willing to consume and the price they are willing to pay for goods at a certain time and place. The theory of demand distinguishes between different types of demand, mainly individual, market and aggregate demand. About the objective of the article, the essential demand is aggregate. Aggregate demand expresses the various quantities of a good that consumers, firms, the government, and the rest of the world are willing to buy at certain price levels. Aggregate demand is, therefore, nothing more than the sum of household consumption expenditure, investment expenditure by firms, government purchases of goods and services and net exports, the amount of which depends on the price level **[11]**.

Household consumption is the most significant component of these aggregate expenditures and the gross domestic product (GDP) in demand, normally reaching around 60.0% of its level. The largest item households demand is food **[15]**, **[16]**.

Consumption represents the expenditure of all households in the economy on short-term and long-term consumption of goods and services. Consumption reaches more than 50% of the gross domestic product. Consumption depends on a number of factors.

The consumption function (Assuming a "two-sector economy") expresses consumption C as the sum of autonomous consumption Ca and the product of the marginal propensity to consume c (indicating how consumption changes if disposable income changes by one unit) and disposable income YD. i.e., the consumption function can be expressed as $C = Ca + cYD$. The basic characteristic of this consumption function is the decreasing share of consumption in total income. Empirical research did not confirm that the share of consumption in income would decrease in the long term, rather, it turned out that consumption shows a stable share, and the consumption function is only valid for a short period. An alternative to Keynes' theory is the microeconomic model of intertemporal choice (Irving Fisher: The Theory of Interest (1930)). According to him, the amount of consumption depends on the income in both periods, the interest rate, and the consumer's preferences. Another alternative is, for example, the so-called life cycle theory, which is based on the model of intertemporal choice and explains the long-term stable share of consumption. She claims that people want to maintain a stable level of consumption throughout their lives, and therefore, when they have a low income, they have to borrow in their youth. In their

working age, they spend less than they earn and save more, and in their old age, they have a higher consumption than their pension, and they spend what they have saved in their working age. According to Friedman's permanent income theory, consumption depends only on the so-called permanent income (YP): C = c.YP is the average long-term expected income, which depends on the expected income from labour (from human capital) and the expected income from assets held.

According to **[17]**, the amount of consumption depends on the income in both periods, the interest rate and the consumer's preferences. They recommend considering household consumption in the context of savings, as households are generally an important creator of national savings. According to economic theory, this is the basis of economic growth and prosperity. The starting point for considerations about the economic behaviour of Czech households is the analysis of the development of the consumption structure and the sources of payment for this consumption, i.e., disposable income. Disposable income represents the sum of households' primary and secondary incomes (consumers and entrepreneurs). It is the sum of gross wages and salaries, pensions from business and property, and the sum of the balance of social and other pensions. The balance of social pensions represents the difference between the value of cash social benefits of households from the state, insurance companies and employers, and social contributions (mandatory and voluntary), and benefits that households pay. Other pensions are considered "other ordinary transfers, i.e., net insurance premiums, or reimbursements in non-life insurance, contributions to non-profit organizations, fines and penalties, winnings and bets and other pensions **[17]**." Common taxes that households pay are primarily income and property taxes.

From a macroeconomic point of view, household consumption is expressed as "an indicator of household final consumption expenditure, which includes the value of goods and services (short-term and long-term consumption, except for houses and apartments) purchased by households and also includes part of unpaid consumption **[17]**." Unpaid consumption is represented, for example, by the value of self-supply of agricultural products. Autonomous consumption is part of consumption that does not depend on the size of the income and is financed from earlier savings. Saving is generally interpreted as the difference between disposable income and final consumption (According to Hronová and Hindls, the specific value of savings is, however, still influenced by the value of savings that households created in pension funds during the given period (so-called changes in the net share of households in).

Empirical research on consumer demand is almost overwhelmingly based on the neoclassical theory of consumer behaviour: the choice of the best consumer basket by the relevant consumer entity. Within this theory, two approaches can be distinguished in the search for the optimal consumption basket. First, the consumer strives for the most useful consumption basket that he can buy from his income and at a certain level of market prices. Or else secondly, the consumer chooses among useful baskets the one with the lowest expenses **[18]**.

During the last two to three decades, there have been significant changes in food consumption, structure and volume. These changes were influenced by various factors, primarily the development of consumer prices for food and non-food products and services, the development of the population's income, advertising and promotion, and the offer and availability of products on the market concerning the development of the distribution network. In addition to the factors as mentioned earlier, food consumption is also affected by, for example, the extent of self-supply, the development of quality, the degree of saturation of needs, etc. The biggest influence on food consumption was primarily the development of consumer prices of food and industrial goods and services in relation to the development of incomes, i.e., purchasing power demand. Currently, however, there is a noticeable tendency to reduce the influence of prices on food consumption, and many consumers consider non-financial factors when purchasing **[19]**.

Some consumers began to feel responsible for the negative impact of consumer goods on the environment. It began to purposefully monitor purchased goods' composition, components, packaging, entire distribution chain, etc. In this context, new value attitudes are created based on the principle of sustainable development. An integral part of these changes can also include the application and creation of decision-making methods for individuals in the area of consumer choice. This is not only an issue of choosing the optimal consumer basket from the perspective of the traditional price-quality ratio but also the creation of preferences concerning ecologically suitable consumer goods, for example, organic food **[20]**.

Published studies dealing with macroeconomic determinants of food consumption in general and organic food are not frequent topics in professional journals. Some of the published articles dealing with consumption usually look at the issue from the point of view of malnutrition and famine rather than the consumption of energy-rich foods produced in an environmentally friendly way. Even though organic food is a phenomenon whose importance has been growing significantly recently, the issue of organic food production and consumption and its conditioning variables and barriers on the part of producers are still largely unexplored. After all, finding out consumers' values and attitudes about the environment and organic food consumption is a more frequent topic. As regards the issue addressed in this article, i.e., the investigation of the influence of macroeconomic indicators

on the consumption of food and organic food, existing published studies usually examine the influence of the population's income (respectively the amount of GDP) or the level of the price level (respectively the rate of inflation). In contrast, other macroeconomic variables such as unemployment are not given attention.

The Intergovernmental Panel on Climate Change **[21]** states that adaptation to global climate change aims to reduce risk and vulnerability to climate change, strengthen resilience, improve well-being and the ability to anticipate and successfully respond to changes. Existing international frameworks provide a high level of direction for coordinating, financing and evaluating progress toward these goals. Specifying goals for specific adaptation activities is not easy to define because the impacts of climate change affect people and nature in many ways that require different adaptation measures. Therefore, the goals relate to health, water or food safety, jobs and employment, poverty eradication and social equality, biodiversity and ecosystem services at international, national and local levels.

The most frequently mentioned determinants concerning malnutrition and bad eating habits are primarily the level of GDP and the growth of food prices, i.e., inflation of this group of goods **[22]**, **[23]**. According to their findings, the intrinsic price elasticity of most food groups is close to one, indicating a high response of consumers to a change in food price. However, lower food prices often lead to reduced agricultural production and lower incomes for farmers and may ultimately contribute to a country's lack of staple foods **[22]**.

Examine the relationship between consumer values and their attitude towards environmental issues in selected economies (Brazil, Czech Republic, Germany, India, New Zealand and Russia). According to their findings, egoism (self-focus) predicts a lack of interest in the environment **[24]** . Conversely, transcending one's ego (caring for others, plants, or animals) predicts interest in environmental issues.

Describe the relationship between attitudes and norms of consumers in Denmark and their consumer behaviour with the purchase of organic food **[25]**. The results of their investigation are represented from a consumer perspective. The entrepreneurial perspective is described by **[26]**, who looks for factors of successful business in the organic food market in the Slovak Republic.

It investigates the functioning of retail chains with organic food in southern Bohemia. However, their attention primarily focuses on sales strategies, marketing mix, conditions, and culture **[27]**.

Also, it analyzes the factors influencing the purchase of organic fruits and vegetables in Istanbul, Turkey, and also conclude that the consumer's concern for their health and food safety is the main factor influencing the consumer's preference towards organic food **[28]**.

The organic food market in the Czech Republic concerning organic farming is analyzed by **[29]**.

Dealing in more detail with the question of how consumers in the Czech Republic perceive the health aspect and benefits resulting from the consumption of organic food **[30]**.

It examines the factors influencing expanding consumer demand for organic food in Saudi Arabia **[31]**. They consider lack of information, poor marketing and their high price to be the main factors preventing the wider spread of organic food. On the contrary, among the important factors supporting the development of organic food production and trade are the support of local producers, the clear declaration of organic food standards and the level of education.

Seeking to describe the dependence of organic food consumption in Turkey on political, economic, social and technological factors **[32]**.

Deal with how consumers perceive various attributes of organic food and how this perception affects consumer demand **[33]**.

Factors influencing the intention to buy organic food are also investigated in Pakistan, Turkey and Iran **[34]**. Their conclusions show that the results may be different for different countries. Nevertheless, they consider consumer care for their health to be an important factor playing a significant role in all three examined economies. Li et al., **[35]** examined the demand for organic dairy food.

Applying the theory of consumer values (healthy lifestyle, sustainability, health care, perceived value of organic food) to predict the purchase intention of organic food in Turkey **[36]**.

However, e.g. **[23]** surprisingly showed that raising the price level unexpectedly reduces malnutrition in developing countries because food prices change the eating habits of poor people. As a result of high prices, they were forced to reorient themselves from many tempting foods that were poor in nutrients to basic foods that are more energy beneficial.

As shown by several studies dealing with the issue of organic food consumption **[37]**, the possibility of changing the dietary regime does not depend only on the individual's will, but above all, on the economic conditions in which he lives **[38]**. He is, therefore, greatly influenced by the macroeconomic environment in his choice **[39]**. The economic situation and food prices are important factors influencing food choices; therefore, changes in the inflation rate can be expected to affect organic food consumption **[40]**.

In their study, looked at the consumption of organic products and the determinants that influence it, confirmed that economic factors such as consumer income, prices of organic products compared to conventional products and inflation as some of the main determinants that influence the demand for organic food. In their analysis, the inflation rate was confirmed as the most important macroeconomic variable influencing the development of the consumption of organic products **[41]**.

GDP is a general indicator of income level in studies that influence food consumption factors. The growing economic performance of the country, expressed by the growth of the gross domestic product, is thus a determinant of higher consumption of organic products **[42]**, **[43]**.

From the above-mentioned follows, those studies dealing with the topic of macroeconomic determinants of food consumption generally view the issue from the point of view of malnutrition and famine, not the consumption of energetically important foods produced in an ecologically considerate manner. Even though organic food is a phenomenon whose importance has been fundamentally increasing recently, the need for organic food and its conditioning variables is still a largely unexplored area. In addition, existing studies on organic food consumption usually examine the influence of the population's income (respectively the amount of GDP) or the price level (respectively the rate of inflation), while other macroeconomic variables are not currently being paid attention to.

To evaluate the current state of knowledge on the issue of organic food, articles were searched in the WOS and Scopus databases for the period 2002-2022 and also for the last five years, i.e., 2017-2022, on topics related to the issue of organic food from the point of view of macroeconomic indicators (see Table 1):

A. A total of 2,725 papers were published in the WOS database and 2923 papers were published in Scopus on the subject of Organic food in the period 2002-2022.
B. A total of 33,860 papers were published in the WOS database on the topic of unemployment in the period 2002-2022, mainly from the fields of Economics (10,006), Public Environment Occupation Health (2,838), Management (1,502), Sociology (1,501), Business (1,365). The largest number of papers came from the USA (7,605), England (3,177), Germany (2,754), and Spain (2001). 918 papers were published in the Czech Republic. In the last five years, 49% of all papers (16,738) were published, representing an increase in interest in this topic in the last five years.
C. If the topic "organic food" is connected with the topic "unemployment," then in the monitored period 2002-2022, only three papers are listed in the WOS database, namely from the field of Business (1), Agricultural Economics Policy (1) and Agricultural Multidisciplinary (1). These papers come from Bosnia Herceg (1), Indonesia (1), and Italy (1). There is no paper from the Czech Republic. Again, an increased interest in these topics can only be seen in recent years, as 67% (2) were published in the last five years.
D. A total of 40,058 papers were published in the WOS database on the topic "inflation" in 2002-2022, of which 49% of the papers were published in the last five years, indicating a growing interest in the topic in recent years. The papers were mainly from the field of Economics (11,158), Physics Particles Fields (6,636), and Business Finance (3,111); they were published mainly from the USA (10,828), England (3,853), China (3,619), Germany 2,829).
E. If we connect the topic "organic food" and "inflation", there is no paper in the WOS database during the monitored period.
F. A total of 42,886 papers were published on the topic "GDP" or "gross domestic product" in the period 2002-2022, while 23,569 were published in the last five years, which indicates a growing interest in the topic. The papers were mainly from the field of Economics (12,974), Environmental Science (5,640), Environmental Studies (2,850), and Green Sustainability Science Technology (2,539). Most papers came from China (9,210), the USA (7,273), England (2,991), and Germany (1,949).
G. The Connection of the theme "organic food" with the theme of GDP (gross domestic product) in the monitored period, only three papers from the field of Business (1), Development Studies (1), and Economics (1) were found in the WOS database. These papers were published in Bosnia Herzegovina (1), Iran (1), and Poland (1). None of these papers were published in the Czech Republic. Again, an increased interest in these topics can only be seen in recent years, as 67% (2) were published in the last five years.

From the results shown in Table 1, it is evident that the topic of organic food is an emerging topic, covered by many articles, but in combination with the topics of macroeconomic indicators such as GDP, unemployment, and inflation, the number of articles is already very low. Thus, this study fills the gap in knowledge and perspective on the issue of organic food consumption through macroeconomic indicators.

Table 1 Bibliographic records on a query in the Web of Science and Scopus 2000-2021.

Search code	Query	Web of Science 2002-2022	Web of Science 2017-2022	Scopus 2002-2022
A	"organic food"	2,725	1,673	2,923
B	"unemployment"	33,860	16,738	
C	"organic food" and "unemployment"	3	2	
D	"inflation"	40,058	19,897	
E	"organic food" and "inflation"	0	0	
F	"GDP" or "gross domestic product"	42,886	23,569	
D	"organic food" and "GDP" or "gross domestic product"	3	2	

Note: Sources: Calculated by authors, access 2023/03/16.

According to **[44]** priorities for supporting rural development are fulfilled through measures (17 measures) together with the initiative LEADER, which mainly covers the following areas:

- dissemination of knowledge, information activities and consulting services,
- programs to support quality products, including promotion and information programs,
- campaigns,
- investments in tangible assets with a higher rate of assistance to young farmers,
- collective and integrated investments - possibilities for irrigation under certain conditions,
- development of agricultural enterprises and trade with extended support for
- small and young farmers and small businesses,
- development and improvement of forest areas,
- support for establishing groups of producers in all EU member states,
- climate-related agri-environmental payments and organic farming:
- greater flexibility and enhanced support for joint activities,
- a significantly strengthened cooperation measure, including pilot projects, short ones,
- supply chains and local promotion,
- a new set of risk management tools,
- strengthening the "LEADER" approach within EU funds.

MATERIAL AND METHODOLOGY

Statistical analysis

Data published by the Czech Statistical Office for the years x-2021 were used to compare the development of the consumption of selected groups of organic foods with selected macroeconomic indicators. The examined indicators were GDP, inflation, and the unemployment rate. Methodologically, GNI and GDP construction, according to the CZSO, is based on GDP from which primary incomes paid by resident units to non-resident units are subtracted, and primary incomes received by resident units from non-resident units are added, thereby obtaining gross national income. Expenditure current transfers paid by resident units to non-resident units are subtracted from it, and current income transfers received by resident units from non-resident units are added to obtain gross disposable national income. Gross disposable income represents the amount that households have left over after paying taxes and current expenses. It is intended to cover final consumption and savings. The result of subtracting final consumption expenditure from gross disposable national income is gross national savings, which are used to finance expenditure on gross capital formation. National disposable income shows the amount that, adjusted for the balance of secondary incomes flowing from/to the rest of the world, economic entities can spend on final consumption and savings.

The time series of the modelled variables were: (1) total consumption of organic food, (2) consumption of organic dairy products and (3) real GDP were tested for stationarity using ADF (Augmented Dickey-Fuller) tests **[45]**. In all cases of time series with original (logarithmic) values, the null hypothesis of the existence of a unit root was not rejected. After the transformation using the first differences (logarithmic) variables, all-time series were already stationary. The results are summarized in Table 2.

Table 2 ADF stationarity test results of the used time series.

ADF stationarity test		Time series (in logarithms)		
		Real consumption of organic food in total (per 1 inhabitant)	Real consumption of organic dairy products (per 1 inhabitant)	Real GDP (per 1 inhabitant)
Undifferentiated data	Test criterion	-1.27	-0.40	-2.10
	p-value	0.63	0.90	0.52
1st difference of variables	Test criterion	-1.97	-4.47	-6.00
	p-value	0.04 **	0.00 ***	0.00 ***

Source: Own results.

Due to the non-stationarity of the investigated quantities, the error correction model is the chosen model for describing the dependence between the total consumption of organic food (or the consumption of organic dairy products) and the real GDP. This model framework makes it possible to eliminate the problem of spurious regression in a model with non-stationary time series and to describe both the long-term equilibrium relationship between the mentioned variables and the short-term dynamics.

However, the formulated error correction model is not econometrically estimated by the standard two-phase Engle-Granger method **[46]**. The chosen methodology of the error correction model is modified, and the assumption of time-invariant coefficients is replaced by time-varying parameters using Hamilton's Markov-Switching Model (MSM) methodology **[47]**. Leaving the assumption of time-invariant coefficients enables a much more realistic description of the evolution of the modelled quantities within the investigated time interval of 1994-2020, in which the global economic crisis of 2008 occurred. Since the standard error correction model with constant parameters is the default model framework, which is modified, this standard model will first be described. Then a specific way of its modification will be shown.

Standard error correction model

The chosen specific form of the error correction model is illustrated here for the explained variable *real consumption of organic food in total* (in logarithms). Nevertheless, an analogous model was also formulated for the explained variable, *the organic dairy products real consumption.*

The econometric estimation of the long-term equilibrium relationship between the consumption of organic food and GDP is carried out in the same way as in the Engle-Granger methodology **[46]** on non-stationary time series using the least squares method (LSM):

$$\ln(C_t) = \alpha_0 + \alpha_1 \cdot \ln(Y_t) + u_t \quad (1)$$

Where:
C_t means the real consumption of organic food per capita; Y_t represents real GDP per 1 inhabitant; u_t is a random error with white noise properties.

Since the time series$\ln(C_t)$ and $\ln(Y_t)$ are non-stationary, this econometric parameter estimation creates a spurious regression problem. Nevertheless, it is possible to use the estimates of the parameters $\hat{\alpha}$, $\hat{\beta}$ using LSM to estimate the deviation from the long-term equilibrium $\hat{u}_t$:

$$\hat{u}_t = \ln(C_t) - \hat{\alpha}_0 - \hat{\alpha}_1 \cdot \ln(Y_t) \quad (2)$$

Applying the error correction model requires that the used variables $\ln(C_t)$, $\ln(Y_t)$ are cointegrated in the first order, i.e. the deviation from the long-term equilibrium $\hat{u}_t$ have to be stationary. The standard ADF test again tested the stationarity, and the results are summarized in Table 3.

Table 3 The stationarity test results of the deviation from the long-term equilibrium using the ADF test estimation.

ADF test stacionarity	Deviation of $\hat{u}_t$ from the long-term equilibrium in the regression with the explained variable:	
	The logarithm of the real consumption of organic food in total	The logarithm of real consumption of organic dairy products
Test criterion	-2.43	-3.10
p-value	0.017 **	0.003 ***

Note: Source: Authors' own calculations.

The null hypothesis is H_0: $\hat{u}_t$ has *a unit root*, which is rejected in both cases at the standard at a 5% significance level. The deviation from the long-term equilibrium $\hat{u}_t$ is therefore stationary, and an error correction model of the form can be applied in the form as follows:

$$\Delta c_t = \beta_1 \cdot \Delta y_t + \beta_2 \cdot \hat{u}_{t-1} + \varepsilon_t \quad (3)$$

Where:
$\Delta c_t \equiv \ln(C_t) - \ln(C_{t-1})$ is the difference in organic food consumption; $\Delta y_t \equiv \ln(Y_t) - \ln(Y_{t-1})$ represents the real GDP difference; $\hat{u}_{t-1}$ means a deviation from the long-term equilibrium relationship in the previous period; ε_t is a random error with white noise properties.

Since the variables are in logarithms, their absolute difference is approximately the relative difference of the original non-logarithmic variables $\ln(X_t) - \ln(X_{t-1}) \cong \frac{X_t - X_{t-1}}{X_{t-1}}$. The parameters β_1, β_2 therefore have the interpretation of the relative elasticity coefficients. The coefficient β_1 shows the percentage change in organic food consumption if GDP increases by 1%. In a completely analogous way, the parameter β_2 expresses the percentage by which organic food consumption will change, if this consumption was 1% above its long-term equilibrium value in the previous period.

The standard model modification

The stated standard error correction model (3) was modified in such a way that the assumption of the constant parameters in time was neglected, which was quite unrealistic due to the global economic crisis of 2008. Therefore, some coefficients of the model will be modelled as time-varying parameters (TVP, Time-Varying Parameters), while specifically for this purpose, the Markov regime change model (MSM, Markov-Switching Model) will be used in the following form:

$$\Delta c_t = \beta_1(S_t) \cdot \Delta y_t + \beta_2 \cdot \hat{u}_{t-1} + \varepsilon_t \quad (4)$$

while the time-varying coefficient $\beta_1(S_t)$ is a function of an unobservable state variable S_t:

$$\beta_1(S_t) = \begin{cases} \beta_{1,1}, \text{ for } S_t = 1 \\ \beta_{1,2}, \text{ for } S_t = 2 \end{cases}$$

The variable S_t is a discrete random variable with only two possible values $S_t = 1$, $S_t = 2$ characterizing the state of the economy, while its development is determined by a Markov chain with transition probabilities:

$$P = \begin{bmatrix} p_{11} & 1 - p_{11} \\ 1 - p_{22} & p_{22} \end{bmatrix}$$

Where:
$p_{ij} = P(S_t = j \mid S_{t-1} = i)$ is the conditional with the probability that the system will be in time t in the state j, provided that in time $t - 1$ was found in the state i.

The parameter β_2 was left in a constant, unchanging form because in the empirical application of the MSM model, the modelling of its variability caused the statistical insignificance of this parameter and other coefficients.

RESULTS AND DISCUSSION

Results estimation, model verification and interpretation

Econometric estimation of the error correction model parameters with time-varying parameters of the form (4) was performed by Hamilton's methodology **[47]**, with the results summarized in Table 4 for both regression equations (with the explanatory variable based on total organic food consumption even with an explained variable based on the consumption of organic dairy products). In addition to the estimation of the parameters ($\hat{\beta}_i, i = 0,1,2$), the table also shows the P-value of the z-statistic testing its statistical significance in parentheses for each estimation.

Table 4 Results of the econometric estimation of the error correction model (4).

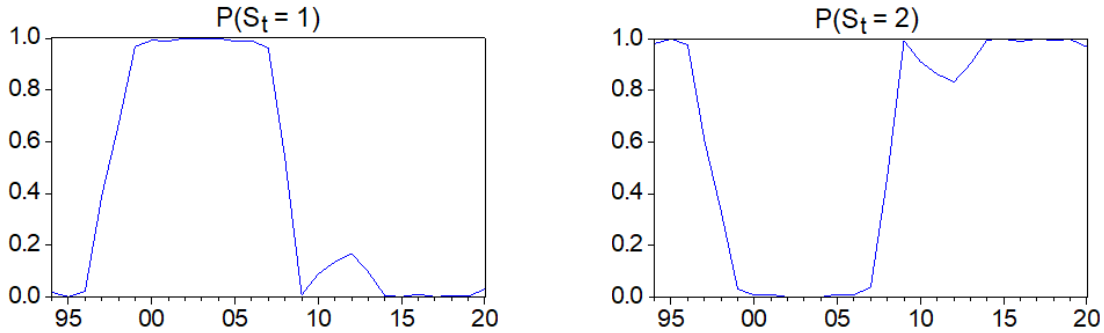
Regression with an explanatory variable	Parameter estimation (p-value of z-statistic)		
	$\hat{\beta}_{1,1}$	$\hat{\beta}_{1,2}$	$\hat{\beta}_2$
The logarithm difference of organic food consumption in total	0.592 (0.000) ***	-0.024 (0.808)	-0.269 (0.004) ***
The logarithm difference between the organic dairy products consumption	0.605 (0.000) ***	-0.346 (0.104)	-0.570 (0.000) ***

Note: Source: Author's own calculations.

The statistical importance of $\beta_{1,1}$ was, according to z-statistics, proven even at the 1% significance level in both cases. Therefore, both regression equations reject the null hypothesis cap H sub 0: beta sub 1,1 equals 0. Thus, if the economy is in the first state, the regressor Δy_t has a statistically significant effect on organic food consumption. In this case, the year-on-year growth of real GDP per capita by 1% leads to a year-on-year increase: the total real consumption of organic food per 1 inhabitant by 0.592 percentage points; real consumption of organic dairy products per 1 inhabitant by 0.605 percentage points.

However, if the economy finds itself in its second state, this statistical dependence of organic food consumption on real GDP disappears, which is proven by the statistical insignificance of the parameter $\beta_{1,2}$, in both considered regression equations.

A smoothed probabilities estimation of the individual modes $S_t = 1$, $S_t = 2$, using all the information from the entire data set, is shown for both estimated regression equations in Figure 1 below.



a) Regression equation with the organic dairy food consumption

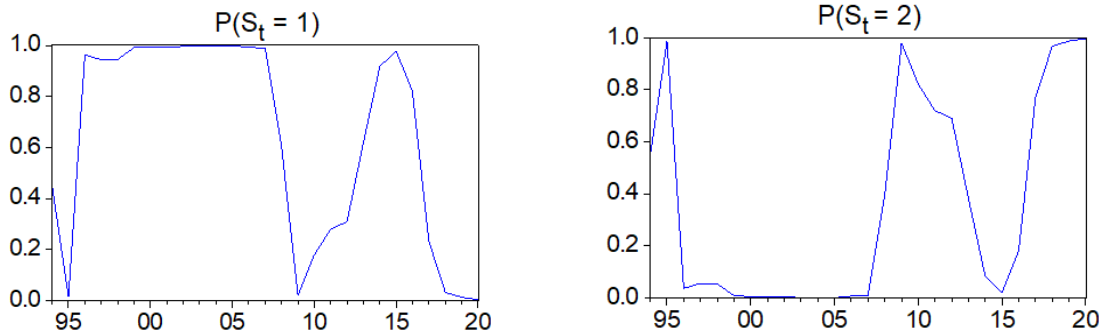
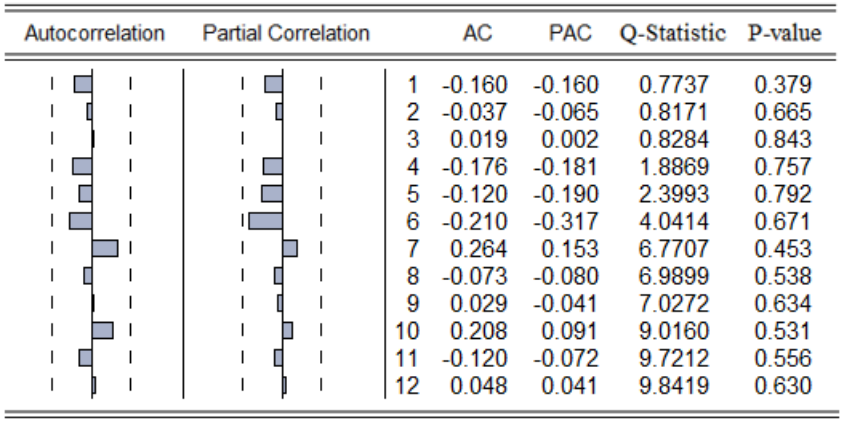


Figure 1 Smoothed probabilities estimation $P(S_t = 1), P(S_t = 2)$ for both regression relationships. Regression equation with the organic food total consumption.

The above graphs demonstrate that in 2008 there was a change in the dependence of organic food consumption on GDP in connection with the global economic crisis. This change turned out to be more pronounced and more permanent in the regression in the total consumption of organic food. For the regression equation with the consumption of organic dairy products, the system tended to return to its original first regime around 2015. However, this tendency did not prevail in the end, and in 2000 the system found itself again in its second regime, just as in the case of the first regression. It can therefore be summarized that the statistical significance of the dependence of organic food consumption on GDP disappeared after the economic crisis in 2008.

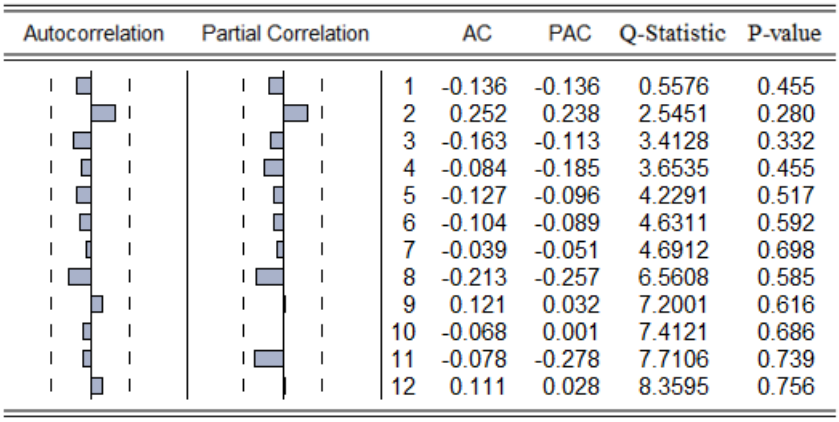
The coefficient β_2 fulfills a priori condition $\beta_2 \in (-1,0)$ in both considered regression equations. The fulfillment of this condition ensures that the consumption of organic food partially returns to its long-term balance if it has deviated from the balance in the previous period. The statistical significance of this adjustment mechanism, which keeps the given variables close to equilibrium, was demonstrated in both considered regressions, even at the 1% level of statistical significance. Therefore, if the total consumption of organic food (or milk consumption) deviates from its equilibrium value by 1 percentage point, then in the following period, the total consumption of organic food (or milk consumption) will decrease by 0.269 (or 0.570) percentage points.

In time series regression models, the problem of autocorrelation of random errors is very common. For this reason, the estimated error correction model (4) with time-varying parameters according to the Markov methodology was statistically tested for autocorrelation. Autocorrelation was tested using the Q-statistic as part of the correlogram analysis. The results are shown in Figures 2 and 3.



Autocorrelation	Partial Correlation		AC	PAC	Q-Statistic	P-value
		1	-0.160	-0.160	0.7737	0.379
		2	-0.037	-0.065	0.8171	0.665
		3	0.019	0.002	0.8284	0.843
		4	-0.176	-0.181	1.8869	0.757
		5	-0.120	-0.190	2.3993	0.792
		6	-0.210	-0.317	4.0414	0.671
		7	0.264	0.153	6.7707	0.453
		8	-0.073	-0.080	6.9899	0.538
		9	0.029	-0.041	7.0272	0.634
		10	0.208	0.091	9.0160	0.531
		11	-0.120	-0.072	9.7212	0.556
		12	0.048	0.041	9.8419	0.630

Figure 2 Autocorrelation (AC) and partial autocorrelation (PAC) functions of the regression model residuals (4) with the explained variable logarithm of the difference in total organic food consumption.



Autocorrelation	Partial Correlation		AC	PAC	Q-Statistic	P-value
		1	-0.136	-0.136	0.5576	0.455
		2	0.252	0.238	2.5451	0.280
		3	-0.163	-0.113	3.4128	0.332
		4	-0.084	-0.185	3.6535	0.455
		5	-0.127	-0.096	4.2291	0.517
		6	-0.104	-0.089	4.6311	0.592
		7	-0.039	-0.051	4.6912	0.698
		8	-0.213	-0.257	6.5608	0.585
		9	0.121	0.032	7.2001	0.616
		10	-0.068	0.001	7.4121	0.686
		11	-0.078	-0.278	7.7106	0.739
		12	0.111	0.028	8.3595	0.756

Figure 3 Autocorrelation (AC) and partial autocorrelation (PAC) functions of the regression model residuals (4) with the explained variable logarithm of the difference in organic dairy foods consumption.

The results in Figures 2 and 3 show that the null hypothesis of no autocorrelation was not rejected in both regression equations.

The results of the econometric analysis of non-stationary time series proved the existence of a long-term equilibrium relationship between organic food consumption and real GDP, both in the case of the indicator of the total consumption of organic food and the indicator of consumption of organic dairy products. The econometric estimation of the modified error correction model in both of these cases showed:

1) The statistical significance of the adjustment mechanism towards long-term equilibrium, even at the 1% level of statistical significance.
2) The intensity of this mechanism action was quantified as follows: If the total consumption of organic food (or milk consumption) deviates from its equilibrium value by 1 percentage point, then in the following period, the total consumption of organic food (or milk consumption) will decrease by 0.269 (or 0.570) percentage point.
3) In the case of both regression equations, the statistical significance of the dependence of real consumption of organic food on real GDP was proven before 2008, even at the 1% significance level.
4) The intensity of this mechanism action before the year 2008 was quantified as follows: Year-on-year growth of real GDP per 1 inhabitant by 1% leads to year-on-year increase:
   - the total real consumption of organic food per 1 inhabitant by 0.592 percentage points;
   - the real consumption of organic dairy products per 1 inhabitant by 0.605 percentage points.
5) However, in 2008, in connection with the global economic crisis, there was a regime change, and the statistical significance of this relationship disappeared. This conclusion turned out to be more permanent in the case of the relationship between total organic food consumption and GDP. In the case of the consumption of organic dairy foods, this dependence also changed in 2008, but it turned out to be of a less permanent nature. Around the year 2015, a short-term tendency to return to the original regime was characterized by a statistically significant dependence between the consumption of organic dairy products and GDP, and it was detected using the Markov models of regime change methodology.

The topic of this paper opens up the discussion of a whole range of other contexts and possibilities of investigating the economic and monetary policy influence as a tool for solving global climate change and also the inflation estimates or the search for efficient organic farming systems to ensure sustainable consumption of the world.

A number of economic schools dealt with consumption. According to classical economics/neoclassical economics, consumption depends on the real wage C= C(W/P); households decide on both consumption and labour supply (employment and real wages are endogenous from the model's point of view). According to Keynes, real consumption depends on current real income C = C(Y). Keynes analyzed the components of effective demand, such as household consumption, company investment, government spending on purchasing goods and services, transfer payments, autonomous taxes, and the income tax rate, and imports and exports in an open economy. His model addressed how to use this aggregate expenditure to stimulate real output growth to approach or reach the level of potential output and ensure full employment. Keynes's linear consumption function (the consumption theory: 45°) considers households' disposable income for consumption and savings. Total consumption increases as income increases but also regularly increases by a certain percentage of the additional product. For example, for every additional CZK 100 million in income, households spend CZK 80 billion and save CZK 20 billion. This stable share is called the marginal propensity to consume. Indicators measuring the size of these aggregates and their dynamics are usually used to monitor the development of final consumption expenditure and disposable income, i.e., the propensity to consume. Disposable income can be considered gross (including consumption of fixed capital) or net (without consumption of fixed capital). It is also possible to compare the development of final consumption expenditure and disposable income using indicators measuring absolute and relative increases in the values of these indicators. In the first case, it is the marginal propensity to consume, and in the second case, the elasticity coefficient.

Reviews how climate change and its policies may affect the macro economy in ways that are relevant for central banks' monetary policy assessment of the inflation outlook **[48]**. This review concludes with evidence regarding the potential channels of transmission and economic impacts of climate change and climate mitigation policies with potential significance for macroeconomic policymakers. **[49]** even describes how monetary policy can react to climate change and underlines the impact on key economic variables. Therefore **[50]** emphasises that the costs and benefits of any action to proactively mitigate climate change must be carefully balanced. Organic agriculture can be a part of the solution and help tackle climate change through its ability to reduce greenhouse gas emissions, store away huge amounts of carbon, and enable farmers to be resilient in an evolving climate **[51]**. According to **[52]** organic agricultural systems' mitigation and adaptation potential along three main features: I.) farming system design, II.) cropland management and III.) grassland and livestock management. These authors also confirmed that an important potential contribution of organically managed systems to climate change mitigation is identified in the careful management of nutrients and, hence, the reduction of N_2O emissions from soils. Another high mitigation potential of organic agriculture lies in carbon sequestration in soils. The Strategies for feeding the world more sustainably with organic agriculture is also discussed by **[53]**, where it is stated that

an organic agriculture is proposed as a promising approach to achieving sustainable food systems, but its feasibility is also contested. Meanwhile, biodiversity loss due to food production has increased by 50% in freshwater ecosystems. Agriculture accounts for some 70% of freshwater withdrawals worldwide and contributes to water pollution from agrochemicals, organic matter, drug residues, sediments and saline drainage into water bodies **[54]**. Finally, **[55]** provided a high-level overview of the evidence favouring nature-positive food systems, discussing opportunities and challenges associated with sustainable, efficient agricultural production with a view towards concrete policy suggestions. This study concludes that, on average, and particularly in temperate zones with highly intensive agriculture, conversion to nature-positive systems typically results in a reduction of yields that must be compensated by cost savings, higher product prices, or other support measures as to ensure the economic viability of the farms. This is particularly true in the case of organic farming **[56]**, **[57]**, but much less distinctive for integrated production systems with restrictions on plant protection and nitrogen fertilization **[58]**. Therefore ecosystem-based adaptation, defined as the 'use of ecosystem management activities to increase the resilience and reduce the vulnerability of people and ecosystems to climate change' **[59]**, has its core recognition that unexploited synergies in agricultural systems can increase productivity and resilience. These can result from increasing biodiversity, adding organic matter to soils, integrating livestock and aquatic species, including aquaculture, into farming practices, broadening landscape practices to exploit crop–forestry synergies, supporting beneficial insect populations and altering pest management practices that have unintended negative consequences **[60]**. The review of **[61]** also determines that transformative adaptation is characterized as restructuring, path-shifting, innovative, multiscale, systemwide, and persistent. Despite several barriers to implementing transformative adaptation, policymakers and practitioners should consider this option in adaptation plans to help societies to anticipate, guide, or recover from radical climate change impact. Using transformative adaptation to navigate shifts driven by climate change can increase the efficiency and sustainability of climate solutions. Another area for discussion related to assessing the influence of macroeconomic indicators and consumption, including organic food, is the relevance of these indicators. The findings are based on statistical data related only to data for the Czech Republic. To confirm the correctness of the answer to the research question, it would therefore be appropriate to investigate other similarly developed EU countries, possibly for the EU countries as a whole or even for non-EU countries. The authors examined only a selected group of organic foods, and the findings would be appropriate to analyse for other groups of statistically reported organic foods.

One of the certainly significant factors influencing organic food consumption are the customers' attitudes towards green marketing and the actual purchase and consumption of organic food. The measurement of consumer attitudes was not the subject of research, but the authors should certainly investigate this issue, and search based on the results of already conducted research abroad and in the Czech Republic.

Based on the results obtained in this research, it can be concluded that organic food has recently been considered trendy and, therefore, fashionable. However, organic products and products must comply with all generally valid hygiene and food standards for ordinary foods, and, in addition, they must comply with the rules for organic agriculture. Therefore, organic farming is a key ally in the transition towards a more sustainable food system and better biodiversity protection. The main reasons for its application are **[62]**:

1.) Organic farming reduces the number of greenhouse gases;
2.) Organic farming improves soil carbon sequestration;
3.) Organic farming increases the resilience of farms by building healthy soil and crops that allow them to adapt better to a changing climate.

The **[21]** emphasizes that changes in people's lifestyles and consumption patterns are crucial for climate action **[63]**, **[64]**. With rapidly changing dietary habits, increasing purchasing power, and lifestyle changes in countries across Asia, especially those with large populations such as China and India, contributing to global climate solutions will be critical **[64]**. Lifestyle changes that can help with adaptation include:

- Engaging in urban agriculture through rooftop gardening, building community gardens in urban and suburban areas **[65]**, **[66]**, **[67]**;
- Moving towards organic farming and creating demand for organic food and other raw materials;
- Moving towards water conservation, such as rainwater harvesting, water conservation, reducing water consumption, etc.

The transition to organic farming and agroecological practices is the key to reducing and adapting to the adverse impacts of climate change. The transition to sustainable food systems must occur now and cannot be delayed any longer **[68]**.

Understanding the motivations and processes underpinning decisions to adapt or not is key to enabling adaptation (see: **[63]**, **[69]**, **[70]**, because how and why certain people adapt is shaped by sociocultural factors, ways of making sense of risks and uncertainty, and personal motivations to undertake action **[71]** in **[21]**. The IPCC's Assessment Report 5 was critiqued for silences on how perceptions shape climate action and the behavioural drivers of adaptation responses **[71]**. Addressing this gap and assessing the growing literature from social sciences, notably psychology, behavioural economics and risk perception studies, the IPCC Special Report on 1.5 °C **[63]** comprehensively assessed behavioural dimensions of CCA for the first time; however, compared with studies on mitigation behaviour, the literature on what motivates adaptation remains incomplete **[69]**.

According to **[21]**, there are three key aspects of adaptation to which psychology and behavioural science contribute: understanding perceptions of climate risk, identifying the behavioural drivers of adaptation actions and analysing the impacts of climate change on human well-being **[69]**. Overall, there is growing acknowledgement that individual adaptation is significantly shaped by perceptions of risk, perceived self-efficacy (i.e., beliefs about which options are effective and one's ability to implement specific adaptation interventions), sociocultural norms and beliefs within which adaptation decisions are taken, past experiences of risk management and the nature of the intervention itself **[70]**, **[71]**, **[72]**, **[73]**. This is in addition to more commonly understood factors shaping adaptation behaviour, such as technical know-how and the cost and benefits of an individual alternative.

CONCLUSION

The changes in the approach to organic food require a fundamental shift in the paradigms that are the basis of the current dominant thinking and action. However, this approach will provide an innovative, proactive stance, enabling and facilitating meaningful change for sustainable agriculture. This paper provides a basic framework for developing effective local, contextual, collaborative, integrated planning and action to achieve sustainability within the food systems, which is critical nowadays. The literature as mentioned earlier source also presents the consequences of the transition from current conventional agriculture and so-called shallow organic production to more sustainable deep organic production, using social ecology as a tool for their implementation. When evaluating macroeconomic indicators for the consumption of selected crops, it is, therefore, necessary to emphasize the need to understand and solve the psychological and psychosocial roots of the challenges of unsustainability for many contemporary modern societies.

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Polymer selection for microencapsulation of probiotics: impact on viability, stability, and delivery in functional foods for improved manufacturing and product development in the food industry

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ABSTRACT

Probiotics have won considerable interest in the food industry because of their health benefits. However, ensuring probiotics' viability, stability, and effective delivery in functional ingredients constitute a major concern. Microencapsulation is a promising method to ensure probiotic viability and stability. The best polymer for microencapsulation of probiotics is a determining factor. This paper presents an overview of the impact of polymer selection on probiotic viability, stability, and delivery in functional foods. It discusses numerous microencapsulation techniques and factors influencing polymer selection. It further explores the consequences of various polymers on probiotic viability, highlighting their protecting mechanisms. Additionally, it examines the role of polymer selection in enhancing probiotic stability during delivery, launch kinetics, storage and processing. The business packages of microencapsulated probiotics in foods and case studies on precise polymer choices for probiotic product improvement are also presented. Finally, we present challenges and future directions in using polymers for probiotic microencapsulation in the food industry. This review thus presents insights to enhance manufacturing tactics and product development within the food industry.

Keywords: Polymer selection, microencapsulation, probiotics, viability, stability, delivery, functional foods, product development

INTRODUCTION

Probiotics, defined as stay microorganisms that confer health benefits whilst fed on in adequate amounts, have received much interest within the food industry. The idea of using useful microorganisms for promoting health and wellbeing may be traced back to centuries of using fermented ingredients and conventional remedies. However, recent medical studies have shed light on the mechanisms of motion and ability applications of probiotics **[1]**, **[2]**, **[3]**. Functional ingredients, also called nutraceuticals, are food products that provide extra health benefits beyond basic vitamins. They are designed to optimize physiological functions and reduce the danger of certain diseases. Probiotics constitute one of the key components in the development of useful ingredients because of their ability to modulate the intestine microbiota, improve digestion, enhance immune features, and exert anti-inflammatory outcomes **[4]**. The intestine microbiota, a complicated community of microorganisms residing within the gastrointestinal tract, performs an essential role in human health. Disruptions inside the gut microbiota have been associated with diverse fitness situations, which include gastrointestinal disorders, metabolic problems, and immune dysregulation. Probiotics taken orally can engage with the gut microbiota and affect its composition and interest, consequently resulting in useful effects on host health **[4]**. The

significance of probiotics in foods lies in their capacity to provide a handy and centered approach to deliver specific beneficial microorganisms to the intestine. However, there are challenges associated with the delivery of probiotics, along with their survival during processing, storage, and passage through the cruel situations of the digestive tract. These challenges have explored microencapsulation as a strategy to shield probiotics and enhance their viability, stability, and delivery in functional food merchandise **[5]**, **[6]**.

By understanding the function of different polymers in protecting probiotics, researchers and manufacturers within the food industry can improve the producing approaches and product quality. Information from this paper will contribute to the advancement of the food industry, which should effectively release quality probiotic products in the marketplace.

The main objective of this review is to assess the impact of polymer selection for microencapsulation on the viability, balance, and delivery of probiotics in functional foods. It offers an overview of various microencapsulation techniques used for probiotic delivery and highlights the importance of polymer selection in these strategies. Additionally, it seeks to portray the factors influencing the choice of polymers for probiotic microencapsulation, including their physicochemical properties, biocompatibility, and capability. Furthermore, the review aims to study and analyze present literature related to the outcomes of different polymers on probiotic viability, stability, and release kinetics. It reveals the protective mechanisms supplied by way of selected polymers and their contributions to improving probiotic survival and capability. The commercial packages of microencapsulated probiotics in functional foods are discussed, and case studies on using unique polymers for probiotic product improvement are addressed **[20]**. The also reveals gaps in information and spotlight areas for future research and improvement within polymer selection for probiotic microencapsulation. It thus provides insights and tips for enhancing production tactics and product improvement in the food industry, aiming to optimize the viability, balance, and delivery of probiotics in functional foods. By addressing these objectives, this paper contributes to the know-how of the function of polymer choice in microencapsulation for probiotics. It aims to provide valuable insights for researchers, producers, and stakeholders in the food industry, guiding the selection and alertness of appropriate polymers for probiotic microencapsulation. Ultimately, the review seeks to facilitate the improvement of functional foods with greater probiotic efficacy and customer attractiveness.

Literature Search Strategy

A comprehensive literature search was realized to explore relevant studies and statistics related to polymer preference for microencapsulation of probiotics and its effect on viability, balance, and delivery in functional ingredients. The search was done using virtual databases: PubMed, Scopus, Web of Science, and Google Scholar. The search terms and keywords utilized in several mixtures covered "probiotics," "microencapsulation," "polymer preference," "viability," "balance," "delivery," "functional food," and associated phrases **[21]**. The search was limited on articles posted in English and focused on studies on food technology, food generation, microbiology, and biotechnology. The work was narrowed only to consider articles posted within the last ten years to ensure the inclusion of latest improvements and applicable research. In addition to the virtual database search, relevant references were also screened to include extra research that might have been omitted in the virtual research. This method, known as backward citation tracking, helped to ensure a holistic review of the applicable literature **[22]**.

Challenges in the Delivery of Probiotics

Probiotics have received huge interest due to their numerous advantages, but their successful delivery through the gut remains a challenge. Several factors hinder probiotics' viability, balance, and efficacy in functional foods. The challenges in probiotic delivery include viability throughout processing, shelf balance, acid and bile tolerance, colonization and persistence inside the intestine, interaction with the food matrix, and regulatory concerns. During processing, probiotics are exposed to conditions that could damage their cells, together with heat, shear forces, and pH modifications. Manufacturing techniques like drying, freezing, and excessive-pressure homogenization can reduce probiotics viability. Thus, retaining probiotics viability at some stage in processing is essential to maintain their functionality in the final product **[7]**. Shelf stability is another issue as probiotics have a constrained lifespan because of sensitivity to environmental elements like moisture, temperature, and oxygen. Over time, probiotics' viability can decline, reducing their efficacy. Therefore, ensuring the stability of probiotics during the shelf life of functional food products is important to maintain their potency **[8]**, **[9]**. Probiotics need to live on the acidic conditions of the belly and the bile salts within the small intestine through to the colon, where they exert their beneficial properties. However, many probiotic lines have low tolerance to those harsh situations, ensuing in massive losses of possible cells throughout gastrointestinal transit. For probiotics to offer long-term health benefits, they should be able to colonize and persist in the intestine. However, most probiotics are temporary and do not establish a long-lasting presence in the gastrointestinal tract. Enhancing probiotic survival and colonization inside the intestine is critical to ensure sustained efficacy. Probiotics are often integrated into food matrices, that

could affect their viability and capability. Factors including pH, moisture content, and the presence of other food components can affect probiotic survival and function. Understanding the interaction among probiotics and the food matrix is critical for optimizing delivery and maintaining probiotic viability. Furthermore, regulatory concerns pose challenges for developing and commercialising probiotic-containing functional ingredients. Compliance with labeling necessities, fitness claims, and safety exams is important for successfully marketing probiotic products. Microencapsulation is a promising method to cope with these challenges, since it helps to reinforce probiotic viability, balance, and delivery. By encapsulating probiotics within protective polymers, microencapsulation provides a barrier against harsh environmental situations, improves survival during processing and storage, and complements acid and bile tolerance, while permiting targeted delivery to the intestine. Choosing an appropriate polymer for microencapsulation is critical in overcoming these demanding situations and maximizing the capacity and advantages of probiotics in functional ingredients **[10]**, **[11]**.

Role of Microencapsulation in Improving Probiotic Viability, Stability, and Delivery

Microencapsulation is a valuable technique for enhancing probiotics' viability, stability, and delivery in functional foods. It involves the encapsulation of probiotic cells within protective polymeric materials, forming microspheres or particles that act as a physical barrier against environmental stresses. This protective barrier is crucial in improving probiotic viability, stability, and delivery. One of the key benefits of microencapsulation is its ability to protect probiotics against harsh conditions during processing. The encapsulating polymers create a barrier that reduces the exposure of probiotic cells to heat, shear forces, and pH changes. This protection minimizes cell damage and improves the viability of probiotics during processing, ensuring a higher number of viable cells in the final product **[12]**, **[13]**. Microencapsulation also enhances the shelf stability of probiotics in functional foods. The encapsulating polymers create a microenvironment that helps maintain probiotics viability by reducing moisture uptake, preventing oxygen exposure, and minimizing interactions with other food components. This increased stability allows for a longer storage period without significant losses in probiotics viability, ensuring the product's efficacy over time. Another important aspect of microencapsulation is its impact on probiotics survival in the gastrointestinal tract. As mentioned earlier, the encapsulating polymers provide a physical barrier that protects probiotic cells from the acidic conditions of the stomach and the presence of bile salts in the small intestine. This barrier reduces cell damage and increases the survival rates of probiotics, enabling a larger number of encapsulated cells to reach the colon, where their beneficial effects are exerted **[14]**, **[15]**. Microencapsulation also enables the controlled release and targeted delivery of probiotics. The encapsulating polymers can be designed to release probiotics in a controlled manner, allowing for sustained release over time. This controlled release ensures prolonged exposure of probiotics to the gut environment, increasing their chances of colonization and persistence. Additionally, microencapsulation facilitates targeted delivery to specific sites in the gastrointestinal tract, optimizing the therapeutic effects of probiotics **[16]**, **[17]**. Furthermore, microencapsulation offers compatibility with various food matrices, allowing the incorporation of probiotics into a wide range of functional food products. The encapsulating polymers can be tailored to withstand the specific conditions of the food matrix, maintaining probiotics viability and functionality. This versatility enables the development of probiotic-enriched foods with diverse textures, flavors, and processing requirements **[18]**, **[19]**.

Overview of Microencapsulation Methods

Figure 1 depicts the material used in the microencapsulation mechanism. Microencapsulation strategies involve the encapsulation of probiotic cells within defensive polymeric substances, forming microspheres or debris. These encapsulating systems act as a barrier, providing safety to the probiotics and enabling managed launch and focused delivery. Several microencapsulation methods have been developed and utilized for probiotics delivery **[23]**, **[24]**. One normally used approach is spray drying, which includes atomizing a probiotic-containing suspension right into a drying chamber. The droplets come in contact with a hot air circulation, resulting in fast evaporation of the solvent and the formation of dried particles. These debris encompass probiotic cells embedded within the polymer matrix **[25]**.

Another technique is the extrusion technique, in which a mixture of probiotic cells and a polymer solution is extruded through a small orifice, forming continuous strands. These strands are then cut into smaller debris to obtain microspheres or beads containing the probiotics. The coating technique includes coating probiotic cells with a polymer layer. Some techniques include fluidized bed coating, pan coating, or electrostatic coating. Multiple layers of polymers are deposited onto the probiotic cells, growing a shielding barrier. The emulsion approach includes the formation of an emulsion gadget comprising a probiotic-containing water segment, a polymer answer, and an emulsifier. The emulsion is then subjected to solvent evaporation or crosslinking to solidify the polymer matrix and encapsulate the probiotics **[26]**, **[27]**. Coacervation is a phase separation approach wherein a polymer answer is delivered into contact with a non-solvent, forming a polymer-rich coacervate phase.

The probiotic cells are then suspended or dispersed inside this coacervate segment and hardened to shape microcapsules.

Each microencapsulation method offers specific benefits and disadvantages. Some benefits of microencapsulation include the protection of probiotic cells from harsh environmental situations, controlled release of probiotics through the years, targeted delivery to unique regions of the gastrointestinal tract, and enhanced stability all through storage and processing **[28]**, **[29]**. However, there are also barriers and challenges related to microencapsulation techniques. Some techniques can be expensive and require specialised device, making them less economically feasible for large-scale manufacturing. Certain methods, such as coacervation and emulsion strategies, may be complicated and require precise control over manner parameters. The encapsulation process may additionally exert stress on probiotic cells, potentially resulting in a loss of viability. Scaling up microencapsulation techniques from the laboratory to business scale can also pose demanding situations in terms of process scalability, reproducibility, and cost-effectiveness. Despite these challenges, microencapsulation strategies remain precious for protecting and delivering probiotics. Ongoing research keeps optimizing and developing those techniques for various packages **[30]**.

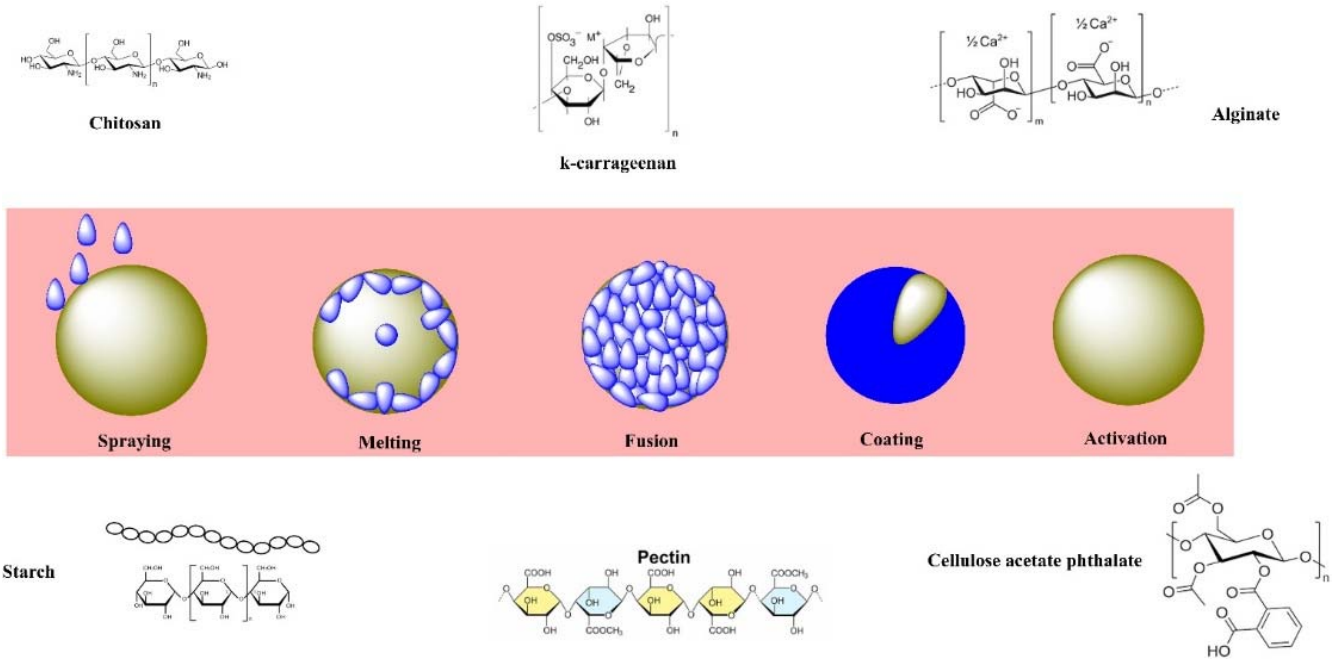

Figure 1 Scheme of used materials in microencapsulation.

Impact of Polymer Selection on Probiotic Viability

The choice of the suitable polymer for microencapsulation essentially impacts the functionality of probiotics. The epitome prepares and the properties of the chosen polymer can impact the survival and usefulness of probiotic cells amid capacity, preparing, and gastrointestinal travel. Here are a few key impacts of polymer choice on probiotic functionality:

1. Protection against Natural Push: The essential part of the polymer is to provide a defensive boundary around the probiotic cells, protecting them from unfavourable natural conditions. The appropriate polymer ought to be able to anticipate or minimize introduction to variables such as warmth, dampness, oxygen, and light, which can antagonistically influence probiotic functionality. It makes a difference by keeping a more favourable microenvironment for the probiotics, protecting their functionality over time **[31]**, **[32]**.

2. pH and Corrosive Resistance: The chosen polymer should display resistance to acidic conditions, especially within the stomach. Gastric corrosive is known to hinder probiotic functionality. The polymer ought to act as a boundary, deferring or lessening the introduction of probiotics to the acidic environment, prolonging their survival amid gastric travel.

3. Protection against Enzymatic Debasement: The polymer should protect against enzymatic action by stomach-related proteins within the gastrointestinal tract. Proteolytic chemicals, bile salts, and other chemicals can possibly debase probiotic cells, lessening their functionality. The polymer ought to repress or moderate enzymatic attack, guaranteeing that the probiotics stay viable until coming to the target location within the intestine.

4. Moisture Control: The chosen polymer should have great dampness control properties. Dampness can lead to the development of microorganisms, among which are potential pathogens and can adversely affect the functionality of probiotics. The polymer should avoid dampness take-up or discharge, keeping up an ideal dampness substance inside the microcapsules to back probiotic functionality **[26]**.

5. Oxygen Boundary: Oxygen can cause oxidative push, causing a loss of probiotic functionality and usefulness. typifying good polymer ought to act as a viable oxygen boundary, minimizing oxygen infiltration into the microcapsules and securing the probiotics from oxidative harm.

6. Release Characteristics: The polymer's discharge characteristics are critical in probiotic functionality. It ought to empower controlled and supported discharge of probiotics within the gastrointestinal tract, permitting for delayed presentation to the target location. This controlled discharge increases probiotic survival and colonization within the intestine **[33]**, **[34]**.

7. Interactions with Probiotics: The polymer should not display any hindrance with the probiotic cells. A few polymers may have antimicrobial properties or associate with the probiotic surface, compromising their functionality. Compatibility between the polymer and probiotics is fundamental to guarantee the ideal embodiment and functionality of the probiotic cells.

By carefully selecting a reasonable polymer with the vital defensive properties, the functionality of probiotics can be enhanced amid handling, capacity, and conveyance in utilitarian nourishments. Proper polymer determination can improve the survival and usefulness of probiotics, leading to expanded buyer benefits and better product quality **[35], [36]**.

Factors Affecting Probiotic Viability During Microencapsulation

One key aspect is the choice of microencapsulation approach. Different strategies, including spray drying or extrusion, can subject probiotic cells to high temperatures or shear forces that may potentially harm them. It is crucial to optimize the procedure parameters and situations to minimize stress and ensure minimum impact on probiotic viability **[37]**. The selected polymer must match well with probiotics and not cause toxicity or harm. Therefore, it is important to choose a biocompatible polymer with good enough protection and that does not compromise probiotic viability **[38]**. Encapsulation performance, which refers to the proportion of probiotic cells efficaciously encapsulated inside the microspheres or particles, can also affect probiotic viability. Low encapsulation efficiency means that many probiotic cells remain unprotected and are prone to environmental stresses. Hence, optimizing the encapsulation technique to gain high efficiency is crucial for optimum probiotic encapsulation and protection. The preliminary concentration of probiotic cells used during microencapsulation can also affect viability. High cellular concentrations can result in improved cell-to-mobile interactions, resulting in clumping or aggregation that can reduce viability. Therefore, it is important to optimize mobility to decrease aggregation and ensure uniform distribution in the microcapsules **[39]**, **[40]**. The use of shielding products, such as cryoprotectants or osmoprotectants, in microencapsulation can also impact probiotic viability. These products help mitigate the stress on probiotic cells at some point of processing and storage **[41]**. If the microencapsulation technique involves drying, the drying conditions can also affect probiotic viability. Controlling temperature, airflow, and drying time is important to decrease warmness and oxidative stress all through drying, as a result assisting to keep probiotic viability and capability. Proper storage conditions, such as temperature, humidity, and exposure to mild, significantly impact probiotic viability at some stage in storage. Implementing appropriate storage conditions, together with refrigeration or freeze-drying, is important to ensure long-time viability and stability of the microencapsulated probiotics **[42]**, **[43]**. Lastly, the aim of microencapsulation is also to decorate probiotic viability at some point of gastrointestinal transit. Factors such as resistance to acidic situations in the stomach, and protection in opposition to enzymatic degradation. Therefore, the microencapsulation method should ensure most probiotic viability and colonization inside the gastrointestinal tract. By thinking about and optimizing those factors for the duration of microencapsulation, the viability of probiotics may be better, ensuring their efficacy and functionality in functional foods. Thorough research and optimization studies are vital to discover top-quality situations for precise probiotic lines and encapsulation strategies **[44]**.

Evaluation of Different Polymers for Probiotic Viability

When choosing a polymer for the microencapsulation of probiotics, it is important to assess the potential effect of various polymers on probiotic viability. Various polymers have been investigated for their suitability in shielding and preserving probiotics during encapsulation. The following are a few commonplace methods for comparing the effect of different polymers on probiotic viability:

Viability Assays: Viability assays are normally used to evaluate the survival and viability of probiotic cells after encapsulation with distinctive polymers. These assays can encompass techniques like plate counting, fluorescence-based total staining strategies (e.g., stay/useless staining), or metabolic interest assays (e.g., MTT

assay). By evaluating the viability of probiotics encapsulated with one-of-a-kind polymers, researchers can decide the impact of each polymer on probiotic survival **[45]**, **[46]**.

Microscopic Examination: Microscopic examination, including light microscopy or scanning electron microscopy (SEM), can offer visual data about the morphology and integrity of encapsulated probiotics. It permits researchers to observe the physical interaction among probiotics and the encapsulating polymer, investigate cellular damage or aggregation, and examine the general encapsulation performance **[47]**, **[48]**.

Release Studies: Release studies assess the managed release of encapsulated probiotics from exceptional polymers. This research involves monitoring probiotics' discharge kinetics from the microcapsules under simulated gastrointestinal conditions or in specific food matrices. By evaluating the release profiles of probiotics encapsulated with extraordinary polymers, researchers can examine the effect of each polymer at the viability and capability of launched probiotics **[49]**, **[50]**.

Stress Testing: Stress testing includes subjecting the microencapsulated probiotics to simulated harsh conditions, such as excessive temperature, low pH, or exposure to digestive enzymes. This testing helps to examine the protective impact of different polymers on probiotic viability under tough conditions. Probiotics' viability and survival cost after pressure testing may be assessed using viability assays or suitable techniques **[51]**.

Shelf-Life Stability: Stability research is carried out to evaluate the long-term viability and balance of microencapsulated probiotics stored under specific conditions over a prolonged period. By tracking the viability of probiotics encapsulated with special polymers through the years, researchers can determine the polymer's effect on keeping probiotic viability throughout storage. These evaluation techniques offer precious insights into the influence of different polymers on probiotic viability and assist in choosing the most suitable polymer for microencapsulation, ensuring the maintenance and viability of probiotics throughout their lifecycle **[52]**.

Mechanisms of protection provided by selected polymers

Different polymers used for microencapsulation protect probiotics through various mechanisms, contributing to the preservation of probiotic viability and functionality during processing, storage, and gastrointestinal transit. One of the mechanisms is the physical barrier. Polymers act as a protective coating or matrix around the probiotic cells, creating a physical barrier. This barrier prevents direct contact between probiotics and external stressors such as moisture, oxygen, and enzymes, which can compromise probiotic viability. The probiotics are shielded from detrimental factors by forming a polymer barrier, reducing their exposure and preserving their integrity **[53]**, **[54]**. Moisture control is another important mechanism offered by many polymers used for microencapsulation. These polymers exhibit moisture control properties, allowing them to absorb or release moisture based on environmental conditions. By regulating moisture levels within the microcapsules, the polymers help to maintain an optimal moisture content for probiotic survival. This moisture control minimizes the risk of microbial growth and prevents dehydration or damage to probiotic cells **[55]**. Selected polymers also act as an effective oxygen barrier, which is crucial because oxygen exposure can lead to oxidative stress and damage to probiotic cells. By preventing oxygen penetration into the microcapsules, these polymers reduce oxygen availability and minimize oxidative damage to probiotics, ensuring their viability and functionality are maintained **[56]**. Some polymers provide resistance against acidic conditions and enzymatic degradation in the gastrointestinal tract. They can withstand low pH environments, protecting probiotics during gastric transit. Additionally, these polymers resist the action of digestive enzymes, such as proteases and bile salts, which can otherwise degrade probiotic cells. The acid and enzyme resistance provided by these selected polymers enhance probiotic survival in the harsh conditions of the gut **[57]**. Controlled release is an essential mechanism facilitated by selected polymers. These polymers allow for a controlled and sustained release of probiotics in the gastrointestinal tract. The encapsulated probiotics are gradually released, providing a continuous supply of viable cells to the target site. Controlled release enhances probiotic survival, colonization, and functionality in the gut **[58]**. Polymers used for microencapsulation should also be compatible with the specific food matrices in which the microencapsulated probiotics will be incorporated. The selected polymers should not adversely affect the final product's sensory attributes, texture, or stability. Compatibility with food matrices ensures the successful integration of microencapsulated probiotics into various functional food formulations, maintaining their viability and functionality **[59]**. Moreover, the selected polymers need to be biocompatible, meaning they are safe for human consumption and do not cause toxicity or adverse effects on probiotics. Biocompatible polymers are well-tolerated by the gastrointestinal tract, minimizing any potential harm to probiotic cells. This biocompatibility ensures the viability and functionality of probiotics during their journey through the gut **[60]**. By employing these protection mechanisms, selected polymers effectively safeguard probiotics during microencapsulation. The combination of the survival and functionality of probiotic cells and the polymers' physical, chemical, and barrier properties ensures the survival and functionality of probiotic cells. The polymers' physical, chemical, and barrier properties ensure probiotic cells' survival and functionality, enhancing their potential health benefits when incorporated into functional foods. The specific

mechanisms may vary depending on the characteristics of the chosen polymers and the encapsulation technique employed **[61]**.

Impact of Polymer Selection on Probiotic Stability

The choice of the proper polymer for microencapsulation has a big impact on the stableness of probiotics. Probiotic stability refers back to the ability of probiotic cells to keep their viability, functionality, and favored traits over the years. The choice of polymer can have an impact on different factors that affect probiotic balance during processing, storage, and product development. Here are some key influences of polymer choice on probiotic stability **[62]**.

1. Protection against Environmental Factors: Polymers act as a protective barrier, defending probiotics from environmental factors that can compromise their stability. Factors consisting of temperature, moisture, oxygen, light, and pH can adversely have effect on probiotic viability and capability.

2. Temperature Stability: Polymers can provide thermal protection to probiotics by insulating them from high temperatures encountered during processing, storage, and product management. High temperatures can result in probiotic mobile dying, reduced viability, and lack of capability. The selected polymer must have warmth resistance and thermal stability, stopping thermal degradation and making sure probiotic balance throughout warmness exposure **[63]**.

3. Moisture Control: Excessive moisture can promote microbial increase and compromise the stability of probiotics. The selected polymer should have moisture-limiting properties, either by preventing moisture uptake. This allows maintaining an ultimate moisture-content material in the microcapsules, minimizing the chance of microbial contamination and ensuring the long-term stability of probiotics **[64]**.

4. Oxygen Protection: Oxygen exposure can cause oxidative pressure that may harm probiotic cells and reduce their stability. The selected polymer needs to act as a powerful oxygen barrier, preventing oxygen penetration into the microcapsules. By minimizing oxygen exposure, the polymer maintain probiotic balance and preserves their viability and functionality.

5. Light Protection: Light, mainly UV radiation, can result in oxidative harm and reduce the probiotic balance. The decided-on polymer needs to offer safety against mild, appearing as a light barrier to limit UV penetration. This safety maintains probiotic's viability and functionality, ensuring their stability throughout product storage and manipulation **[65]**.

6. PH Stability: The gastrointestinal tract presents a variety of pH situations that probiotics ought to resist for powerful delivery and functionality. The selected polymer has to show off pH balance, permitting probiotics to continue to exist and maintain their balance under acidic situations inside the belly and alkaline situations inside the intestines. The pH balance guarantees that probiotics remain viable and useful throughout their transit inside the gastrointestinal tract **[66]**.

7. Long-Term Storage Stability: The balance of microencapsulated probiotics during storage is critical for product improvement and business viability. The selected polymer needs to contribute to the long-term stability of probiotics, making an allowance for prolonged shelf life without extensive lack of viability and functionality. This stability ensures the product can maintain its favoured probiotic content material and efficacy during its shelf life **[67]**.

Factors influencing probiotic stability during storage

In addition to the factors mentioned earlier (temperature, moisture, oxygen exposure, pH), processing techniques like freeze-drying and extrusion have potential to affect probiotics.Various processing techniques, namely freeze-drying, spray drying, and extrusion, can potentially expose probiotics to thermal, atmospheric, and mechanical stressors. Inadequate process parameters or excessive stress may decrease viability and stability, underscoring the importance of employing optimized processing methodologies **[68]**, **[69]**. Protective formulations incorporating cryoprotectants, prebiotics, or antioxidants can bolster probiotics' stability by alleviating stress and imparting supplementary protection. The selection and optimization of the protective agents have been evidenced to effectively enhance probiotics' survivability. Packaging materials are of utmost importance in the preservation of the stability of probiotics during the storage process **[70]**. Moreover, the stability of probiotic products can be influenced by the general composition of their formulation. In order to ensure the preservation of probiotic stability and viability, it is crucial to consider the potential for interactions with other dietary components, including prebiotics, fibers, and vitamins. The compatibility of probiotics with such ingredients should therefore be carefully evaluated to mitigate any adverse effects on their functionality. Optimization of the formulation is a crucial step in ensuring the stability of probiotics throughout the product's shelf life. The stability of probiotics during storage and processing can be considerably improved by meticulously considering and optimising these factors. Comprehensive investigation, suitable methodologies, proper storage

methods, and optimized compositions are imperative to attain utmost probiotic stability, guaranteeing their effectiveness and functionality in functional edibles and dietary supplements **[71]**.

Evaluation of polymer effects on probiotic stability

The assessment of polymer outcomes on probiotic stability is a complete method that involves examining a couple of components to ensure a complete understanding of how specific polymers impact probiotics' viability, functionality, and standard stability. One crucial aspect of evaluation is viability evaluation, where various strategies such as plate counting, fluorescence-primarily based staining techniques, or metabolic activity assays are applied to decide the survival and viability of probiotics encapsulated with special polymers. These viability exams permit researchers to evaluate the protecting results of every polymer and discover those that satisfactorily maintain probiotic stability **[72]**. Another essential attention is the evaluation of functionality, which includes assessing the ability of encapsulated probiotics to exert their unique health advantages or carry out metabolic activities. Functional assays and enzyme hobby assays or adherence assays are performed to observe any changes in probiotic functionality attributable to distinct polymers. By evaluating the impact of polymers on probiotic functionality, researchers can determine the volume to which polymer selection influences probiotic stability **[73]**.

Morphological evaluation: gives valuable insights into probiotics' bodily traits and structural modifications when encapsulated with special polymers. Techniques including light microscopy or scanning electron microscopy (SEM) permit researchers to observe encapsulated probiotics' morphology, cell structure, and aggregation styles. Morphological analysis aids in the assessment of the way polymers have an effect on probiotic stability on a visual level **[74]**. To evaluate the resistance of encapsulated probiotics to environmental stressors, researchers face difficulty in simulating conditions that mimic temperature variations, pH tiers, moisture exposure, or oxygen availability. These stress tests assist in determining the stability of probiotics under challenging conditions and decide how specific polymers contribute to their resilience **[75]**.

Release research: is performed to research probiotics' release kinetics from diverse polymers. By monitoring the release profiles of encapsulated probiotics under simulated gastrointestinal conditions or in particular foods matrices, researchers can examine the impact of polymers on the managed release and stability of probiotics during their adventure through the gastrointestinal tract **[76]**. Long-term balance research is essential to assess the viability and functionality of probiotics encapsulated with extraordinary polymers over an extended length of time. These studies contain storing encapsulated probiotics under unique conditions and periodically comparing their viability and functionality. By monitoring the long-time period stability, researchers can gain insights into probiotics' shelf lifestyles and apprehend how exceptional polymers contribute to their preservation **[77]**. Comparative studies are often conducted to immediately compare the performance of different polymers in phrases of probiotic balance. Encapsulating probiotics with various polymers and subjecting them to equal assessment techniques and situations permits researchers to compare and rank the polymers based on their impact on probiotic stability. Comparative research offers precious statistics for deciding on the maximum appropriate polymer for specific applications in the food industry. Researchers can gain comprehensive information on how specific polymers influence probiotic balance by evaluating viability, capability, resistance to stressors, launch profiles, long-term stability, and comparative performance. This knowledge guides the choice of the most appropriate polymer for keeping probiotics' viability, capability, and basic stability, permitting their successful integration into purposeful food products and dietary supplements **[78]**, **[79]**.

Influence of polymer properties on probiotic release and survival

The houses of the chosen polymer play a crucial role in probiotics' release and survival. The porosity of the polymer matrix is essential, as fairly porous polymers facilitate probiotic release but might also lead to an initial burst release that reduces viability. Balancing the porosity is vital. The degradation fee of the polymer affects launch kinetics, with quick degradation inflicting speedy release and potentially lowering survival. Optimizing the degradation rate ensures a managed launch. The polymer's water solubility or swelling capability influences launch, and adjusting these homes allows for controlled and extended release. Biocompatibility is crucial, as a few polymers may be cytotoxic or induce an immune reaction, leading to decreased viability. The pH sensitivity permits centered launch in unique intestine areas, protecting probiotics within the belly and liberating them inside the intestine. Mechanical strength is essential to defend probiotics at some stage in processing, storage, and transit. Interactions between the polymer and probiotics, which include electrostatic or binding interactions, affect release kinetics and viability. By thinking about and manipulating these polymer homes, it's possible to optimize probiotic launch and decorate their survival, ensuring controlled, targeted, and sustained launch while simultaneously ensuring controlled, targeted, and sustained launch while retaining viability and capability.

Evaluation of different polymers for probiotic delivery

Evaluating different polymers for probiotic delivery entails assessing their suitability and overall performance in probiotic viability, release traits, and basic delivery efficacy. Viability evaluation techniques, which include plate counting, fluorescent staining, or metabolic activity assays, are used to determine the quantity of feasible probiotics released from the polymer matrix and investigate the protecting impact of the polymer on probiotic survival. Release kinetics research assists in understanding the discharge profiles and controlled launch capabilities of different polymers, evaluating the concentration of launched probiotics at one-of-a-kind time factors. Controlled launch is evaluated by becoming release records to mathematical fashions and comparing the rate and volume of probiotic launches among distinct polymers. The stability of probiotics throughout encapsulation is assessed by tracking their viability and capability earlier than and after encapsulation. The compatibility of polymers with probiotics is examined for adverse outcomes on probiotic viability, capability, or morphology. Comparative studies directly compare the overall performance of different polymers in terms of probiotic delivery, assisting in identifying the most suitable polymer(s) for particular packages. These assessment methods and parameters guide the selection of the most effective polymers for maintaining probiotic viability, attaining managed launch, and improving usual delivery efficacy in useful foods and nutritional supplements.

Challenges and future directions

The use of polymers for probiotic microencapsulation in the food industry has shown outstanding potential in improving probiotic viability, balance, and delivery. However, numerous demanding situations need to be addressed, and there are future directions to explore to enhance the effectiveness of this technique. One vast task is deciding on the most suitable polymer for probiotic microencapsulation. The desire of polymer relies on various factors, including compatibility with the probiotic strain, processing conditions, desired release profiles, and regulatory concerns.

Maintaining high probiotic viability during processing, storage, and gastrointestinal transit is challenging. Despite the safety provided by encapsulating polymers, factors like moisture, oxygen, temperature, and mechanical stresses can nonetheless impact probiotic survival. Further studies are needed to optimize the system and processing conditions to maximize probiotic viability and increase the shelf life of microencapsulated probiotic merchandise. Scaling up the microencapsulation process for business production while maintaining the viability and capability of probiotics is also a mission. Developing cost-effective production techniques and scalable processes is important for the tremendous adoption of probiotic microencapsulation in the food industry. Exploring strategies which include non-stop production, automation, and enhanced equipment layout can contribute to addressing this mission. Achieving precise management over probiotic release profiles and focused delivery to precise sites inside the gastrointestinal tract is an ongoing venture.

The interplay between encapsulated probiotics and the food matrix can also affect probiotic viability and capability. Understanding the compatibility of encapsulating polymers with distinctive food matrices and the impact on food components, processing conditions, and storage situations on the release and pastime of encapsulated probiotics is important. Further research must discover these interactions to optimize the overall performance of microencapsulated probiotics in diverse food products.

Addressing health claims and regulatory concerns is vital for successfully implementing microencapsulated probiotics in food products. Clear pointers and regulations should allow for safety and efficacy of probiotic microencapsulation techniques. Future research needs to focus on setting up standardized protocols for evaluating the pleasant, stability, and capability of microencapsulated probiotics and understanding the effect of encapsulation on probiotic fitness benefits. Furthermore, developing superior characterization techniques is important for a better knowledge of encapsulated probiotics' conduct and overall performance. Techniques like imaging technology, molecular biology tools, and *in vitro* digestion models can provide valuable insights into the discharge kinetics, survival charges, and functionality of probiotics inside the encapsulation matrix and within the gastrointestinal surroundings. Addressing those challenges and exploring the suggestions mentioned above will contribute to successfully implementing polymer-based totally microencapsulation techniques for probiotics within the food industry.

CONCLUSION

In conclusion, selecting suitable polymers for microencapsulation is essential in enhancing probiotics' viability, stability, and delivery in functional foods. The encapsulation of probiotics using polymers gives numerous advantages, such as enhanced protection against environmental factors, controlled launch, and targetted delivery to the gastrointestinal tract. This technology has the potential to revolutionize the food industry by incorporating probiotics into a wide range of products, preserving their capability and fitness advantages. This paper has highlighted the importance of polymer choice in probiotic microencapsulation. Factors which include polymer biocompatibility, mechanical strength, and stability affect the performance of encapsulated probiotics. Various polymers, including alginate, chitosan, gelatin, and their mixtures, have been investigated for their suitability in probiotic microencapsulation. However, demanding situations still exist within the area of polymer-based probiotic microencapsulation. Overcoming problems associated with viability and survival in processing and storage, scaling-up and cost-effectiveness, particular manipulation over launch profiles and targetted delivery, interactions with the food matrix, regulatory concerns, and advanced characterization techniques require similar research and development. Despite these challenges, using polymers for probiotic microencapsulation holds wonderful promise for the food industry. It permits the production of functional foods, dietary supplements, animal feed, prescribed drugs, and beauty products with enhanced probiotic viability, balance, and delivery. The advancements in polymer choice and microencapsulation techniques contribute to developing progressive probiotic products that provide more advantageous health benefits, convenience, and customer acceptability. In the long run, research and collaboration between academia, industry, and regulatory bodies might be critical to overcome the challenges and explore new possibilities within the polymer-primarily based probiotic microencapsulation discipline. This will facilitate the development of secure, effective, and commercially viable probiotic products, leading to enhanced manufacturing processes, product quality, and better fitness results for clients in the long run.

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Advancements in nano bio sensors for food quality and safety assurance – a review

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ABSTRACT

Nano-biosensors are rising as a promising technology for ensuring the protection and high-quality of meals merchandise. They offer excessive sensitivity, selectivity, and speedy reaction, making them ideal for detecting contaminants, pathogens, and first-rate signs in meals samples. This up to date evaluate affords a complete evaluation of recent improvements in nano-biosensor technology for meals great and safety warranty. The evaluate covers the essential standards and kinds of nano-biosensors typically utilized in meals evaluation, exploring various nanomaterials and their unique homes and sensing talents. It also discusses mixing nanomaterials with biological reputation elements, antibodies, enzymes, and DNA aptamers to enhance sensor performance. The software of nano-biosensors in detecting chemical contaminants, which includes pesticides, heavy metals, and mycotoxins, is drastically protected. Nanomaterials allow ultrasensitive detection of these contaminants, even at trace stages, ensuring the protection and compliance of meal products. The review also explores the usage of nano-biosensors for rapid identification and quantification of foodborne pathogens, such as microorganisms, viruses, and parasites, allowing on-web page pathogen detection and timely interventions to prevent outbreaks. Additionally, the review highlights the tracking of meals satisfactory signs of using nano-biosensors, including freshness, spoilage, and dietary composition. Accurate assessment of those parameters offers treasured information to manage and predict shelf-life. Overall, the advancements in nano-biosensor generation maintain high-quality promise for ensuring the integrity of meals products, defensive public fitness, and assembly regulatory standards.

Keywords: nano-biosensors, food safety, food quality, contaminants, pathogens, nanomaterials

INTRODUCTION

Nanobiosensors are a promising tool for enhancing meals quality and safety assurance. These sensors use nanomaterials and biological recognition elements to locate and analyze food samples' specific biological or chemical components. They offer numerous blessings over traditional strategies, including rapid response, high sensitivity, and miniaturization. Nanomaterials have been utilized in various programs, including food production, processing, packaging, labeling, transporting, tracing, and maintaining excellent food products. They have also been used to broaden green optical sensors to discover hint pollution stages from various sources. Additionally, nanomaterials have increased nanobiosensors for food safety, enabling rapid and touchy detection of food-borne pathogens. Using nanocomposite materials in meal packaging has also made for a better great food product, with enhanced antimicrobial, mechanical, thermal, and barrier properties against external factors that could affect the food packaging system **[1]**, **[2]**, **[3]**, **[4]**, **[5]**.

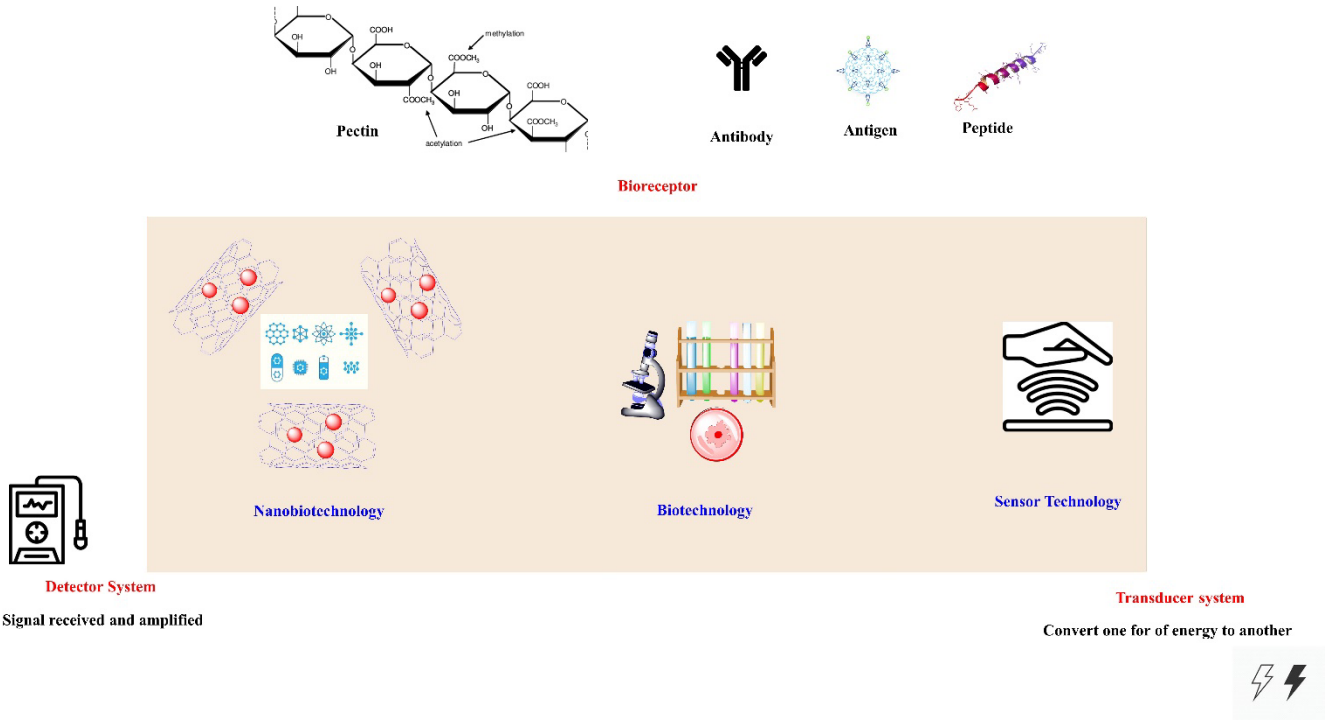

Figure 1 Scheme of different applications of nanobiosensors.

Food quality and safety assurance are essential elements of the food industry, ensuring that our food is safe, nutritious and free from contamination. Traditional approaches to food quality and safety assessment often require time-consuming and labour-intensive processes, which can delay the identification of potential hazards and compromise consumer health In order to overcome these challenges on, researchers and scientists have turned to nanotechnology, especially nano biosensors, as a promising solution. Nano biosensors combine nanomaterials with biological sensors to detect and analyze specific biological or chemical compounds in food samples. These sensors offer several advantages over conventional methods, making them an attractive tool for improving food quality and safety **[2]**, **[6]**, **[7]**.

One of the main advantages of nano biosensors is their fast reaction time. Traditional methods typically require extensive sample preparation and analysis time, which can take several hours or even days. In contrast, nano biosensors offer real-time monitoring and detection, providing almost immediate results. This allows quick decisions and interventions to prevent further contamination or food waste. Nano biosensors also exhibit high sensitivity, enabling the detection of trace contaminants or pathogens in food samples. Their ability to detect and quantify specific substances at trace levels is critical in identifying potential hazards and ensuring compliance with regulatory standards Upon reaching high sensitivity, nano biosensors can reduce false positives, negative and false positives, to give accurate and reliable results **[8]**, **[9]**, **[10]**.

Furthermore, the miniaturization of nano biosensors allows them to be integrated into portable handheld devices. This portable unit allows for easy on-site testing and monitoring across the entire food supply chain, from fields to pans. Farmers, food manufacturers, and distributors can use these devices for real-time analysis, spoilage detection, and detection of harmful substances Nanomaterials have been widely used in a variety of applications in the food industry. For example, processing, manufacturing, labelling, transportation and inspection of food products to ensure the quality and safety of foods are used to obtain the environment of mature consciences the development of the enabled pollution is enhanced and protected. Specialized nano(bio)sensors have been developed to ensure food safety. These sensors allow rapid and sensitive detection of food-borne pathogens, such as Salmonella, *Escherichia coli (E. coli*), and *Listeria monocytogenes*. Their high sensitivity enables rapid detection, allowing for early intervention to prevent the spread of infection and reduce the risk of foodborne illness **[2]**, **[3]**, **[10]**, **[11]**, **[12]**.

The use of new nanocomposite materials in food packaging has also contributed significantly to the quality of the food. Nano composite materials have desirable properties, such as high antimicrobial properties, mechanical strength, thermal stability, and barrier properties to prevent migration in food packaging systems and these properties can help extend the shelf life of perishable foods, reduce food waste and reduce the need for chemical preservatives **[3]**, **[13]**, **[14]**, **[15]**.

This review aims to provide a comprehensive understanding of the progress of nano biosensors for food quality and safety assurance. By highlighting the capabilities of these sensors and discussing their applications, limitations and prospects, it seeks to contribute to the growing knowledge base in this fascinating area and to stimulate further research and innovation to promote nanobiosensors for food technology.

Challenges in Shelf-Life Monitoring

Accurate and predictive analysis of the shelf life of food products is essential to ensure safety and quality throughout storage and distribution. However, there are many challenges to this task. One of the major challenges is the complexity of food systems, which have different components and characteristics. Factors such as moisture, pH, fat content, and the presence of additives or preservatives can significantly impact storage time. Understanding the interconnections and dynamics of these complex matrices is essential to monitor shelf life accurately. Another challenge is the inherent variability in the material properties. Food products can exhibit variations in microbial loads, enzymatic activity and chemical reactivity due to factors such as raw materials, processing methods and storage conditions. These variations make it difficult to establish statistics fixed retention period **[3]**, **[15]**, **[16]**.

Microbial spoilage and decomposition are important challenges in storage time management. Microorganisms play an important role in food packaging, affecting storage and safety. Accurate identification and detection of microbial organisms is essential for effective management. Furthermore, microbial adaptation and evolution can lead to the emergence of new strains or pathogens, further complicating monitoring efforts. Time and cost are additional challenges in determining shelf life. Traditional methods typically require time-consuming and expensive procedures such as sensory testing, microbiological testing, and chemical analysis. These methods may not be suitable for real-time monitoring, making it difficult to respond quickly to product quality or safety changes. The lack of standardized methods for predicting the shelf life of foods is another challenge. Each production stage may require specific procedures, making it difficult to establish standardized quality control guidelines and procedures **[2]**, **[16]**, **[17]**.

Furthermore, interactions between packaging and food products can affect shelf life. Process efficiency and stability can be affected by factors such as air permeability, moisture permeability, and the presence of volatiles. Understanding and managing these interactions is essential to maintaining shelf life accuracy. Addressing these challenges requires developing and adopting advanced technologies such as nano biosensors. By integrating this technology into food packaging design, it is possible to improve the accuracy, efficiency and reliability of inspection practices through the limitations of traditional methods, controlling limitations of traditional methods using nanobiosensors also provide real-time precision monitoring capabilities, timely intervention and enhanced quality control. They provide valuable insights into changes in the food chain, enabling better decision-making and reducing food waste **[3]**, **[11]**, **[13]**, **[14]**, **[15]**, **[16]**.

Need for Advanced Monitoring Systems

The demand for superior monitoring systems in food packaging and shelf-lifestyles management has grown because of the increasing want for stronger product quality, safety, and efficiency throughout the supply chain. Traditional tracking methods frequently fail to provide real-time data and comprehensive insights into product conditions. Therefore, implementing superior tracking systems, including nano biosensors, is important for numerous key motives. Firstly, advanced tracking systems allow extra correct shelf-life determination. These structures offer actual-time information by constantly tracking essential parameters like temperature, humidity, fuel composition, and microbial hobby, allowing for a more precise shelf life assessment. This is an extensive improvement over conventional techniques that depend upon fixed expiration dates. Secondly, superior tracking structures facilitate prompt intervention and quality control. Real-time monitoring enables the early detection of deviations or adverse conditions, allowing for prompt moves to save you spoilage, maintain product high-quality, and reduce food waste. By figuring out capacity troubles quicker, businesses can take corrective measures to ensure product integrity **[11]**, **[13]**, **[14]**, **[16]**, **[18]**.

Third, these programs contribute to enhancing food safety. Advanced monitoring systems can identify potential sources of contamination, microbial growth, or degraded conditions that could compromise food safety. Early identification of such risks enables appropriate action to prevent the distribution and consumption of unsafe products, thereby protecting consumer health. Another advantage of a comprehensive monitoring system is effective supply chain management. Real-time information about product conditions, such as fluctuations in temperature, helps identify points that can affect product quality or shelf life. This information simplifies logistics planning, reduces losses, and improves inventory management, leading to more efficient and cost-effective supply chains **[3]**.

Additionally, the improved surveillance system contributes to customer confidence and transparency. By incorporating smart labels or QR codes, consumers can receive real-time information on renewal, safety and storage recommendations. This transparency enables consumers to make informed purchasing decisions and builds consumer confidence in the products they purchase. Sustainability and waste reduction are addressed through advanced monitoring systems. By more accurately determining the remaining life of consumer goods, premature disposal of still-safe consumer goods can be reduced, including food waste, consumables role and environmental reduction. These programs help companies comply with regulatory standards. Monitoring companies provide precise timekeeping control and management. Advanced monitoring systems enable product inspection, data recording and proof of compliance with food safety standards to help businesses comply with regulatory requirements **[19]**, **[20]**, **[21]**, **[22]**, **[23]**.

Principles of Nano Biosensors

Nano biosensors are state-of-the-art devices that use the principles of nanotechnology and biotechnology to detect and analyze specific biological, chemical, or physical agents. These sensors work based on several fundamental operating principles. First, nano biosensors incorporate a detector responsible for operating principles selectively interacting with the target probe. This sensing agent can be a biological molecule, such as an enzyme, antibody, nucleic acid, or receptor, or a synthetic material with a specific binding ability. The selectivity of the sensor's specificity and ability to distinguish between the target analyte and other factors. Second, nano biosensors use a transducer, which converts the binding reaction between the detection element and target analyte into a detectable signal. The transducer can be based on physicochemical principles, including electrical, optical, light energy, or piezoelectric effects are included. Converts a biological or chemical discovery process into a quantifiable form that can be detected and analyzed accurately. Nano biosensors typically incorporate amplification techniques to increase the sensitivity and detection capability of the target molecule. These methods can include enzymatic reactions, signal amplification based on nanomaterials, or signal amplification by electronic or optical methods. Amplification techniques increase the signal-to-noise ratio, enabling the analyst to detect even trace amounts **[23]**, **[24]**, **[25]**, **[26]**, **[27]**, **[28]**, **[29]**.

Signals generated by nano biosensors are often read and detected using specialized instruments or devices. This can be electronic devices, optical detectors, imaging systems, or remote sensing methods. The readout system converts the sensor output into meaningful data that can be analyzed and interpreted further. Moreover, nano biosensors are characterized by miniaturization and integration abilities. Using nanoscale materials and manufacturing techniques, these sensors can be made extremely small, allowing them to be incorporated into platforms. They can be incorporated into lab-on-chip devices, wearable sensors, or products that packaging in combinations. This miniaturization enables mobility, adaptability, and integration into complex systems. Concepts of ecological and environmental analysis, importance, and time savings across various industries include those used in various industries, and it lacks the positive role of unique, acceptable and transformative specificity. To analyze implications and emotions, for Shelf-Life decision-making and to provide greater control **[22]**, **[30]**, **[31]**.

Introduction to Nano Biosensors

Nano biosensors are advanced devices that combine the principles of nanotechnology and biotechnology to detect and analyze specific biological, chemical, or physical properties of target molecules. These sensors work at the nanoscale, leveraging the unique properties of nanomaterials to enable highly sensitive and selective detection. Nano biosensors have received significant attention and applications in various fields, such as healthcare, environmental monitoring, and food safety. The key components of a nano biosensor are a detector, transducer, and readout system. The sensing agent, typically a biomolecule or synthetic material with the ability to selectively bind to a specific target, selectively interacts with the target probe. This binding process generates a signal that the converter converts into a detectable output such as an electrical, optical, or chemical signal, and the readout system detects and interprets the result, providing valuable information regarding the presence and quantity of the target molecule **[32]**.

As nanotechnology continues to evolve, nano biosensors have the potential to revolutionize food packaging and shelf-life by improving product quality, reducing waste, increasing consumer safety, and maintaining the supply chain. However, challenges related to standardization, integration, and regulatory considerations must be addressed for wider adoption and commercialization of nano biosensors in the food industry **[30]**, **[31]**, **[32]**, **[33]**, **[34]**.

Nano biosensors have several applications in food packaging, including the following:

- Monitoring key parameters: Nano biosensors can be integrated into food packaging materials or devices to monitor critical parameters such as temperature, humidity, gas concentration, and microbial activity in real-time.
- This enables accurate product freshness, quality, and safety determination, enabling timely interventions and informed decision-making throughout the supply chain.
- Detection of pathogens: Nano biosensors can be used to detect pathogens in food packaging, which can help prevent foodborne diseases.
- Shelf-life monitoring: Nano biosensors can be used to monitor the shelf-life of food products by detecting changes in the chemical composition of food, such as the presence of volatile organic compounds (VOCs) that indicate spoilage.
- Quality control: Nano biosensors can be used for quality control in food packaging by detecting contaminants or adulterants present in food products.

Working Mechanisms of Nano Biosensors

Nano biosensors work based on various techniques in which nanomaterials and biological recognition elements are used to detect and analyze target analytes. The working mechanism of a nano biosensor depends on its design and configuration. Some common applications are optical detection, electrochemical detection, piezoelectric detection, magnetic detection, and nanomechanical detection. optical, electrochemical, piezoelectric, magnetic, and nanomechanical detection techniques. Changes in the optical properties of these nanomaterials, such as fluorescence intensity or surface plasmon resonance, occur when the detection element binds to the analyte. On the other hand, electrochemical nano biosensors lead to changes in electrical properties such as conductance or impedance changes used to investigate binding events **[35]**, **[36]**, **[37]**.

Piezoelectric nano biosensors use nanomaterials with piezoelectric properties, causing changes or deformations in mechanical stress due to binding phenomena magnetic nano biosensors use magnetic nanomaterials, where changes in magnetization or magnetic field response indicate that analyte it binds to nano biosenicsers they measuring structures like mass or resonance frequency to detect binding events, in order to determine bonding phenomena **[38]**.

These working mechanisms show the various approaches nano biosensors use to detect and measure target analytes. By using the distinctive properties of nanomaterials and their interaction with recognition elements, nano biosensors offer high sensitivity and selectivity in detection. They can be customizable for specific analytes and applications, enabling real-time monitoring of essential parameters in food packaging and shelf-life management. Figure 2 displays the mechanism of how nano biosensors working.

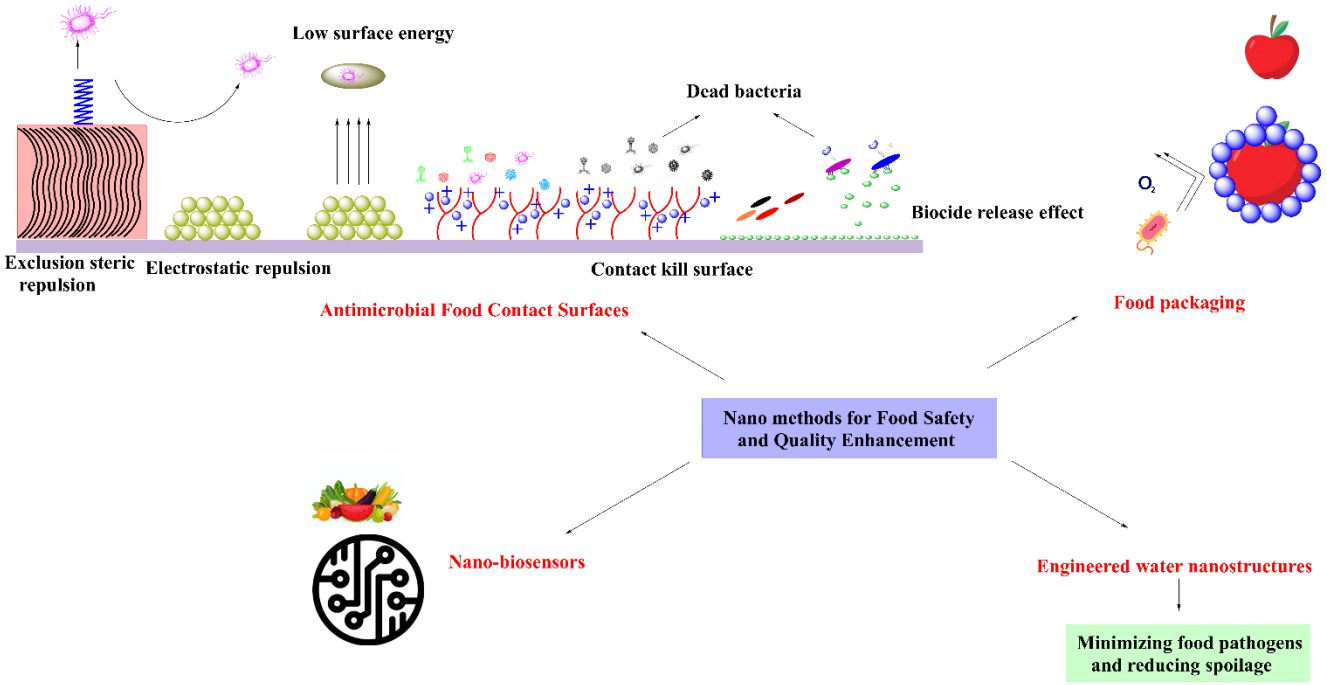

Figure 2 Scheme of nanobiosensors working mechanism.

Types of Nano Biosensors

Nano biosensors include various types of systems, each designed for analyzing specific materials. Some types of nano-biosensors commonly used in various applications, including food packaging and shelf-life monitoring, are nanoparticle-based biosensors, and nano-wire biosensors based on carbon and porous materials. Availability of Raman scattering (SERS) biosensors and nanoimprint biosensors. Nanoparticle-based biosensors use nanoparticles such as quantum dots, gold, or magnetic nanoparticles as transducer components. A detector such as antibodies, enzymes, or DNA probes is used is attached to the surface of the nanoparticles. When the detector binds to the target probe, it induces changes in the optical and electromagnetic properties of the nanoparticles, allowing the analyte to be concentrated and quantified **[39]**, **[40]**, **[41]**, **[42]**.

Nanowire biosensors are nanoscale wires made of silicon or carbon nanotubes. The recognition element is located on the surface of the nanowire, and upon binding to the target analyte, causes a change in electrical conductivity or impedance. These changes are measured, and the concentration of the analyte is determined based on the electrical signal. Carbon-based biosensors use carbon nanotubes, graphene or other carbon-based nanomaterials. These materials exhibit excellent electrical conductivity and a large surface area, making them suitable for sensing applications. The recognition element is embedded in a carbon-based nanomaterial, and the binding event with the target analyte induces changes in electrical conductivity or electrochemical properties, allowing detection and analysis **[43]**, **[44]**, **[45]**.

Nanopore biosensors utilize tiny pores, typically at the nanoscale, to detect and analyze analytes. The recognition element is placed inside the nanopore, and when target analytes pass through the pore, there is a change in ionic current or electrical impedance. These changes are measured, and the concentration or type of analyte is determined based on the electrical signal. Surface-enhanced Raman scattering (SERS) biosensors use metallic nanostructures, such as silver or gold nanoparticles, to enhance the Raman scattering signal of target analytes The identification element is attached to the nanoparticle surface at the Raman scattering signal or spectrum or spectrum in which it binds to the analyte or conversion occurs These conversions are measured using Raman spectroscopy, allowing sensitive detection and identification of the analyte **[46]**, **[47]**, **[48]**.

Nanoimprint biosensors use nanoimprint lithography to create nanoscale patterns or features on a substrate. The recognition element is immobilized on a patterned surface, and binding to an analyte causes changes in optical and electromechanical properties these changes are measured, and the analyte is quantified or characterized appropriately. These types of nano biosensors provide unique benefits and are appropriate for specific analytes or sensing requirements. An appropriate type of nano biosensor is selected based on factors such as target analyte, sensitivity, selectivity, intended use in food packaging and shelf-life monitoring **[49]**, **[50]**, **[51]**, **[52]**, **[53]**.

Nano Bio Sensors for Food Packaging

Nano biosensors are highly advanced sensing devices that use the principles of nanotechnology and biotechnology to monitor and manage food quality, safety, and shelf life. These sensors are incorporated into packaging materials or devices, continuous monitoring of important parameters such as temperature, humidity, gas concentration and microbial activity and accurate Provision of Nano biosensors enables timely intervention and manufacturing controlled to ensure the freshness and safety of packaged food products. Sensors use a variety of sensing techniques based on optical, electrochemical, or nanomechanical principles to detect and analyze specific materials of interest. Furthermore, nano biosensors contribute to intelligent packaging design by integrating sensors into packaging or devices to ensure food integrity and quality. This allows for improved monitoring and control of material conditions, including detecting increased temperatures, decreased ventilation, or bacterial growth and taking appropriate action to maintain optimal conditions **[1]**, **[12]**, **[54]**, **[55]**, **[56]**, **[57]**.

One of the most important advantages of nano biosensors is their ability to improve quality and safety assurance in food packaging. They enable early detection of potential sources of contamination, microbial growth, and conditions that could compromise food safety and freshness. This enables prompt action, preventing the distribution of unsafe or substandard products that have been eaten. Moreover, nano-bio sensors play an important role in the remaining shelf life of food products. This sensor provides accurate and dynamic information about material conditions by continuously monitoring parameters such as temperature, humidity and air content. This accurate determination of shelf life decreases unnecessary waste and enables better inventory management. Nano biosensors also contribute to traceability and transparency in food packaging by incorporating technologies such as RFID or QR codes. These sensors provide consumers with information on product history, shelf life, and expiration dates, enabling them to make informed purchasing decisions and increase confidence in the food supply chain. Additionally, nano biosensors contribute to sustainability efforts by reducing food waste. By precise control of production conditions, unnecessary disposal of still-safe consumables is prevented by accurately determining the remaining shelf life This waste reduction not only reduces consumption but also meets sustainability goals **[3]**, **[4]**, **[58]**, **[59]**.

Current State of Food Packaging

The current state of the food packaging industry is characterized by continuous developments and innovations driven by consumer needs, regulatory requirements, and technological advancements. Safety and security are top priorities, with continuous enhancements in food packaging materials and technologies to safeguard foods from contamination, moisture, oxygen, light, and physical damage. Convenience drives adopting eco-friendly solutions that reduce waste, enable recycling and promote recycling. Proactive intelligent packaging technology is gaining popularity due to the ability to interact with products, monitor conditions, improve quality, and prolong shelf-life. Packaging is a crucial marketing tool, offering branding opportunities and effective communication with consumers through attractive design, labelling, and storytelling elements on the packaging **[53]**, **[60]**, **[61]**, **[62]**, **[63]**, **[64]**, **[65]**.

Convenience drives the adoption of eco-friendly solutions, including standards and regulations for materials, additives, labelling claims, and recycling practices to guarantee food safety, labelling precision, and environmental compliance. E-commerce has also led to specific packaging material requirements for shipping, protecting products during transportation, and promoting efficient resource management. The current state of food packaging reflects a dynamic state driven by safety, sustainability, convenience, branding, and regulatory compliance. Technological advances and evolving consumer preferences continue to shape the industry, while future growth may focus on additional environmental sustainability, the incorporation of intelligent packaging technologies, and addressing emerging challenges related to food waste and traceability **[66]**, **[67]**, **[68]**, **[69]**.

Role of Nano Biosensors in Food Packaging

Nano biosensors play an important role in enhancing the efficiency and effectiveness of food packaging by integrating the principles of nanotechnology and biotechnology. The main application is continuous real-time monitoring by nano biosensors to monitor the temperature, humidity, and gas concentration and microbial activity in the packaging. This allows for immediate detection of deviations and timely corrective action to ensure quality and safety. Nano biosensors also contribute to quality assurance by detecting degradation indicators such as volatile organic compounds (VOCs) or microbial activity. This early detection enables immediate decision-making and mitigates the risk of substandard or unsafe products. For shelf-life extension, nano biosensors offer precise and real-time information about packaging conditions. This helps to identify and address spoilage factors more quickly, facilitating interventions to enhance product freshness and prolong shelf life. Nano biosensors contribute to food safety by detecting harmful contaminants, pathogens, and allergens. They can be designed to specifically target and detect these objects, enhancing security assurance. Furthermore, nano biosensors can be integrated into actual packaging materials for quality and authenticity control. For example, sensors embedded in packaging films can detect breakage or leaks, preventing physical damage, contamination, or exposure to adverse conditions. Nano biosensors also help enable examine and honest in packaging. Including unique indicators enables the tracking of product origin, manufacturing and storage of products, enhances visibility and prevents counterfeiting and tampering. In terms of waste reduction, nano biosensors contribute to reducing food waste by providing accurate information on freshness and shelf life. This allows for better inventory management and prevents the premature disposal of safe and edible products **[33]**, **[70]**, **[71]**, **[72]**, **[73]**.

Enhanced Integrity and Barrier Properties

Nano biosensors are important in enhancing food packaging integrity and barrier properties. Several strategies for incorporating nano biosensors can be used to enhance the safety potential of food packaging using nanotechnology-based materials. On the one hand, nano biosensors enhance integrity by providing mechanical strength to the packaging. They can be incorporated into packaging films or coatings to provide durability and resistance to tears and physical damage during controlled storage. Nano biosensors also contribute to the gas barrier properties of materials, a packaging is excellent. Nanocomposite films can be produced by incorporating nanoparticles or nano clays into the packaging material, significantly reducing oxygen and water permeability. This provides the desired environmental properties of the packaging material to remain intact, preventing food contamination, oxidation or water-related non-degradation **[33]**, **[70]**, **[71]**, **[72]**, **[73]**.

Another advantage is the ability to deliver antibiotics. Nano biosensors can incorporate antimicrobial compounds or nanomaterials with inherent antibacterial properties into the packaging. This actively inhibits the growth of bacteria, fungi, and other microorganisms, extends the shelf life of foods, and ensures safety. Nano biosensors can also serve as an anti-pollution barrier. The application of nanocoatings or nanoporous membranes on packaging materials prevents unwanted ingredients such as chemicals or fragrances in the food, thereby enhancing product safety, quality, and consumer acceptability. Nano biosensors can also contribute to UV protection. Packaging containing UV-repellent nanoparticles or coatings effectively protects photosensitive foods

from harmful UV rays, while retaining their sensory properties and nutritional value. Nano biosensors can also provide waterproofing solutions. Nanomaterials with absorbent or water-repellent properties can be incorporated into films or packaging bags to maintain optimal moisture content for moist products. This helps prevent texture changes, microorganisms growth and migration edges. In addition, nano biosensors help protect the taste and aroma of foods. Packaging films can be incorporated with nanomaterials containing barrier anti-taste or odour molecules to prevent loss or contamination and preserve the sensory properties of the product **[72]**, **[74]**, **[75]**.

Active Packaging Systems

Dynamic packaging design involves packaging technology that interacts actively with packaged food to extend shelf life, enhance safety, and improve overall effectiveness. Nano biosensors consume plays an essential role in designing and implementing dynamic packaging systems. This framework employs various techniques and applications to create a dynamic environment that adapts to packaging material or product changes. Nano biosensors and gas laws enable a key feature of dynamic packaging. Nano biosensors enable systems to monitor and control the gas content of packaging materials. It includes the detection of oxygen, carbon dioxide, or ethylene levels, which are important factors affecting food quality and shelf life. Based on sensor readings, the packaging system can release or absorb specific gases to maintain optimal airspace for the product, thus reducing waste and preventing widespread product contamination. Hydration is another crucial aspect of dynamic packaging. Nano biosensors embedded in these systems can monitor the moisture content of the packages. Excess moisture or changes in humidity can be detected and trigger mechanisms to release or absorb moisture in the packaging. This helps prevent moisture-related issues such as microbial growth and alteration that results in moulding or spoilage, thereby improving the quality and safety of packaged foods. They observe **[71]**, **[76]**, **[77]**, **[78]**, **[79]**.

Nano biosensors can also be active packaging materials with antimicrobial agents or nanomaterials with innate antimicrobial properties. These biosensors detect the presence of harmful microorganisms or signs of spoilage and trigger the release of antimicrobial agents to prevent microbial growth. This tight control of microbial activity helps to prolong the shelf life of the product and enhances security. Temperature monitoring and control is another function nano biosensors enable in dynamic packaging materials. These sensors can monitor temperature changes in the packaging and detect deviations from the desired temperature. If necessary, the packaging system can trigger storage cooling and heating mechanisms to maintain optimal storage conditions. Thermal insulation helps preserve the quality and freshness of the product, especially for hot items. Nano biosensors facilitate the integration of time-temperature records into dynamic packaging materials. These signals provide visual or electronic information about the aggregation behaviour of the product under adverse temperature conditions. By looking at the temperature history, customers and stakeholders can assess the time remaining in a product and make informed decisions about its suitability **[76]**, **[77]**, **[78]**, **[79]**, **[80]**.

Nano biosensors continually monitor various parameters in dynamic packaging systems, such as pH, colour change, or volatile emissions. Nano biosensors detect indicators of quality degradation and distribute real-time information to stakeholders. This information enables prioritization of actions, such as rearrangement, debris removal, or quality assessment. Moreover, dynamic packaging can offer an intelligent feedback mechanism that employs nano biosensors to provide stakeholders in the supply chain. Such feedback may comprise information on heat transfer, changes in air composition, or product quality conditions. These feedback systems expedite decision-making and improve supply chain management **[76]**, **[78]**, **[79]**, **[80]**.

Nano Bio Sensors for Shelf-Life Monitoring

Determining shelf life is crucial in food production and quality control. It evaluates when a food can maintain its desired quality, safety and performance under specific storage conditions. Nano biosensors are valuable in determining shelf life by offering real-time and accurate data on parameters that affect product stability. To determine shelf life, it is essential to consider a broad range of factors that may change over time. These parameters include sensory attributes such as taste, texture, colour, and nutrient content, microbial load, chemical composition, and processing characteristics. Periodic analysis of these parameters during storage can assess variability and aid in identifying when the product no longer meets acceptable quality standards. Accelerated Lifecycle Testing (ASLT).) is a common method for estimating product durations. ASLT simulates the effects of rapidly changing environmental conditions, such as high temperature, humidity, or light exposure, on a shorter time scale to predict long-term storage effects. Nano biosensors assist in ASLT by continuously monitoring changes in and recording relevant parameters. Nano biosensors offer real-time feedback and valuable insights into product stability and degradation processes, aiding in determining overall shelf life. In summary, nano biosensors enhance shelf-life accuracy and efficiency by providing real-time analysis of critical parameters that enable them to determine shelf life **[71]**, **[77]**, **[78]**, **[79]**.

Traditional Methods vs Nano Biosensors for Shelf-Life Monitoring

Food product shelf-life monitoring traditionally involved manual analysis, laboratory testing, and sensory evaluation. Nano biosensors have revolutionized monitoring by offering significant advantages over conventional methods. Sensory evaluation is a traditional method where trained assessors rate attributes such as taste, smell, appearance, and texture. However, sensory evaluation can be subjective, biased, and limited in information. In contrast, nano biosensors offer real-time and objective information about traits such as volatiles, pH levels, or enzymatic activities. They offer quantitative measurements, reducing subjectivity and providing accurate and consistent information, regardless of changes in innovation and quality **[81]**.

Cytology is another traditional method involving laboratory testing to detect the presence and growth of microorganisms or pathogens. This process can be time-consuming and may not yield immediate results. In contrast, nano biosensors allow real-time monitoring of microbial activity, enabling quick microbial detection and quantification. They offer rapid results and direct detection of signs of damage or pathogens, enabling timely intervention and ensuring microbial safety. This method can be time-consuming and requires sample preparation and complex equipment. In contrast, nano biosensors offer continuous real-time monitoring of chemical parameters such as pH, oxidation markers, or gas content. They offer sensitive and selective detection, enabling immediate detection of chemical changes that affect product properties **[81]**.

Physical testing, including texture, colour and physical properties, is usually performed by visual inspection or instrumental measurement in traditional methods. However, these tests can be subjective, requiring human interpretation, and may not account for subtle changes in material properties. In contrast, nano biosensors can offer accurate and quantitative measurements of physical properties, such as texture, using special nanomaterials or modifiers. They enable objective and sensitive detection, enabling the identification of transcriptional changes that can affect production efficiency. Nano biosensors provide more accurate and consistent results compared to optical or manual inspection. Time-temperature indicators (TTIs) offer an alternative to traditional methods, such as using labels or devices that change colours or provide visual cues based on cumulative temperature changes. However, TTIs provide limited information about changes in the material and require visual inspection. Nano biosensors can include time-temperature monitoring features, offering precise and detailed information on temperature changes. They offer real-time monitoring of temperature history, enhancing our understanding of temperature's impact on product quality and facilitating shelf-life determination **[81]**.

Real-Time Monitoring Techniques

Real-time monitoring techniques are vital to ensuring the quality and safety of food products throughout their shelf life. A common approach involves using nano biosensors, where nanotechnology is utilized to detect and analyze specific compounds in real-time. These sensors can be integrated into food packaging or food processing to offer rapid and precise measurements of temperature, pH, humidity, and microbial activity. Nano biosensors offer accessibility oversight, enabling timely intervention and quality and safety assurance. Another crucial area for real-time monitoring is Internet of Things (IoT) devices. These sensors and connected devices gather and transmit data wirelessly. The food industry employs IoT devices to monitor temperature, humidity, and storage conditions. Stakeholders can monitor product quality in real-time by continuously collecting and sharing information in a centralized system. IoT devices allow for remote monitoring, data analysis, and automated alerts, aiding swift decision-making. Wireless sensor networks (WSNs) are interconnected sensors that wirelessly communicate to monitor and collect data from various points in the system. In food management, WSNs can monitor temperature, humidity, and gas content in storage areas or pipelines. The sensors communicate with a central control system, providing real-time environmental data. WSNs provide scalability, flexibility, and the ability to monitor large areas or multiple locations simultaneously. Imaging and vision systems use cameras, scanners, or spectrometers to capture images or analyze visual information about the food prod **[81]**, **[82]**, **[83]**, **[84]**.

E-nose systems use gas sensor systems to detect and analyze volatile compounds generated by food processing, while e-tongue systems use sensor systems to assess taste profiles. This system provides fast and real-time food taste and aroma analysis, facilitating quality control and sensory monitoring. Data analytics and machine learning algorithms are often combined with real-time analytics techniques to derive meaningful insights from the collected data. These techniques analyze patterns, look for anomalies, and predict future events based on real-time data. Using historical data and real-time measurements, combined with data analytics and machine learning for predictive modeling and optimizing decision support systems, enables food manufacturers to monitor priorities continuously, identify deviations from desired situations, and optimize decision-making. By implementing these real-time analytics techniques, technology acts as an active enabler, facilitating real-time decision-making, increasing productivity, reducing inventory spending, and ensuring customer satisfaction **[85]**.

Detection of Spoilage Markers

The detection of spoilage indicators plays an important role in assessing the freshness and durability of food products. Spoilage markers are specific products or symbols produced by spoiled food, indicating the presence of spoilage. Several methods are available for determining these markers, including gas chromatography (GC) for identifying markers of thermal degradation and high-performance liquid chromatography (HPLC) for identifying nonvolatile compounds associated with degradation, such as organic acids or biogenic amines. GC works well for identifying markers of thermal degradation. At the same time, HPLC is suitable for identifying nonvolatile compounds associated with degradation, such as organic acids or biogenic amines Enzyme-based assays are often used to identify markers of damage associated with enzymatic degradation. These assays use specific enzymes or cofactors that react with the target compound, producing a measurable signal. Biosensors, including nano biosensors, are increasingly used for rapid and real-time detection of damage markers. Nano biosensors incorporate nanomaterials or nanoscale structures to enhance sensitivity, selectivity, and real-time diagnostic capabilities. Markers of degradation such as volatile compounds, specific enzymes and microbial metabolites can be detected with high specificity and sensitivity **[86]**, **[87]**, **[88]**, **[89]**.

The Electronic Nose (E-Nose) system uses an array of gas sensors to detect volatile organic compounds (VOCs) associated with food spoilage, providing a unique pattern or "scent print" for identification purposes. Mass spectrometry (MS) is a powerful identification and annotation recognition alternative. MS provides detailed information on the sample's chemical composition, facilitating the identification of specific markers of degradation. The discovery method depends on the drugs of interest, the required sensitivity and selectivity, and the need for real-time monitoring. Nano biosensors offer advantages such as early detection, high sensitivity and can be incorporated into food packaging or manufacturing processes, enabling and contributing to real-time monitoring of spoilage signals so they are assured of food quality and safety **[90]**.

Emerging Trends in Nano Biosensors for Food Packaging and Shelf-Life Monitoring

The field of nano biosensors for food packaging and shelf-life monitoring is developing rapidly, with many emerging products shaping their future applications. One trend is to miniaturize and embed nano biosensors as much as possible for incorporation into packaging materials or directly on food surfaces. These small sensors are cost-effective, disposable, and highly sensitive, allowing real-time temperature, humidity, pH, and microbial activity monitoring. Another trend is wireless communication technology connectivity, enabling remote monitoring of foods. This wireless and remote monitoring capability provides immediate access to critical information, enhances analysis, and facilitates quick decision-making. The development of multi-parameter sensing is also an important factor, enabling nano biosensors to detect multiple quality indicators simultaneously. By combining multiple sensors or using multiple sensing channels, these sensors can monitor various components in a single pipeline, providing a comprehensive view of nutritional quality and enhancing quality control. These systems offer dynamic functions such as real-time analysis, freshness indicators, and intelligent release of additives or pesticides. Nano biosensors detect signs of degradation, triggering alerts or interventions to extend shelf life and enhance product safety. Advances in nanomaterials are leading to the developing of highly sensitive and selective nano biosensors. Nanostructured materials can be adapted to interact with specific analytes, improve sensor performance, and enable accurate detection of dissolution markers. Integrating nano biosensors into food packaging and shelf-life monitoring systems is an emerging trend **[91]**, **[92]**.

Integration of Wireless and IoT Technologies

The integration of wireless communications and Internet of Things (IoT) technologies is transforming the use of nano biosensors for food packaging and shelf-life analysis. This integration allows for real-time data collection, transmission, and analysis, providing valuable insights into product quality and safety. One key aspect of this integration is wireless sensor networks (WSNs), where nano biosensors communicate wirelessly to monitor various parameters such as temperature, humidity, vibration, and more at different points in the packaging or warehouse. The data collected by these sensors are transmitted wirelessly to a central control system for remote monitoring and management of food products. IoT systems provide a centralized data storage and analysis system, facilitating seamless integration and communication between nano biosensors, data storage systems, and analytical tools. This connectivity enables stakeholders to access real-time information, receive alerts, and make informed decisions based on collected data. IoT technologies, combined with nano biosensors, can revolutionize the food industry by improving product quality and safety, reducing waste, and increasing efficiency in the supply chain.

Cloud computing is key in integrating nano biosensors, enabling flexible and efficient data processing and analysis. Sensor data collected from multiple sources can be stored and processed in the cloud efficiently, eliminating the need for local storage and audit resources. Cloud-based data analytics provide real-time insights,

trend analysis, and predictive models for shelf-life estimation and quality management. The combination of wireless and IoT technologies enables sensor data visualisation through a user-friendly interface. Stakeholders can access aggregated data through web-based dashboards or mobile applications, facilitating real-time analysis and enabling informed decision-making. Active alerts and reports can be generated when nano biosensors detect parameter deviations, enabling timely intervention and prompt product development to ensure product quality and safety. Data analysis and machine learning algorithms can be applied to collect sensor data, using historical data to identify patterns and assess product quality and shelf life and potential risks. Predictive models can be used to optimize storage conditions, predict the end of shelf life, and implement targeted quality control strategies for stakeholders **[93]**, **[94]**, **[95]**, **[96]**, **[97]**.

Smart Packaging Solutions

Smart packaging solutions in the food industry use nano biosensors to provide proactive functionality and real-time monitoring capabilities. These solutions include advanced technologies to improve the quality of food packaging. Real-time monitoring is a key enabler of nano biosensors integrated into smart packaging, allowing continuous data collection on parameters such as temperature, humidity, gas content, microbial activity etc. The immediate delivery of this information allows for timely interventions and changes to help ensure product quality and safety. Quality indicators are often combined in smart packaging to inform consumers and stakeholders about the freshness and quality of food. Specific changes detected by nano biosensors and triggered by these signals can be colour change labels or visual signs of deterioration, alerting consumers to potential hazards. Smart packaging solutions can also be active release systems with additives to extend the shelf life of food products. They can provide antibiotics or preservatives. Nano biosensors monitor environmental conditions, detect signs of pollution, and trigger reactions that occur as needed. This proactive approach protects food quality and safety in the long term **[98]**.

Authentication and traceability are important components of a smart packaging solution. An RFID tag or QR code can be inserted to verify the authenticity and origin of the food. Nano biosensors monitor and record environmental conditions during storage and transport, ensuring that the product remains within specified parameters. Storing and accessing this information through a trust system provides traceability and transparency throughout the supply chain. Smart packaging solutions also improve customer engagement through interactive features and information. QR codes on packaging provide consumers with product descriptions, nutrition information, or cooking instructions. Nano biosensors can track consumer usage patterns, providing insights into product development and marketing strategies. Nano biosensors enable the prediction of shelf life by continuous monitoring of the composition of the packaging. This information helps in inventory management, waste reduction, and supply chain efficiency. Smart packaging solutions with shelf-life prediction can improve planning and decision-making and ensure efficient use of resources. Promoting sustainability and reducing waste is another goal of smart packaging solutions. Nano biosensors prevent premature removal of safe-to-consume products by monitoring them in real-time. Smart packaging provides information about optimal storage conditions, helping consumers reduce food waste at home. Combining biodegradable materials and environmentally friendly designs contributes to environmental sustainability **[99]**.

Advanced Sensing Approaches

In nano biosensors for food packaging and life cycle monitoring, significant progress is being made in advanced detection techniques to improve the efficiency and effectiveness of these biosensors. Notable advanced sensing techniques include surface-enhanced Raman spectroscopy (SERS), which combines nanomaterials with Raman spectroscopy to amplify signals and provide accurate detection without labelling. Plasmonic sensing uses metallic nanostructures to interact with target molecules, resulting in higher sensitivity and faster response times. Electrochemical sensing measures electrical signals generated during analyte-electrode contact, enabling sensitive and selective detection. Microfluidics-based sensing combines nano biosensors into a microfluidic system for efficient sample handling and analysis. Nanobar codes use encoded nanostructures with recognition elements for multiple signal recognition. Bio-inspired sensing draws inspiration from biological systems to develop highly sensitive and selective biosensors. Smartphone-based sensing uses smartphone capabilities for spatial analysis and data processing. These advanced detection techniques push the limits of nano biosensor technology, enabling improved accuracy, efficiency and real-time analysis to ensure food quality, safety and shelf life expansion **[71]**, **[100]**, **[101]**.

Nanomaterials and Surface Modifications

Nanomaterials and surface modification are essential in improving the performance and efficiency of nano biosensors used for food packaging and shelf-life monitoring. These advances improve sensitivity, selectivity,

and stability, and they contribute to improved sensing with different analytes. Nanomaterials, such as nanoparticles, nanowires, and nanofilms, offer unique properties that can be tailored for specific sensing applications. By mechanical means, these materials can selectively interact with target analytes, leading to increased sensitivity and selectivity of nano biosensors. In addition, the nanomaterial offers a high area-to-volume ratio, amplifying the sensing signal and overall biosensor performance. Surface modification is important for immobilization of biomolecules such as antibodies or enzymes on the sensing surfaces of nano biosensors. These modifications facilitate the specific identification and binding of targets, allowing them to be identified. Techniques such as self-assembly of monolayers, chemical reactions, and layer-by-layer deposition are being used to achieve stable and continuous biomolecules, thereby increasing the sensitivity and specificity of biosensors **[102]**, **[103]**, **[104]**, **[105]**, **[106]**.

Bioconjugation techniques are used to covalently attach biomolecules to nanomaterials or surface coatings, creating a working interface for sensing. This adds specific receptors or recognition elements to the biosensor surface, enabling detection and monitoring of target analytes. Scripting and other bioconjugation techniques, as well as nano functionality and nanocoatings, are applied to the surface of biosensors to improve their stability, selectivity, and overall performance **[107]**, **[108]**, **[109]**, **[110]**, **[111]**.

Quantum dots (QDs) and fluorescent probes are used as labels or tags in nano biosensors to enable sensitivity and multiplex detection. These semiconductor nanocrystals and fluorescence probes offer unique optical properties, high signal-to-noise ratio, long-lived fluorescence, and compatibility with detection methods. When conjugated with biomolecules, QDs and fluorescence probes provide unique recognition and fluorescence-based readout. Incremental, nanostructured surfaces, obtained by patterning, nanoprinting, or nanotexturing, improve the sensing performance of biosensors. This surface modification increases the effective site sensing, increases analyte capture efficiency, and enhances binding specificity. In addition, nanostructured surfaces facilitate complex biomolecules, enhance their stability and contribute to the overall sensitivity, reproducibility and performance of nano biosensors **[109]**.

Technical Challenges

The successful implementation of nano biosensors for food packaging and shelf-life monitoring faces several technical challenges that need to be addressed. These challenges include ensuring the stability and durability of the sensors to withstand the harsh conditions encountered during food processing, transportation, and storage. Standardization and reproducibility are crucial to ensure consistent and reliable sensor performance. Establishing standardized processes, materials, and characterization methods is essential to achieve comparable and consistent sensor performance, enabling widespread adoption and consumption of this technology in the market. Furthermore, the sensitivity and selectivity of the sensors need to be improved to ensure accurate and reliable monitoring of food products, and the scalability and manufacturing cost must be optimized to make the technology economically viable. Addressing these technical challenges is critical to successfully applying nano biosensors for food packaging and shelf-life monitoring.

Achieving high sensitivity and selectivity is an ongoing challenge in biosensor development. A major research objective is to increase the sensitivity of nano biosensors to detect low concentrations of analytes while maintaining their selectivity to distinguish between specific markers and interfering substances. Limits to improve portable detection and reducing false positives or false negatives are important. Scalability and manufacturing cost are important considerations for adopting nano biosensors. Ensuring that manufacturing becomes cost-effective on a large scale requires developing processes that can be manufactured, optimizing material costs, and simplifying manufacturing. Such efforts will enable the integration of nano biosensors into food packaging and monitoring systems, making them commercially viable and accessible **[38]**, **[112]**, **[113]**.

Future Prospects and Opportunities

Nano-biosensors for food packaging and shelf-life monitoring hold great promise for the future, offering many possibilities and opportunities for development. One exciting opportunity is that artificial intelligence (AI) technology and nano biosensors will be combined. Using AI algorithms, analyzing complex data generated by sensors to predict shelf life, optimize warehousing conditions, and provide real-time feedback, machine learning models can be seen modelling and correlating data to improve nutrition management and decision-making. Another promising possibility is the development of multiple sensing mechanisms. Integrating multiple sensing methods such as optical, electrical, and biological, nano biosensors provide increased performance and versatility. These sensing techniques provide information that enables accurate and comprehensive monitoring of nutrition data. It opens up new possibilities for detecting analytes and monitoring multiple parameters simultaneously. The combination of wireless communication and Internet of Things (IoT) technologies is another area of great potential. Integrating nano biosensors into wireless networks allows real-time data to be transmitted and analyzed

remotely. This connectivity allows continuous monitoring of foods throughout the supply chain, providing valuable insights into their status and enabling prompt production in case of delays. The IoT integration also facilitates seamless communication between various stakeholders in the food industry, fostering collaboration and effective decision-making.

Conclusion

Nano biosensors offer exciting properties that can revolutionize food packaging and shelf life. These advanced sensing technologies have the potential to improve food safety, quality and sustainability by providing real-time, accurate and reliable information about the state of a of packaged foods. In this paper, we investigated the aspects of nano-biosensors for food packaging and storage time monitoring. Integrating nano biosensors into food packaging enables improved integrity and barrier characterization and provides improved protection against external factors that can affect food quality and shelf life, extending the life of the food products for storage. For shelf-life monitoring, nano biosensors offer advantages over conventional methods by providing real-time non-destructive monitoring of key parameters such as temperature, humidity, air content, and signals that indicate spoilage. This enables the sensor to detect quality and deterioration quickly, enabling timely intervention and reducing food waste. However, several challenges must be addressed to apply nano biosensors in the food industry successfully. Additional research and development efforts are needed for technical challenges such as sensor stability, standardization, feed matrix absorption, and scalability. Regulatory considerations for safety assessment, labelling, and appropriate regulation are also needed to ensure the safe and responsible use of nano biosensors. The future prospects and opportunities for nano biosensors on food packaging and shelf life are promising. Artificial intelligence (AI) technology can improve data analysis and decision-making, while multi-sensing techniques provide complete and accurate information. Wireless connectivity and IoT integration enable real-time and remote monitoring food packaging systems, improving efficiency and quality control. . In conclusion, nano biosensors represent a transformative technology in food packaging and shelf-life monitoring. By addressing the technical challenges, regulatory considerations and leveraging emerging trends, nano biosensors can revolutionize the food industry, ensuring safer, longer-lasting, and higher-quality food products while reducing waste and promoting sustainability. Continued research, innovation, and collaboration among scientists, engineers, regulators, and industry stakeholders will pave the way for the successful adoption and implementation of nano biosensors in the food packaging and monitoring landscape.

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The Research of Whey Permeate Mineral Profile at Different Stages of Membrane Filtration

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ABSTRACT

Whey permeate powder is widely used in technologies of various line groups of food products, but the main limiting factor of its application is its high ash content. This research aimed to establish the efficiency of ash reduction and change of mineral profile at various stages of production for obtaining demineralized whey permeate powder suitable for further usage in technologies of lactose. The experiments were carried out following the referee method and the common methods used in research practice. The objects of research were cheese whey and its concentrate and permeate obtained in the process of ultrafiltration (UF), nanofiltration (NF), electrodialysis (ED), vacuum-evaporating and spray drying. UF made it possible to remove partially Ca^{2+}, total phosphorus, and Mg^{2+} from cheese whey, NF was effective in removing part of K^{+}, Ca^{2+}, Fe^{2+}, Mg^{2+}, Cu^{2+}, Cl^{-} and total phosphorus from UF-permeate. Using polymer membranes made it possible to obtain the NF-concentrate containing mainly lactose and increase the efficiency of ED due to their high permeability relative to water, as well as their ability to eliminate proteins and partially some ions of mineral salts. The mass fraction of ash in the finished product decreased by 93.0% compared with cheese whey, as well as Na^{+} and K^{+} by 89-94%, and Ca^{2+} and Mg^{2+} by 60-75%; the total phosphorus – by 78%; chlorides – by 70%. The obtained results allow to justify the technological operation sequence to produce a product suitable for further usage as a raw material for highly purified lactose.

Keywords: ash content, electrodialysis, nanofiltration, ultrafiltration, whey

INTRODUCTION

In the past year the cheese world market increased by 2.0% and became 25.3 mln. tons accordingly, milk whey amount also increased, its unique chemical composition (50% of milk dry solids, including 95% of lactose, 80% of minerals, 20% of proteins and 10% of milk fat **[1]**) allows to produce new ingredients on its basis, both using membrane methods of separation (microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO)) due to pressure gradient and osmotic membrane. This fractionates solution components by size and molecular structure **[2]**, **[3]**. Membrane methods are used for obtaining from milk whey or milk of high molecular concentrate of proteins, fat and lactose (retained by membrane) and low molecular fraction containing lactose and ash, in this case, the moderate temperature conditions allow the components to remain as native as possible and preserve their functional-technological properties **[4]**, **[5]**. The raw milk permeate contains lactose, mineral salts, and other low molecular compound solutions (Table 1).

The world's production and consumption of whey protein concentrates is constantly growing. According to the analytic center Fortune Business Insights data in 2022-2029, at an average annual rate of 7.4%, it will reach $18.12 bln. **[6]**. However, the permeate market accompanying the production of milk protein concentrates is developing not so actively despite its unique functional-technological properties: in 2022 it was $836.5 mln, which meant an annual increase by 4.5% **[6]**.

Table 1 Chemical composition of whey permeates.

Parameter description	Value
Mass fraction of moisture in terms of dry solids, not more than, %	2-5
Mass fraction of lactose in terms of dry solids, not more than, %	76-92
Mass fraction of fat in terms of dry solids, not more than, %	0-1.5
Mass fraction of protein in terms of dry solids, not more than, %	1.2-4.0
Mass fraction of ash in terms of dry solids, not more than, %	
Demineralized permeate	
DD 25%	7.0
DD 50%	4.0
DD 70%	2.5
DD 90%	1.0
Don-demineralized permeate	9.0

The typical whey powder is widely used both as an independent ingredient in technologies of various product line groups of food products (dairy, confectionary, baking, grocery production, milk replacer, feed for farm animals etc.) and as a raw material for lactose production, including pharmacopoeia. The production of whey permeate powder is rapidly developing in our country, but there are no technologies for obtaining highly refined lactose. One of the main limiting factors for applying whey permeate for food and feed purposes is the mass fraction of minerals **[7]**. The number of food technologies excludes the application of ingredients with high ash content. The mineral salt content in cheese whey varies from 0.3 to 0.8%, up to 15.0% in terms of dry matter. The quantitative ratio of anions (5831 g/l) and cations (3323 g/l) of whey is the same as that of milk. Na, K, Ca, Mg, Cl, P predominate among minerals **[8]**. Sodium, potassium and chloride are electrolytes that belong to the diet's excessive macronutrients. Their high concentration in tissues and blood of the body disrupts water balance, osmotic pressure, acid-base balance (pH) and results in the development of diseases of kidneys, heart, gastrointestinal tract, liver, etc., so limiting their content in food products is worth limiting.

In accordance with the standard for dry permeates of raw milk (CXS 331-2017), the maximum ash content in whey permeate powder should not exceed 12.0%. Membrane methods allow fractionation of the raw materials and refining of the finished product from minerals, which prevent its application in food technologies. The permeate powder production includes the following operations: ultrafiltration, nanofiltration, and electrodialysis, which are widely used methods of demineralization, allowing the decrease in ash content at various technological stages **[9]**.

Ultrafiltration membranes with pore sizes from 0.01 to 0.1 μm retain fat and almost all the whey proteins. In the process of ultrafiltration, ions and minerals presented in the concentrate are connected with proteins (calcium, magnesium, phosphates and citrates), and free ones are fully transferred to the permeate (sodium, potassium, chloride).

Nanofiltration membrane with pore size from 0.001 to 0,01 μm makes it possible to retain substances with molecular weight from 100 to 300 Da, in this respect, minerals, some organic acids, non-protein nitrogen and a small amount of lactose (0.07%) pass into the nanofiltration permeate. The permeate production according to the known technology (Table 1) using ultra- and nanofiltration allows to obtain the finished dry whey permeate with partial demineralization, in which about 25% of salts are removed, and the mass fraction of ash in terms of dry solids is not more than 7% **[10]**.

Electrodialysis may reduce the minerals content due to ions separation by transportation through the osmotic membranes of cation and anion exchange under the influence of direct current, which results in a high degree of demineralization **[11]**. Unlike nanofiltration, electrodialysis does not require high hydrostatic load of the inlet solution to induce the mass exchange processes. The electric field provokes ion migration, the charged particles released from the soluble salts are easily removed from solution, and the uncharged ones, such as sugars and lactose, remain in the solution. The barriers that carry out ion transportation are ion-exchange membranes of various types and selective characteristics.

The research aims to establish the efficiency of ash reduction and change of mineral profile at various stages of production for obtaining demineralized whey permeate powder suitable for further use in technologies of lactose.

The following tasks have been set up to achieve this goal:

- to study the dynamics of ash and mineral profile of cheese whey permeate changes at various stages of production;

- to determine the degree of sample demineralization and the efficiency of sequential application of ultrafiltration, nanofiltration (with polymer membranes) and electrodialysis methods.

Scientific Hypothesis

In accordance with the standard for dry permeates of raw milk (CXS 331-2017), the maximum ash content in dry whey permeate should not exceed 12.0%. Membrane methods allow not only the fractionation of the raw materials but also refining of the finished product from minerals which refrain its application in food technologies. The combination of membrane methods of processing whey in a certain sequence using polymer membranes can provide a high degree of its purification from minerals for further application of highly refined whey permeate in technologies of lactose.

MATERIAL AND METHODOLOGY

Samples

The objectives of research included the whey permeates obtained after ultrafiltration, nanofiltration, electrodialysis and spray drying. The raw material for dry permeate production was cheese whey, produced at the PJSC DP branch office "Voronezhskii" "Kalacheevskii Cheese Factory".

Chemicals

All chemicals purchased by Stock Company "Lenreactiv" (Russia) were of analytical grade quality.

Instruments

The ultrafiltration unit of UF-1 type (DMP Ltd supplier, Stavropol, Russia) with polymer membranes Alfa Laval GR73PE 6338/30 (polyether sulphone with molecular weight cut-off (MWCO) 10kDa, Alfa Laval Corporate AB manufacturer, Lund, Sweden), the nanofiltration unit of NF-1 type of SD-Filtration brand (SiccaDania A/S, Denmark) with polymer membranes DOW FilmTecT Hypershell 245-8038 (MWCO 300Da, DuPont de Nemours, Inc. manufacturer, Wilmington, USA), the electrodialysis unit of ED2*EWDU6*EDR-II/250 type (LLC MEGA ProfiLine, Russia) using ion-selective membranes (RALEX CMH-PES and RALEX AMH-PES, MEGA a.s. manufacturer, Prague, Czech Republic), the vacuum-evaporating unit TH-TVR4 (LLC Kroneswerk Steinecker, Germany) and drying unit VRD5 (VZDUCHOTORG, spol. s r.o., Slovakia) were used for sample preparation.

Laboratory Methods

The experimental research was carried out following the referee method and the common methods used in research scientific practice (Table 2). Physical and chemical parameters of whey permeate were determined in scientific testing laboratories of FSFEI HE "All-Russian Research Institute of Dairy Industry", the Federal Reserve "State Regional Center for Standardization, Metrology and Testing in Moscow and Moscow region", the Federal Reserve "State Regional Center for Standardization, Metrology and Testing in Saint Petersburg and Leningrad region", FSFEI HE "Voronezh State University of Engineering Technologies".

The mass fraction of dry solids was analyzed following GOST 29246-91 **[12]**, **[13]** using oven drying; mass fraction of total protein was determined according to GOST 34454-2018 **[14]** with help of Kjeldahl method; mass fraction of lactose was analyzed following GOST 33958-2016 **[15]** using polarimetry; mass fraction of ash was determined according to GOST R 56833-2015 **[16]** with help of dry combustion method; chlorides content was analyzed following GOST R 54045-2010 **[17]** using capillary electrophoresis; calcium content was determined according to GOST R 55331-2012 **[18]** with help of the atomic absorption spectrometry; total phosphorus content was analyzed following GOST 31980-2012 **[19]** using capillary electrophoresis; sodium content was determined according to GOST EN 15505-2013 **[20]** with help of the atomic absorption spectrometry; potassium content was analyzed following ISO 8070:2007 **[21]** using atomic absorption spectrometry; iron content was determined according to GOST EN 14084-2014 **[22]** with help of the atomic absorption spectrometry; magnesium content was analyzed following MG 4.1.3606-20 **[23]** using atomic absorption spectrometry; copper content was determined according to GOST EN 14084-2014 **[22]** with help of atomic absorption spectrometry.

Description of the Experiment

Sample preparation: The whey was previously purified from fat and casein fume using a vibratory sieve, then pasteurized at a temperature t = (75 ±2) °C for 5 min, then cooled to t = (10-15) °C and sent to the ultrafiltration unit of UF-1 type with polymer membranes. The permeate received was sent to the nanofiltration unit of NF-1 type of SD-Filtration brand at a temperature t = (10 ±2) °C and the process pressure up to 2.5 MPa; in this case it was thickened up to dry solids content of 27.5%. The electrodialysis was carried out at the electrodialysis unit of ED2*EWDU6*EDR-II/250 type at a temperature t = (15 ±2) °C using ion-selective

membranes. Stage I was conducted with negatively charged membrane for 4 h. Stage II was neutralization process up to 7.0 pH for 2 h. Stage III was conducted with charged positively membrane for 25 min until the electrical conductivity of 0.8 $mS.cm^{-1}$. Further, the permeate was thickened up to (54-55%) at vacuum-evaporating unit TH-TVR4 (P = 0.09 MPa, inlet temperature of 70-75 °C, outlet – 40 ±5 °C) and sent to crystallization at 33-35 °C for 3-4 hours and then to 10-15 °C for 10-12 hours. Subsequent drying was carried out at the unit VRD5 at an inlet temperature to the drying tower of 170-200 °C, at outlet – (70-100 °C). Then the whey permeate powder was cooled to 30 ±5 °C.

Number of samples analyzed: 7 samples were analyzed.

Number of repeated analyses: The experimental studies of each sample were carried out 3 - 5 times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was three times.

Design of the experiment: The methodological research strategy realized in this study is represented in Figure 1.

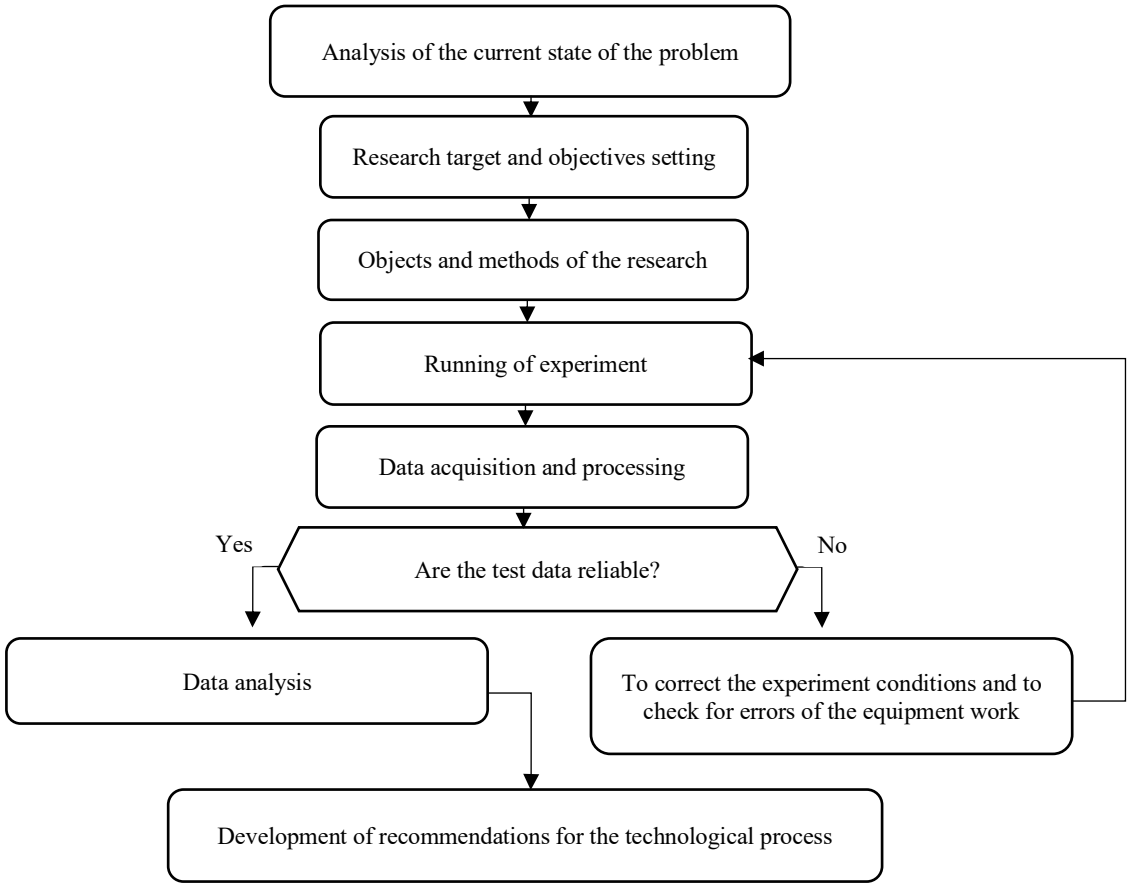

Figure 1 Methodological diagram of conducting the research.

Statistical Analysis

Calculations were carried out by methods of mathematical statistics with the help of Microsoft Office application for home and study 2021 for Mac and Statistica (USA). (Microsoft Corporation, WA, USA). The normal distribution of continuous variables was determined using Shapiro-Wilk test. Data were expressed as mean ± standard deviation and median (minimum value~maximum value) for normally and non-normally distributed data, respectively. The P-value, which was less than or equal to 0.05, was used to determine provided the findings were significant. The limitations of experimental studies included errors and uncertainties of the analysis methods used, which affected the represented results. The results are presented considering the errors found using the least squares method.

RESULTS AND DISCUSSION

Technologies for the production of whey powder **[24]** and its derivatives **[25]** (Figure 2) require the study of mineral composition at UF-filtration, NF-filtration, and electrodialysis stages **[2]**. The chemical composition and mineral profile change of the tested samples at various stages of cheese whey processing were established in the experiments (Table 2). The use of ultrafiltration allowed to concentrate **[9]** and partially purify from minerals the

total protein of cheese whey **[1]**, in this case, the major part of lactose and salt passed into the UF-permeate, predominantly in the ion-molecular distribution **[26]**.

Nanofiltration effectively removes part of mineral salts from UF-permeate and increases lactose mass fraction in NF-concentrate samples **[27]**. The optimal parameters of the process (P = 2 MPa and t = 15 °C) ensured a higher degree of purification from chlorides without significant loss of milk sugar **[10]**. The part of lactose, calcium (30%), phosphorus (20%), sodium (10%), potassium (25%), chlorides (50%), iron (80%), magnesium (70%), and copper (85%) pass into NF-permeate **[28]**, which promotes its application as the raw material for isotonic drinks production **[29]**.

The electrodialysis treatment of NF-concentrate was carried out at a temperature (15 ±2 °C) **[30]** with the subsequent control of electrical conductivity and pH in the process **[31]** (Figure 3). The number of cations in NF-concentrate decreased during stage I of electrodialysis that led to low values of pH. In this case the protein in the NF-concentrate is unstable and may precipitate out **[32]**, which negatively affects the drying and physical-chemical characteristics of the finished product **[33]**. Therefore, the mixture of solutions KOH and NaOH (1:1) was added for further adjustment of this parameter to pH value of 6.2. The process is considered to be finished when the conductivity reaches 0.8 $mS \cdot cm^{-1}$ for the product **[34]** with a demineralization degree of 90%.

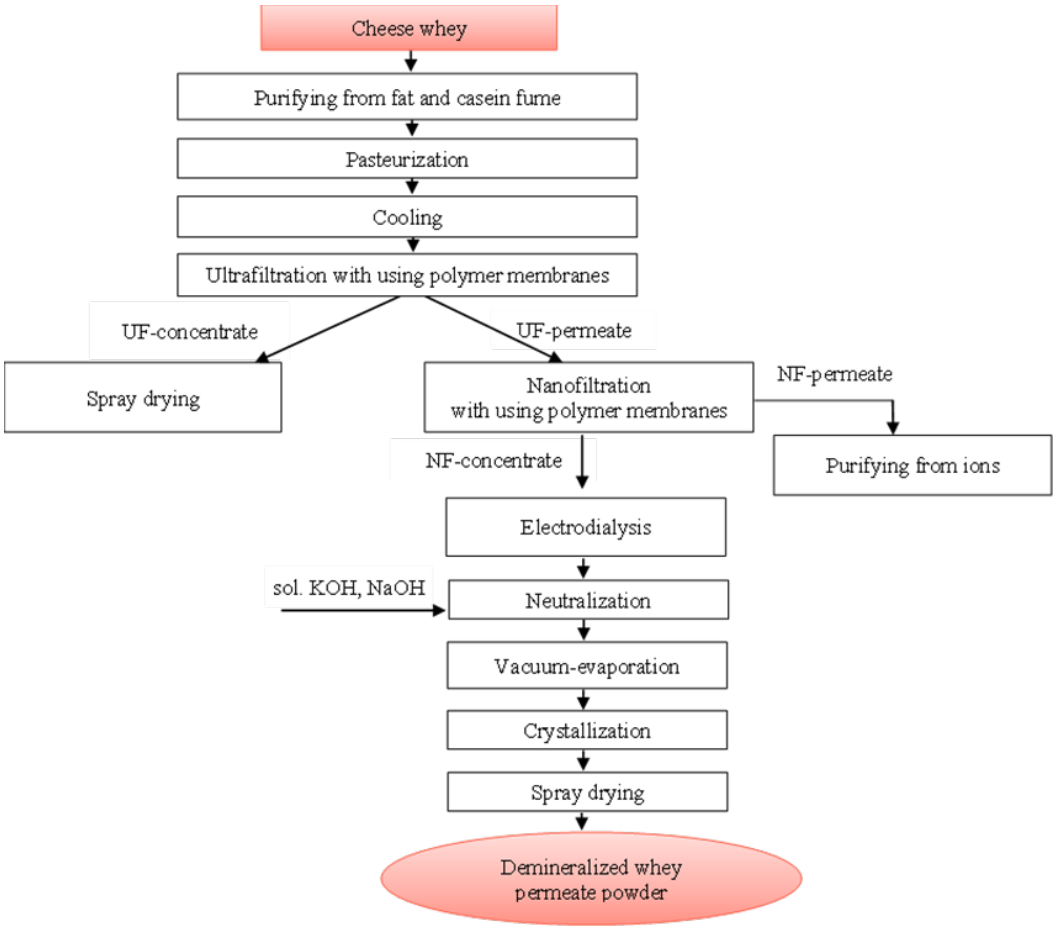

Figure 2 Cheese whey processing scheme.

There was an increase of UF-permeate total ash compared to cheese whey (more than 70%), due to free ion transfer and they were not associated with other components. It decreased by 25.4% for NF-concentrate and the mineral profile changed toward the increase in the concentration of Na^+, K^+, Cl^-. The electrodialysis application for the further demineralization of NF-concentrate of cheese whey made it possible to achieve the removal of Na^+ and K^+ by 89-94%, and of Ca^{2+}, Mg^{2+} by 60-75%; of total phosphorus – by 78%; of chlorides – by 70%.

It is well known that the efficiency of monovalent ion removal (K^+, Na^+ and Cl^-) is higher than that of multivalent ones (Ca^{2+}, Mg^{2+}, SO_4^{2-} and PO_4^{3-}) **[35]**. Several scientists **[36]**, **[37]**, **[38]** established the following deionization scheme for cations: $K^+ > Na^+ > Ca^{2+} > Mg^{2+}$; for anions: $Cl^- > SO_4^{2-} > PO_4^{3-} > LA^- > CA^{3-}$. It is accounted for the smaller hydrodynamic radius [38] and the higher diffusion coefficient of monovalent ions compared to multivalent ones **[39]**. Moreover, the mineral and organic profiles of the initial raw material **[40]** and the permeate technology **[11]** also influence the efficiency of demineralization **[41]**.

Table 2 Chemical composition and mineral profile change of the tested samples.

Parameter description	Cheese whey	Ultrafiltration using polymer membranes		Nanofiltration using polymer membranes		Electrodialysis	Spray drying (whey permeate powder with the DD 90%)
		UF-concentrate	UF-permeate	NF-concentrate	NF-permeate		
Mass fraction of dry solids, %	6.01 ±0.05	27.8 ±0.07	4.21 ±0.03	21.56 ±0.07	0.97 ±0.02	21.40 ±0.09	97.73 ±0.10
Mass fraction of lactose, %,	4.43 ±0.36	7.54 ±0.40	3.48 ±0.33	19.21 ±0.53	0.35 ±0.13	20.93 ±0.51	90.60 ±0.47
including in dry matter, %	73.71 ±0.21	27.12 ±0.24	82.66 ±0.18	89.10 ±0.30	36.08 ±0.08	97.80 ±0.30	92.70 ±0.29
Mass fraction of protein, %,	0.84 ±0.03	19.54 ±0.16	0.16 ±0.02	0.27 ±0.03	-	0.26 ±0.02	2.31 ±0.05
including in dry matter, %	13.98 ±0.04	70.29 ±0.12	3.80 ±0.03	1.25 ±0.05	-	1.21 ±0.06	2.36 ±0.08
Mass fraction of ash, %,	0.42 ±0.02	0.62 ±0.03	0.51 ±0.03	1.95 ±0.04	0.59 ±0.03	0.12 ±0.02	0.55 ±0.03
including in dry matter, %	6.99 ±0.04	2.23 ±0.05	12.11 ±0.03	9.04 ±0.06	60.82 ±0.03	0.56 ±0.06	0.56 ±0.07
Chloride content, mg/100 g,	0.87 ±0.03	1.32 ±0.05	0.86 ±0.04	1.98 ±0.05	2.16 ±0.04	0.58 ±0.02	3.57 ±0.06
including in dry matter, %	0.014 ±2.15 % relative	0.005 ±2.02 % relative	0.020 ±2.68 % relative	0.009 ±1.43 % relative	0.223 ±1.96 % relative	0.003 ±1.94 % relative	0.004 ±0.89 % relative
Calcium content, mg/100 g,	21.32 ±0.15	156.18 ±0.41	48.61 ±0.19	189.79 ±0.44	86.24 ±0.23	17.58 ±0.12	81.7 ±0.21
including in dry matter, %	0.35 ±0.77% relative	0.56 ±0.26% relative	1.15 ±0.55% relative	0.88 ±0.28% relative	8.89 ±1.17% relative	0.082 ±0.55% relative	0.084 ±0.18% relative
Total phosphorus content, mg/100 g,	64.36 ±0.05	138.54 ±0.10	44.39 ±0.03	173.53 ±0.11	76.26 ±0.06	49.19 ±0.05	215.5 ±0.10
including in dry matter, %	1.07 ±0.46% relative	0.50 ±0.16% relative	1.05 ±0.39% relative	0.80 ±0.19% relative	7.86 ±1.07% relative	0.230 ±0.26% relative	0.221 ±0.08% relative

Table 2 Cont.

Parameter description	Cheese whey	Ultrafiltration using polymer membranes		Nanofiltration using polymer membranes		Electrodialysis	Spray drying (whey permeate powder with the DD 90%)
		UF-concentrate	UF-permeate	NF-concentrate	NF-permeate		
Sodium content, mg/100 g,	54.21 ±0.02	141.58 ±0.08	106.96 ±0.06	539.63 ±0.76	183.73 ±0.15	16.35 ±0.003	88.60 ±0.04
including in dry matter, %	0.90 ±0.44% relative	0.51 ±0.16% relative	2.54 ±0.38% relative	2.50 ±0.23% relative	18.94 ±1.07% relative	0.076 ±0.22% relative	0.09 ±0.08% relative
Potassium content, mg/100 g,	193.4 ±0.10	177.1 ±0.09	198.5 ±0.12	794.80 ±0.95	205.28 ±0.16	36.84 ±0.007	159.2 ±0.06
including in dry matter, %	3.22 ±0.44% relative	0.64 ±0.15% relative	4.71 ±0.39% relative	3.69 ±0.22% relative	21.16 ±1.07% relative	0.172 ±0.22% relative	0.16 ±0.07% relative
Iron content, mg/100 g,	0.46 ±0.03	2.3 ±0.05	0.42 ±0.02	0.37 ±0.02	2.26 ±0.11	0.13 ±0.01	less than 1.0
including in dry matter, %	0.0007 ±3.68% relative	0.0008 ±1.21% relative	0.0010 ±2.74% relative	0.0002 ±2.87% relative	0.0233 ±3.47% relative	0.00006 ±4.06% relative	
Magnesium content, mg/100 g,	28.7 ±0.28	6.3 ±0.05	3.71 ±0.05	6.87 ±0.06	9.90 ±0.15	3.9 ±0.04	168.3 ±0.48
including in dry matter, %	0.047 ±0.91% relative	0.002 ±0.52% relative	0.009 ±1.03% relative	0.0032 ±0.60% relative	0.1021 ±1.79% relative	0.018 ±0.73% relative	0.0172 ±0.20% relative
Copper content, mg/100 g,	0.294 ±0.03	0.28 ±0.02	0.276 ±0.02	0.14 ±0.007	0.23 ±0.01	0.05 ±0.004	0.17 ±0.009
including in dry matter, %	0.0005 ±5.52% relative	0.0001 ±3.70% relative	0.0007 ±3.98% relative	0.0001 ±2.66% relative	0.0024 ±3.21% relative	0.00002 ±4.21% relative	0.00002 ±2.70% relative

The conducted research proved that monovalent ions were removed faster than multivalent ones due to their lower mobility. Moreover, considering their ability to form complexes with proteins **[8]**, the preliminary concentration of whey protein via UF or NF polymer membranes significantly affected the speed and process of demineralization by electrodialysis **[42]**. The filter area of the NF-membrane allow to remove most of the multivalent ions from NF-concentrate before ED. The high permeability of polymer membranes relative to water as well as their ability to eliminate proteins and partially some ions of mineral salts made it possible to obtain the NF-concentrate containing mainly lactose and increase the efficiency of electrodialysis due to the fact that the ED is not enough effective for multivalent ions removing. The total content of the inorganic ions was reduced by more than 93.0%. In the vacuum evaporation, crystallization, and drying process, there was no significant change in the mineral composition.

The obtained results proved the high demineralization degree of the samples and the efficiency of sequential application of UF, NF (with polymer membranes) and electrodialysis methods. The additional technological

operations of cheese whey processing allowed to obtain the finished product with a demineralization degree of 90% and bring the mineral profile of dry whey permeate closer to the requirements to obtain pure lactose **[43]**.

The limitations such as concentration polarization during membrane filtrations and possible errors on a measurement could interfere with the validity and interpretation of the obtained data. Increasing of the mass fraction of dry solids in front of the membrane led to reducing of it permeate flux and efficiency.

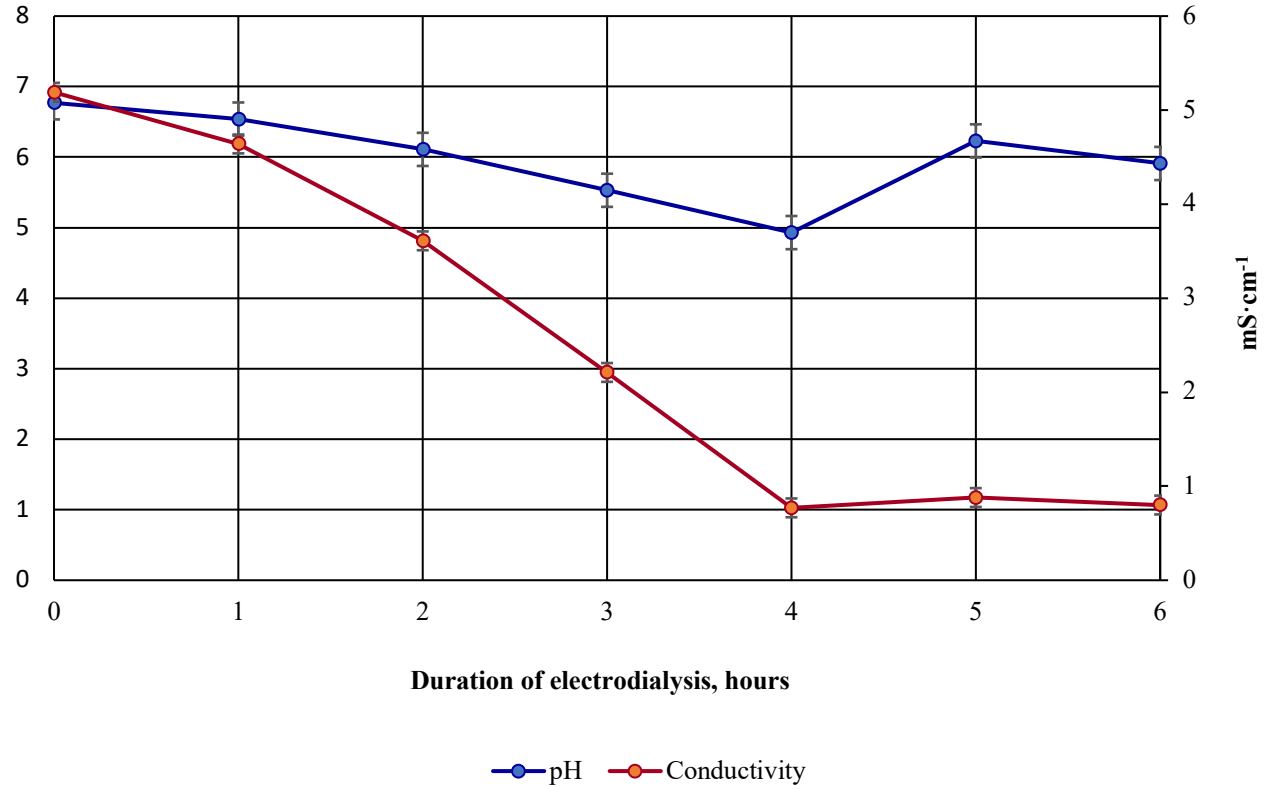

Figure 3 Changing pH and conductivity of NF-concentrate during electrodialysis.

CONCLUSION

The advantage of this study is running experiments in production conditions using industrial equipment. It was found that the UF with polymer membranes made it possible to remove partially Ca^{2+}, total phosphorus, and Mg^{2+} from cheese whey; the NF with polymer membranes was effective in removing part of K^{+}, Ca^{2+}, Fe^{2+}, Mg^{2+}, Cu^{2+}, Cl^{-} and total phosphorus from UF-permeate; the ED allowed to remove residual monovalent ions, and the most of Ca^{2+} and Cl^{-} from NF-concentrate. The theoretical significance of this research is determining the change in the content of different components of cheese whey, including the main macro- and microelements, during technological processing using membrane equipment with only polymer membranes. Practical importance of this research is the justification of the technological operation sequence to obtain a product suitable for further usage as a raw material for highly purified lactose. Further research will be focused on the application of the obtained demineralized whey permeate for lactose manufacture.

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No potential conflict of interest was reported by the authors.

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

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The microscopic structure of pork neck after cooling with showering stiving and processing by culture *Lactobacillus sakei*

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ABSTRACT

Microstructural changes in meat that occur during refrigerated storage depend on the hygiene of slaughtering and primary processing of animal carcasses, their cooling conditions, storage period, and microbial contamination and reflect the processes of meat maturation and spoilage. To extend the shelf life of pork in half-carcasses in a chilled state, 20 heads of 6-month-old large white pigs were used, which were delivered to the meat processing enterprise for slaughter. All half carcasses were cooled in a refrigerating chamber using showering, 1 hour later they were divided into 2 groups: control (without treatment) and experimental with the final treatment with a suspension of lactic acid bacteria of the SafePro® B-2 strain (*Lactobacillus sakei*). It has been found that cooling of pork half-carcasses in a refrigerating chamber with stiving and final processing by a culture suspension of lactic-acid microorganisms of strain SafePro® B-2 (*Lactobacillus sakei*) on the 4th day of storage had a positive effect on the microscopic structure of the pork neck and was characterized by a uniform color distribution when histologic specimens of muscular tissue are colored with hematoxylin and eosin, and minor cracks in the sarcoplasm, preservation of transverse and longitudinal striation of muscular fibers in comparison with that of the unprocessed pork half-carcasses with cultures of lactic-acid microorganisms. The microscopic structure of the muscular tissue of the pork half-carcass neck after cooling with stiving and final processing by a culture of lactic-acid microorganisms of strain SafePro® B-2 for 7 days of storage had a more distinct histoarchitecture in comparison with that of the unprocessed pork half-carcasses, as well as was characterized by insignificant areas of muscular fibers with transverse cracks, suspended development period of autolysis processes, partial preservation of transverse and longitudinal striation of muscle fibers. This points to a positive effect of lactic acid bacteria of strain SafePro® B-2 (*Lactobacillus sakei*) on the quality of the pork meat and contributes to the extension of its shelf life under chilled vintage.

Keywords: pork meat, storage, lactic-acid bacteria, his structure, neck muscles

INTRODUCTION

The quality of fresh pork includes a significant number of properties, which are essential to the suitability of the meat for use as food or the preparation of various dishes **[1]**, **[2]**. Postmortem changes, that occur when the muscles are transforming into the meat, are crucial in developing quality characteristics and the overall consumer comprehension of the fresh product **[3]**, **[27]**. Biochemical processes and structural changes that occur in the muscles during the first 24 hours after slaughtering play a major role in the meat's final quality and taste characteristics and depend upon the cooling processes to which the carcasses are subjected after slaughtering. After the animal is slaughtered, glycogen is anaerobically mobilized in the muscular tissue for maintaining

homeostasis. Lactate and H^+ accumulate in the muscles and cause a decrease in pH value due to postmortem glycolysis. Due to exposure to high temperatures of the muscles and low pH value, the pork must apply a faster cooling process with a recommended core temperature of the muscles of 10 °C after 12 h and 2-4 °C after 24 h. For the achievement of this effect, it is often applied spray cooling (stiving) – a system in which chilled water is supplied to the half-carcasses at the initial stage of post-slaughter cooling, which makes it possible to control the shrinkage of the half-carcasses and increases the cooling rate due to evaporative cooling **[4]**, **[5]**. When postmortem metabolism is ended, structural proteins are proteolytically destructed in the muscles, improving meat tenderness and taste **[6]**, **[28]**.

It has been established that in the process of bleeding, approximately 50% of the total blood volume is removed from the carcass, which amounts to 3.0-3.5% of the body weight of the animal **[7]**, **[8]**, and the blood, which is remaining in the carcass, is a perfect environment for the bacteria growth and spread. Because the fact that chemical agents for the meat to be preserved have several harmful effects on the consumer's body, the need arose to develop and use preservatives **[9]**.

Among these are suspensions of lactic-acid bacteria, particularly *Lactobacillus sakei*, which are capable of inhibiting the growth of related bacteria species by secreting ribosomally synthesized antimicrobial peptides called bacteriocins. Therefore, the use of lactic-acid bacteria *Lactobacillus sakei* for the final processing of the pork half-carcasses during their cooling with stiving is a highly topical issue.

The study aimed to determine changes in the structure of the pork neck muscles in the half-carcasses after cooling in a refrigerating chamber with stiving and final processing by the suspensions of lactic-acid microorganisms *Lactobacillus sakei* during the storage.

Scientific Hypothesis

The scientific hypothesis lies in the fact that the storage duration of the pork half-carcass meat in a chilled condition can be optimized if we apply the cooling in a refrigerating chamber with stiving and final processing of the half-carcass surface by the suspension of lactic-acid microorganisms, which are antagonists of putrefactive and pathogenic bacteria. Such processing will make it possible to extend the shelf life of chilled pork while preserving its quality and safety. Due to such processing, it will be possible to extend the shelf life of chilled pork meat while preserving its quality and safety.

MATERIAL AND METHODOLOGY

Samples

The pork half-carcass meat was obtained when the animals were slaughtered under LLC "Antonivsky meat processing plant" conditions, Kyiv region, Ukraine.

Chemicals

Formalin (analytical grade, LLC "Khimlaborreaktiv" Ukraine).

Paraffin (brand A, analytical grade, LLC "Khimlaborreaktiv" Ukraine).

Ethanol (brand A, analytical grade, LLC "Khimlaborreaktiv" Ukraine).

Hematoxylin and eosin (ready-made solutions produced by "Lieca", Germany).

Animals, Plants and Biological Materials

For research, 20 heads of young fattening pigs of the large white breed aged 6 months were used, which were slaughtered from a private farm in the Kyiv region, Ukraine. To form groups in the experiment, pork half carcasses weighing 43-44 kg were selected.

Natural lactic acid culture (sourdoughs) SafePro® B-2 (LLC "Hr. Hansen Ukraine").

Instruments

Microtome MS-2 (Tochmedprilad, Ukraine).

Microscope Biolam-Lomo (approx. 10, vol. 8; approx. 10, vol. 40) (Medyka, Ukraine).

Microscope Micros MC-50 (approx. 10, vol. 8; approx .10, vol. 40) (Micros, Austria).

Microphotography of the histologic specimens was carried out with the use of a video camera CAM V200 (InterMed, Ukraine)

Mounted in microscope Micros MC-50 (Micros, Austria).

Laboratory Methods

For histological studies, pieces of pork neck muscles (length, width, thickness - 5-15 mm) were selected, which were immediately fixed in a 10% aqueous solution of neutral formalin for 24 hours, after which they were washed with water and dehydrated using ethyl alcohol of increasing strength: 50º, 70º, 80º, 90º, 96º and absolute alcohols. To seal the samples, they were poured into paraffin. To study the morphology of cells and tissues, sections were made, which were deparaffinized, stained with hematoxylin and eosin, and subjected to microscopy **[10]**.

Description of the Experiment

Sample preparation: Pork half-carcasses subjected to carcass ablution with stiving and processing by starters of culture SafePro® B-2 in a dose of 107/cm^2.

Number of samples analyzed: The total number of samples was 52 samples: 26 samples each from half carcasses of the control and experimental groups of pork.

Number of repeated analyses: All measurements were performed 3 times.

Number of experiment replication: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: The control and experimental samples of 10 pork half-carcasses each were formed to experiment (Table 1).

Table 1 Experiment scheme for cooling effect determination of pork half-carcasses with stiving in combination with processing by starters of lactic-acid bacteria.

Sample	Experiment conditions	Collection of samples for histological examination
20 half carcasses	Wet toilet half carcasses of pork by showering with water at a temperature of 2°C	1 hour after showering
Control 10 half carcasses	Storage in the refrigerator	4 days of storage 7 days of storage
Experimental 10 half carcasses	With exceptional treatment and starter culture of strain SafePro® B-2 (*Lactobacillus sakei)* at a dose of 10^7/cm^2 and storage in the refrigerator	4 days of storage 7 days of storage

All pork half-carcasses were stored in the refrigerating chamber at a temperature of 3 ±1 °C until the appearance of the meat deterioration signs carrying out histological studies according to the method **[10]**.

Statistical Analysis

The STATISTICA Microsoft Excel editor combined with XLSTAT processed experimental data using mathematical statistics methods. The accuracy of the obtained experimental data was determined using the Student's t-test with a confidence coefficient ≤0.05 with many parallel definitions of at least 5 (confidence probability $p = 0.95$).

RESULTS AND DISCUSSION

Changes in pH value and increases in solute concentration (i.e., Ca^{2+}), when the meat is stored and aged, can affect protease activity, and physical destruction of the muscle tissue can alter the localization of proteases (i.e., the release of lysosomal cathepsins into cytosol) into myofibrillar cells and disrupt their integrity **[11]**, **[12]**.

One of pork carcasses' most microbially contaminated areas is the neck **[13]**, **[23]**.

Therefore, for the analysis of the histostructural changes that occur in the muscles, when the half-carcasses are cooled and stored, it is the most indicative part of the carcass.

According to the study results, the muscle tissue of the pork neck muscles (MTPNM) in 1 hour after cooling in the refrigerating chamber with stiving was formed by the muscle fibres (MFs) and intermuscular layers, which are formed by the presence of loose connective tissue. Adipose tissue, blood, and lymphatic vessels are found in the intermuscular connective tissue (Figure 1).

MFs had a sharp outline, their sarcoplasm had a uniform color, and under sarcolemma, there was a dark blue ovate-oblong nucleus. MFs of the pork neck had different thicknesses (small, average, and large), but the average-thick fibres were more often detected (Figure 2). When coloring the histologic specimens with hematoxylin and eosin, MFs with large diameters were less colored than average and small ones. It may be because, in MFs of average and small diameter, which are colored more intensively, myofibrils are more densely arranged than those in the fibers of large diameter, which is especially noticeable in transversal sections of the muscle tissue. The layers of the loose connective tissue (endomysium), which is located around skeletal MFs, were weakly expressed (Figure 2). In some places, MFs acquired a wavy appearance.

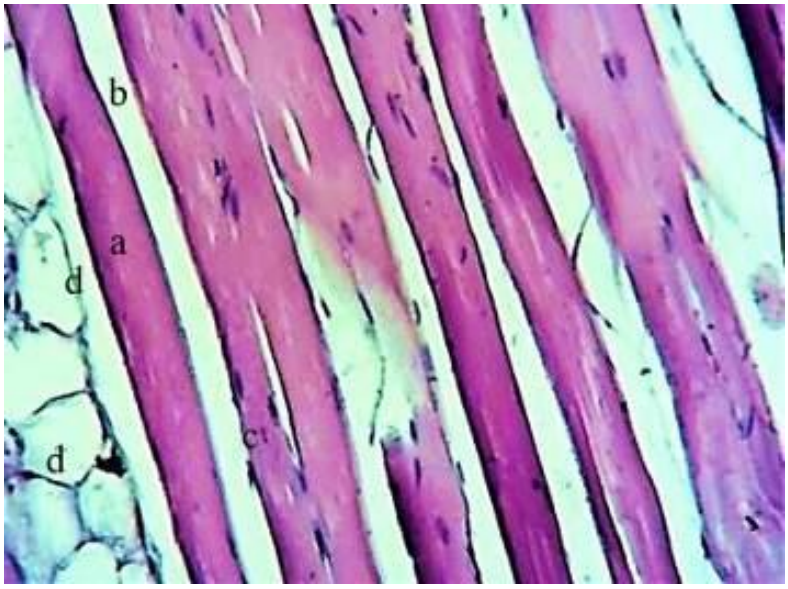

Figure 1 Microscopic structure of MTPNM in 1 hour after stiving: a – muscle fibres; b – endomysium; c – nuclei of muscle fibres; d – fat cells in the premise. Hematoxylin and eosin. X 120.

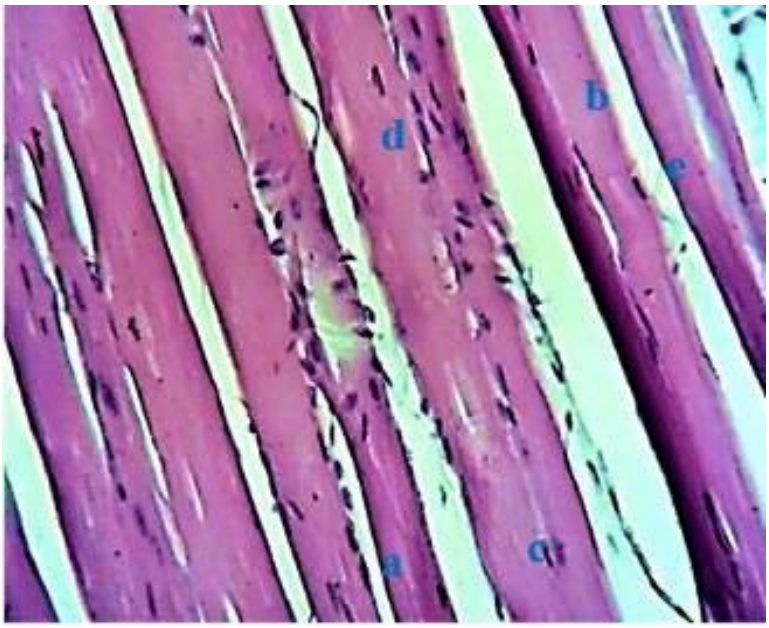

Figure 2 Microscopic structure of MTPNM in 1 hour after stiving: a – small-thick muscle fibre; b – average-thick muscle fibre; c – large-thick muscle fibre; d – nuclei of muscle fibres; e – endomysium. Hematoxylin and eosin. X 120.

At high magnification of the microscope (approx. 10; approx. 40) the transverse striations, which are formed due to the presence of actin and myosin proteins, are visible in MFs of the neck (Figure 3). The nuclei of the muscle fibres were located on the fibre periphery, right next to their sarcoplasm, they were oval and located throughout MV. When coloring the histologic specimens with hematoxylin and eosin, the nuclei were basophilic ally colored in a bluish-purple color. Their nuclear chromatin was equally spaced along the entire perimeter of the karyoplasm, and the contours of the sarcolemma were preserved (Figure 3).

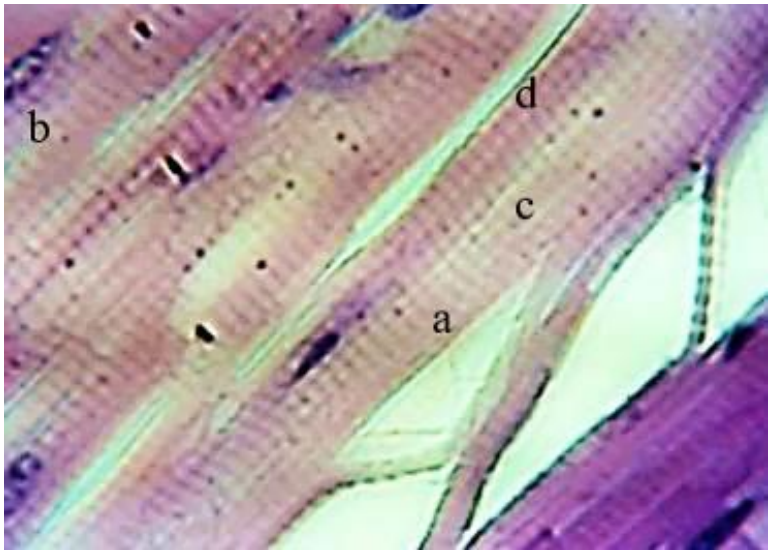

Figure 3 Microscopic structure of MTPNM in 1 hour after stiving: a – muscle fibres; b – nuclei of muscle fibres; c – transverse striation; d – endomysium. Hematoxylin and eosin. X 400.

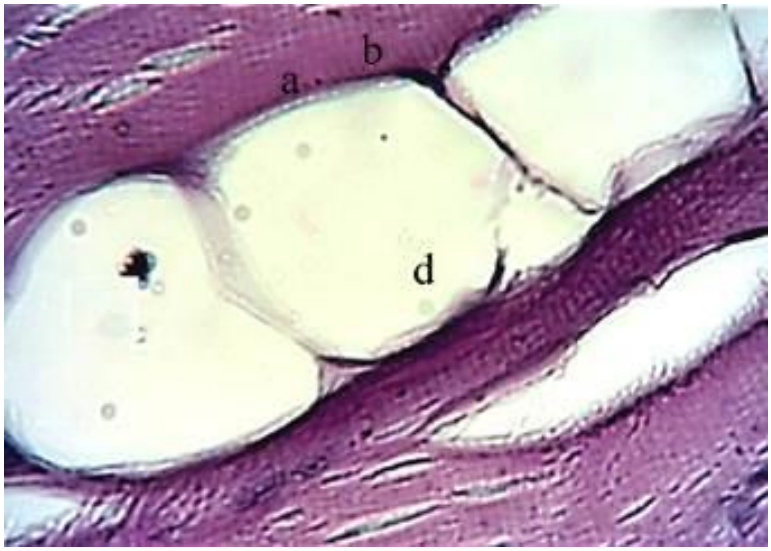

Figure 4 Microscopic structure of MTPNM in 1 hour after stiving: a – muscle fibres; b – transverse striation; c – longitudinal striation; d – fat cells. Hematoxylin and eosin. X 400.

About those mentioned above, the longitudinal striation of the fibres, due to the presence of myofibrils, is somewhat smoothed, but it is sufficiently contoured on the histologic specimens (Figure 4).

The results of the analysis of the histostructure of pig meat are based on the data of other researchers, which mean that the light of the world has a consistently uniform microstructure with slightly separated regular micron fibers, which is a typical form of good-sparing myase cells **[21]**, **[24]**.

According to the results of the histological studies of the muscle tissue, which is selected from the pork half-carcasses on the 4th day of storage, which were subjected to cooling in the refrigerating chamber with stiving, the characteristic structure of the tissue was preserved (Figure 5).

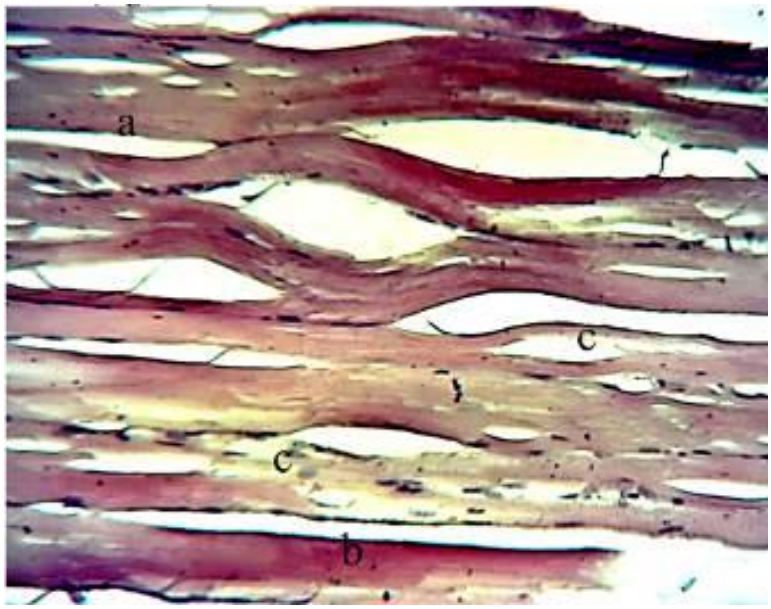

Figure 5 Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 4th day of storage (control): a – muscle fibres; b – nuclei of muscle fibres; c – areas of the uneven coloring of sarcoplasm. Hematoxylin and eosin. X 120.

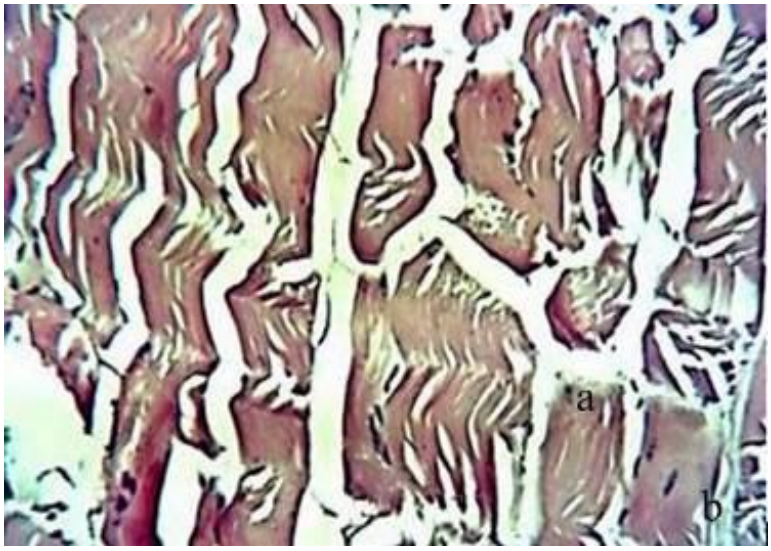

Figure 6 Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 4th day of storage (control): a – rupture of muscle fibres; b – areas of the uneven coloring of sarcoplasm. Hematoxylin and eosin. X 120.

However, transverse cracks and ruptures of muscle fibres were often detected, resulting in cracks and destruction of the sarcoplasm (Figure 6). In these places, the specific histoarchitecture, which is characteristic of the muscle tissue, was destroyed. When the histologic specimens were colored with hematoxylin and eosin, the sarcoplasm of the muscle fibres of the neck was unevenly colored, which indicated the characteristic signs of the beginning of the autolysis process (Figures 5, 7). The deformed muscle fibres and their weakened transverse and longitudinal striations were also detected (Figure 7). At the same time, deformed MFs had a tortuous (wavy) shape. The nuclei in some MFs were deformed, and in some areas, they were in a state of lysis **[29]**. The endomysium and perimysium of the neck muscles were expanded in some places, and their fibrous structures became loose. Such changes could occur with the participation of microflora, which was located both on the meat surface and in its thickness **[14]**, **[25]**.

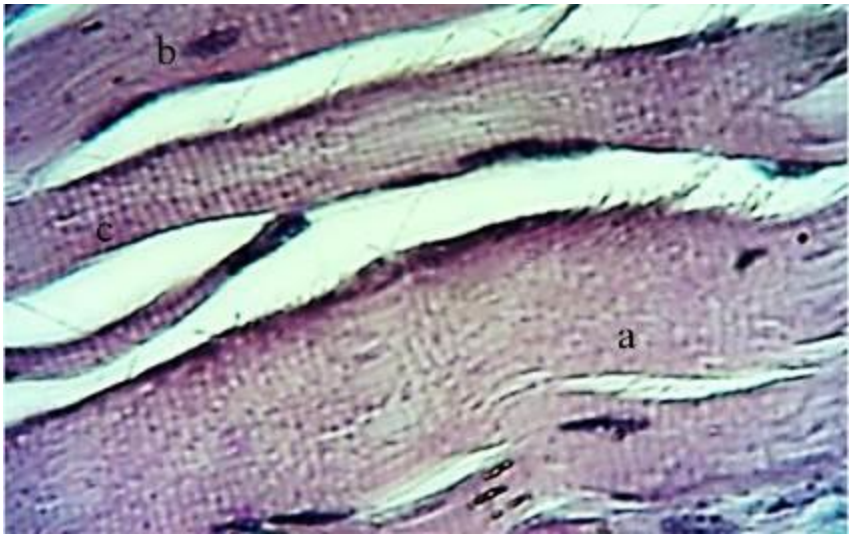

Figure 7 Microscopic structure of muscle tissue selected from the pork half-carcasses subjected to stiving on the 4th day of storage (control): a – muscle fibres; b – nuclei of muscle fibres; c – transverse striation. Hematoxylin and eosin. X 400.

A wide synthetic preservative, including nitrite, nitrate, and sorbate, through its low quality and strong antibacterial activity, is widely used for the growth of micro-organic products in the food industry. At the same time, the majority of consumers will see the use of synthetic chemical preservatives nebazhanim, the stench is not safe for health. It is possible to reduce the intensity of destructive changes in the environment and the continuation of the term of its accessory **[43]**.

According to the results of the histological studies, the muscle tissue selected from the experimental pork half-carcasses had a similar architecture compared to the control (Figure 8).

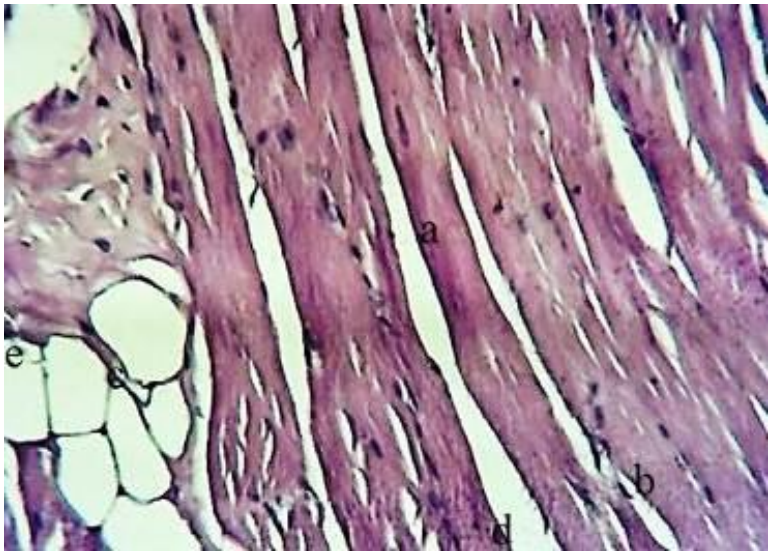

Figure 8 Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the 4th day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – fat cells; d – endomysium; e – perimysium. Hematoxylin and eosin. X 120.

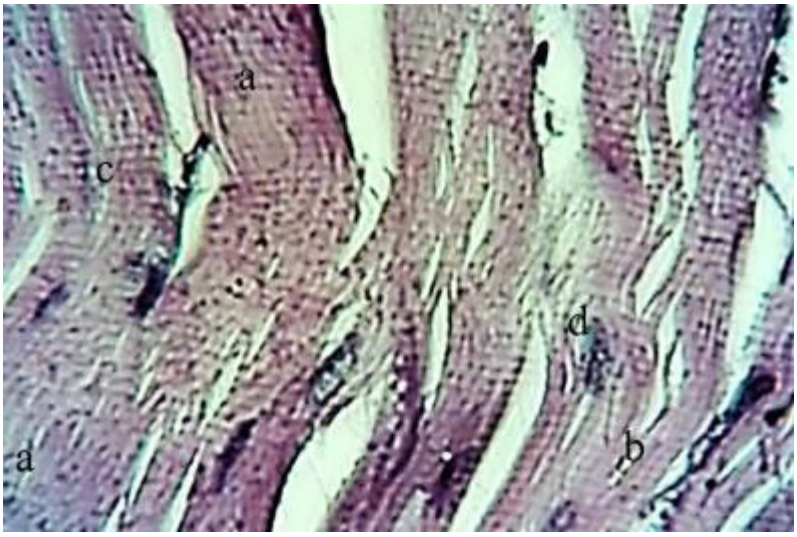

Figure 9 Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the 4th day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – transverse striation; d – longitudinal striation. Hematoxylin and eosin. X 400.

When studying the meat microstructure in the neck area, which was stored in the refrigerating chamber for 4 days, the histoarchitecture of the muscle tissue was formed by the different thicknesses of MFs and the layers of the connective tissue **[32]**. However, compared with the histostructure of this tissue only after stiving, when the histologic specimens are colored with hematoxylin and eosin, the MTPNM of the experimental pork was mostly uniformly colored (Figure 8). In some areas, MFs had a tortuous appearance **[33]**, **[34]**. When the pork half-carcasses were stived, only minor areas with transverse cracks in the sarcoplasm of the muscle fibres were detected. The transverse and longitudinal striations of the sarcoplasm were mostly preserved (Figure 9). It is important to talk about the positive influence of lactic acid microorganisms on the quality of milk **[30]**, **[31]**. At the same time, the nuclei of the muscle fibres had an oval-elongated shape and were basophilically colored. They were located on the periphery of the muscle fibres (Figure 8).

According to the histological studies, the histoarchitecture of the muscle tissue, selected from the pork half-carcasses subjected to cooling with stiving, lost their characteristic structure. Storage of the pork half-carcass meat in the refrigerator for 7 days contributed to the more active development of autolytic processes, which involved many muscle fibres. Thus, when the histologic specimens are colored with hematoxylin and eosin, the sarcoplasm of the muscle fibres is not almost colored, which indicates the characteristic signs of the autolysis process (Figure 10). affect the sensory characteristics of the meat.

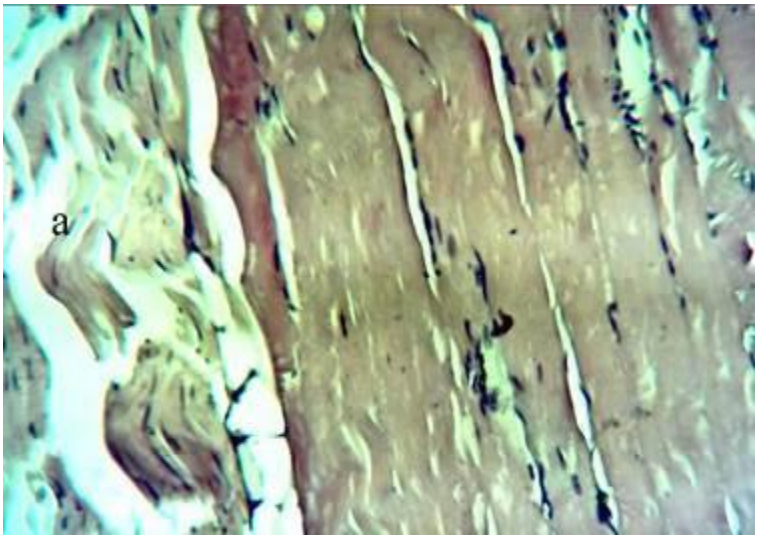

Figure 10 Microscopic structure of muscle tissue selected from the pork half-carcasses subjected to stiving on the 7th day of storage (control): a – rupture of muscle fibres; b – autolysis of muscle fibres. Hematoxylin and eosin. X 120.

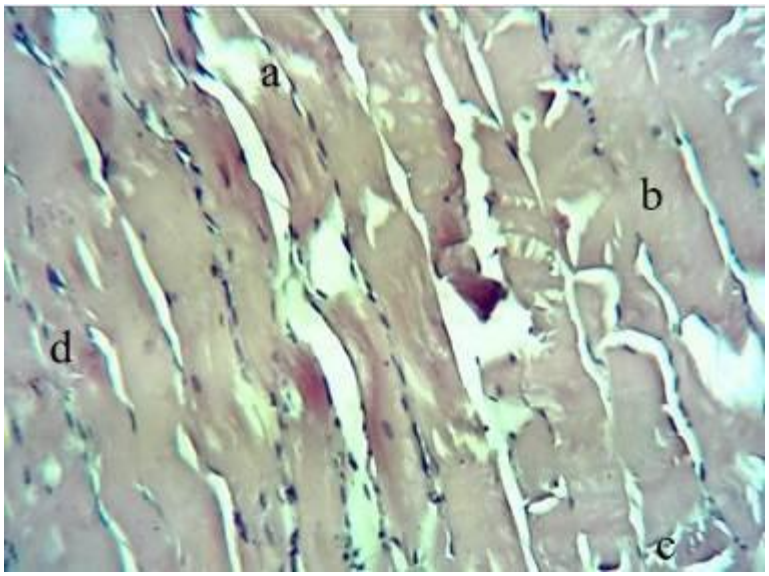

Figure 11 Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 7th day of storage (control): a – rupture of muscle fibres; b – fragmentation of muscle fibres; c – autolysis of muscle fibres; d – nuclei of muscle fibres. Hematoxylin and eosin. X 120.

The obvious transverse cracks of the muscle fibres, their ruptures, and fragmentation, which increased significantly, were detected in many areas of the histologic specimens (Figure 11).

Storage of the meat for 7 days in the refrigerator, compared to the histoarchitecture of the muscle tissue for a storage duration of the meat of 4 days, contributed to the more active development of autolytic processes, which included a significant number of the muscle fibres (Figure 13).

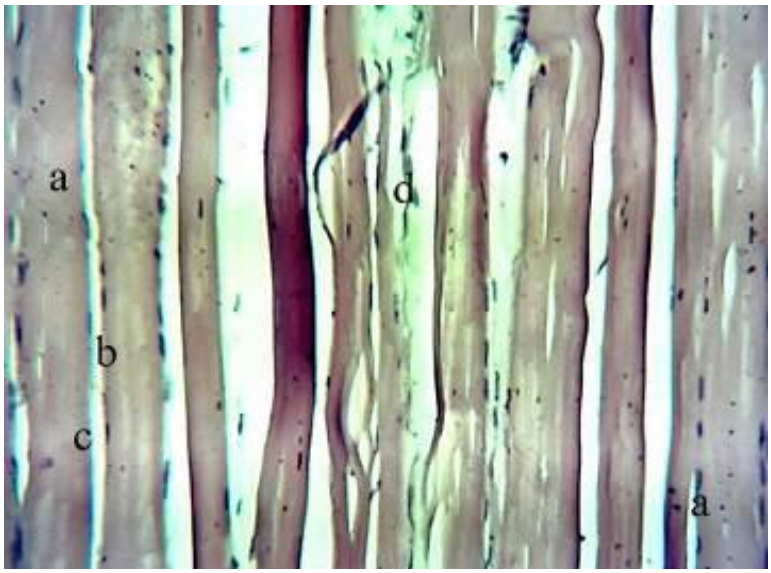

Figure 12 Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 7th day of storage (control): a – muscle fibres; b – nuclei of muscle fibres; c – light coloring of sarcoplasm; d – accumulation of granular protein mass. Hematoxylin and eosin. X 120.

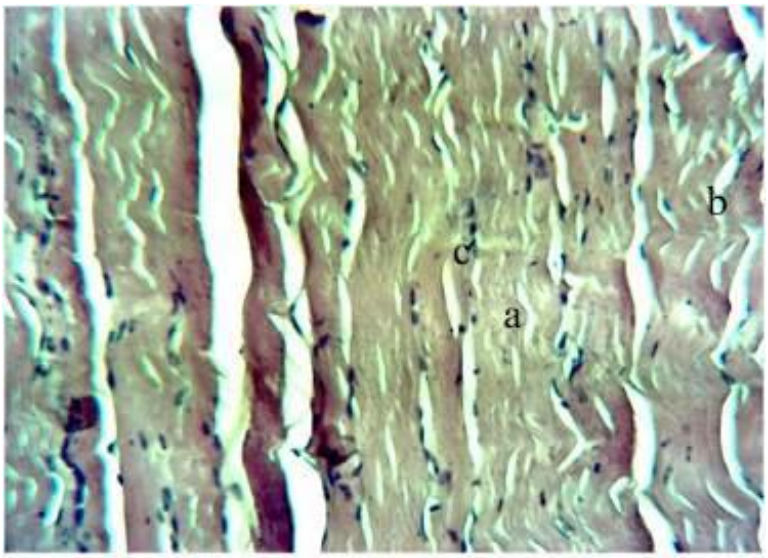

Figure 13 Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 7th day of storage (control): a – autolysis of muscle fibres; b – muscle fibres of tortuous shape; c – nuclei of muscle fibres. Hematoxylin and eosin. X 120.

Deformed MFs of the pork neck had a tortuous shape, and their transverse and longitudinal striations were destroyed (Figure 14). The endomysium and perimysium of the muscle tissue were expanded in some places, and their fibrous structures became loose.

The nuclei of the muscle fibres against the background of the light coloring of the sarcoplasm, due to the autolysis development processes, lost their characteristic structure and, being in a state of lysis, were detected only in the form of shadows or were outlined against the background of the destroyed sarcoplasm (Figures 10-14). Proteolysis of key myofibrillar proteins is the main reason for the ultrastructural changes in the skeletal muscle associated with meat tenderization **[17]**, **[35]**.

According to the results of the histological studies, the microscopic structure of the muscle tissue, which is selected from the experimental pork half-carcasses (with a storage period of 7 days) had a more distinct histoarchitecture compared to that of the unprocessed half-carcasses. This is the case with the biopreservation of *Lactobacillus sakei.* As a result, proteolytic enzymes are produced with the same substrate and are necessary for the growth of *L. sakei*. A special advantage of this microorganism is the growth rate at the time of cooling and in the presence of salt and (3-9% NaCl) in the production of bacteriocins, zocream anti listerial peptide sakacin P. *Sakei* is characterized by the creation of biological pellicle and climatic (auto-co-)aggregation, which allows you to colonize the surface **[22]**, **[38]**.

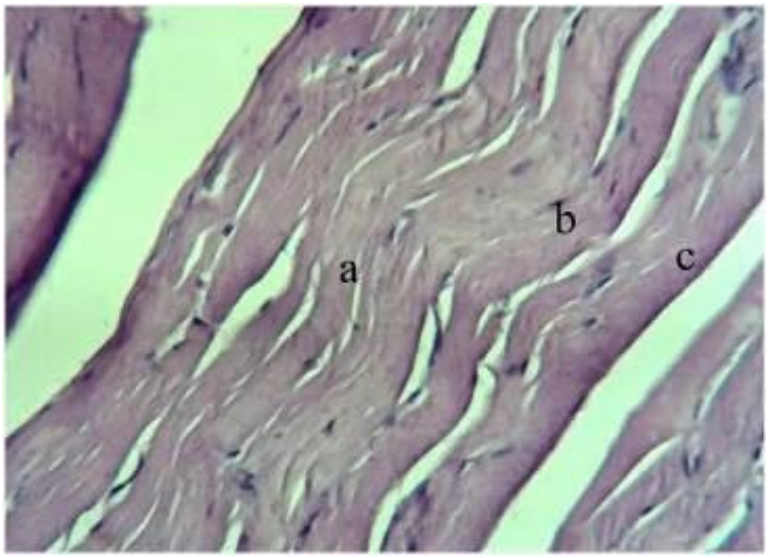

Figure 14 Microscopic structure of muscle tissue, which is selected from the pork half-carcasses subjected to stiving on the 7th day of storage (control): a – muscle fibres of tortuous shape; b – nuclei of muscle fibres in shadow form; c – endomysium. Hematoxylin and eosin. X 120.

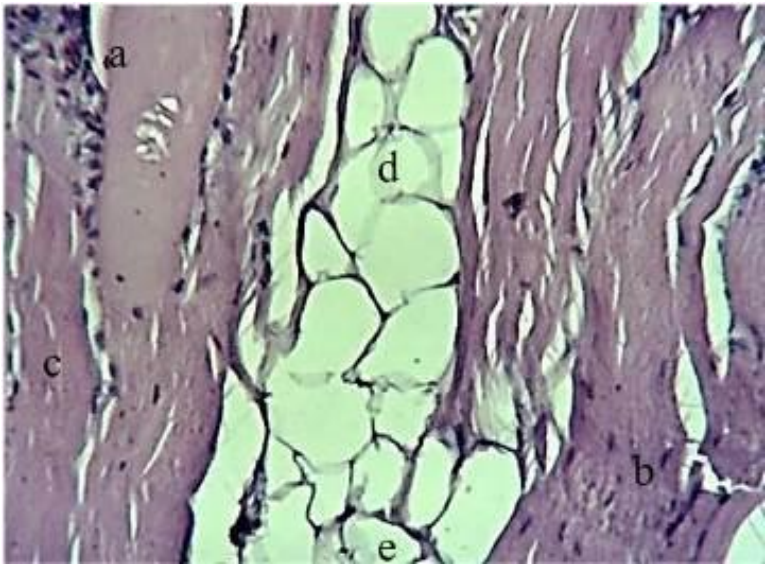

Figure 15 Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the 7th day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – endomysium; d – pyrimidine; e – fat cells in perimysium. Hematoxylin and eosin. X 120.

Such changes in the microscopic structure of the muscle tissue caused the release of the contained muscle fibres into the intermuscular space. Granular masses visible under a light microscope were formed by hydrolytic enzymes (Figure 12). Such changes are associated with aging processes and are related to the most important factors that improve meat tenderness due to the proteolysis of structural proteins by endogenous muscle enzymes **[15]**, **[36]**. In turn, protein degradation leads to the loss of structural integrity and the formation of large peptides and amino acids **[16]**, **[26]**.

This indicates the positive consequences of the influence of cultures of lactic acid microorganisms on the structure of the muscle tissue if the meat is stored for a long time (Figure 15). Thus, the sarcoplasm of MTPNM, which is selected from the experimental pork half-carcasses after a storage period of 7 days, when the histologic specimens were colored with hematoxylin and eosin, was mostly uniformly colored. However, the color saturation was lower than that in MFs of the muscle tissue, which is selected from the processed pork half-carcasses with cultures of lactic acid microorganisms after a storage period of 4 days (Figure 15).

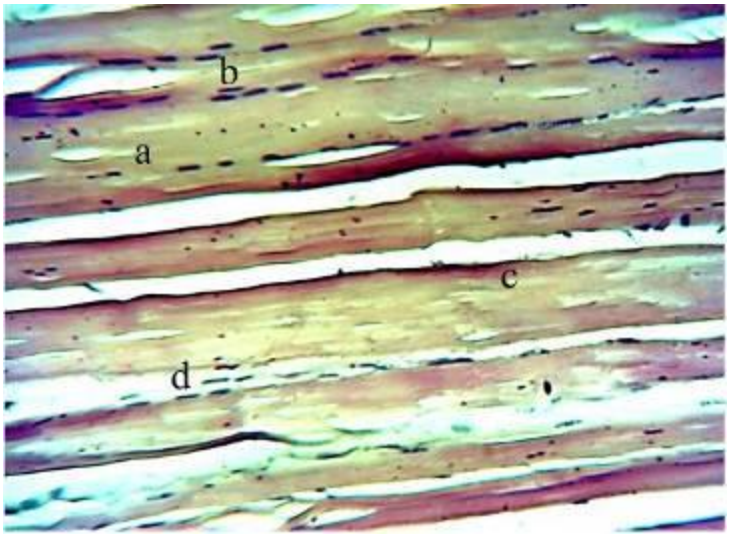

Figure 16 Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the 7th day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – endomysium; d – light lysed areas of sarcoplasm. Hematoxylin and eosin. X 120.

Furthermore, in certain areas of the histologic specimens, MFs were unevenly colored (light-lysed areas of the sarcoplasm were detected), which indicated the characteristic signs of the beginning of the autolysis process (Figure 16). Some of the muscle fibres had a tortuous shape (Figure 17), and their histoarchitecture was destroyed due to minor transverse cracks in their sarcoplasm (Figure 18). Such changes can probably be related to the

proteolytic activity of muscle calpains. The study results, conducted by **[18]**, **[19]**, indicate that calpain-1 and calpain-2 bind to myofibrils, when the meat is stored, and subsequently destroy structural proteins, including desmin.

When analyzing the histologic specimens at a high magnification of the microscope, the transverse and longitudinal striations of the sarcoplasm of individual muscle fibres were partially preserved (Figure 19).

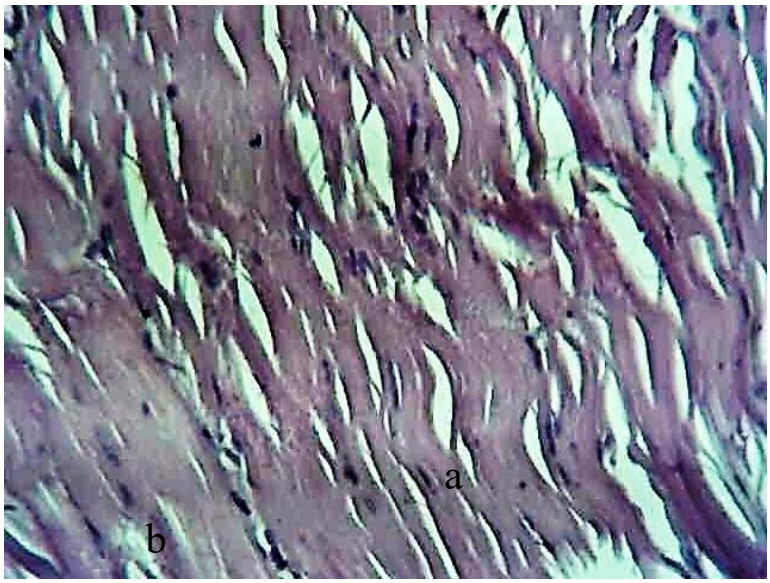

Figure 17 Microscopic structure of muscle tissue selected from the experimental pork half-carcasses on the 7th day of storage: a – muscle fibres of tortuous shape; b – nuclei of muscle fibres. Hematoxylin and eosin. X 120.

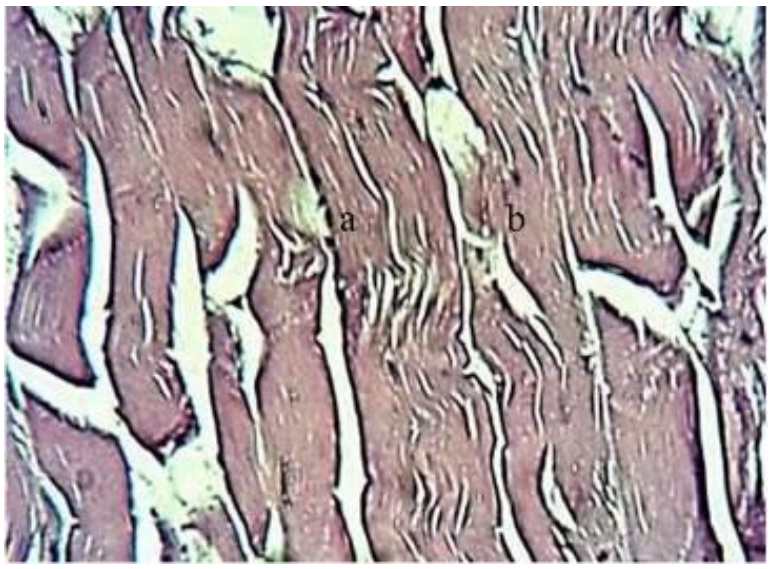

Figure 18 Microscopic structure of muscle tissue selected from the experimental pork half-carcasses on the 7th day of storage: a – muscle fibres of tortuous shape; b – nuclei of muscle fibres. Hematoxylin and eosin. X 120.

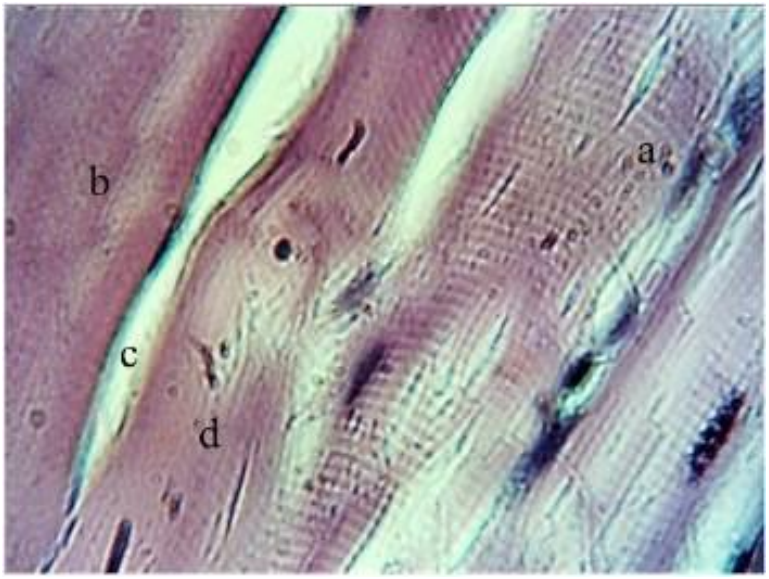

Figure 19 Microscopic structure of muscle tissue, which is selected from the experimental pork half-carcasses on the 7th day of storage: a – muscle fibres; b – nuclei of muscle fibres; c – perimysium; d – transverse striation. Hematoxylin and eosin. X 400.

The nuclei of the muscle fibres had an oval-elongated shape and were basophilically colored. At the same time, the nuclei karyoplasm was not clearly outlined by the nuclear envelope, indicating the initial autolysis processes of the muscle fibre nuclei. Such changes could also be caused by the enzymatic activity of the microflora in the meat, particularly various types of lactic acid microorganisms **[20]**, **[37]**.

It is rather difficult to make a more detailed comparison of the research results obtained by us with the data of other authors, since most strains of lactic acid microorganisms, in particular *L. sakei*, are intended for the technology of fermented meat products (sausages, ham) **[38]**, **[44]** and for raw pork, shock chilling or freezing is most often used, which ensures the reduction of microbial contamination during meat storage **[39]**. However, freezing of meat causes its quality to deteriorate due to protein oxidation and ice crystal formation, which occurs during freezing/thawing and frozen storage, which leads to irreversible physicochemical changes and quality deterioration **[40]**. Therefore, most researchers prefer to improve the methods of storing pork in a chilled state over freezing, but histological studies on this issue are very small and they are performed in laboratory conditions, and not in the conditions of meat processing plants. In addition, most studies used individual muscles or groups of muscles in which histological sections were made in a transverse perspective, which does not give a complete idea of the structural changes in muscle tissue **[41]**. As can be seen from the obtained data, the histostructure of the muscle tissue of the experimental pork neck was less damaged as a result of the autolysis process, which contributed to the extension of its storage period up to 7 days. The data, we obtained, are compliant with the study results of other scientists **[42]**, which indicate that 8 days are enough for pork meat to acquire the sensory characteristics that satisfy the consumer requirements.

We have analyzed the results of the study of the fermentation of lactic acid microorganisms. The SafePro® B-2 can be used as a promising tool for the use of pork refrigeration in the refrigeration chamber with showers, but for this, it is necessary to increase the possible risks of microflora-control, which will be characteristic of the skin-specific micronutrient enterprises and will be characteristic of the skin-specific micro-processing enterprises.

CONCLUSION

Thus, the microscopic structure of the muscle tissue of the pork half-carcasses neck, which was subjected to cooling in the refrigerating chamber with stiving on the 4th day of storage, was characterized by a slight deformation of the muscle fibres, their weakened transverse and longitudinal striations. Transverse cracks and ruptures of the muscle fibres were often found in it. The sarcoplasm of the muscle fibres was unevenly colored, which indicated the beginning of the autolysis process. The nuclei of individual neck MFs were in a state of lysis. The microscopic structure of the muscle tissue of the neck, which is selected from the experimental pork half-carcasses on the 4th day of storage, was characterized by more positive characteristics of the microscopic structure (uniform coloring of the sarcoplasm, minor cracks in the sarcoplasm, preservation of the transverse and longitudinal striations of the muscle fibres, etc.) compared to that of unprocessed pork half-carcasses with cultures of lactic-acid microorganisms, which indicated the positive effect of lactic-acid bacteria on the meat quality and, as a consequence, the extension of its shelf life in a chilled condition. Therefore, when the results of the

histological studies are analyzed, it is worth pointing out that the microscopic structure of the muscle tissue of the pork half-carcass neck after cooling in the refrigerating chamber with stiving with an increased storage period of up to 7 days underwent a deformation of the muscle fibres, shown as the increased number of the transverse cracks and ruptures of the muscle fibres, characteristic signs of the autolysis process of the muscle tissue. The histoarchitecture of the pork half-carcass neck muscles after cooling in the refrigerating chamber with stiving and final processing of the surface with cultures of lactic-acid microorganisms of strain SafePro® B-2 was characterized by insignificant areas of the muscle fibres with transverse cracks, suspended development of the autolysis processes, partial preservation of the transverse and longitudinal striations of the muscle fibres, which indicates the positive effect of lactic-acid bacteria on the meat quality and contributes to the extension of its storage in a chilled condition up to 7 days. The proposed treatment makes it possible to extend the shelf life of chilled pork meat while maintaining its quality and safety. The development of research in the direction of using lactic acid micro-organisms for the production of refrigerated pork is necessary with the improvement of microbiological and physicochemical indicators, as well as in the form of a mixture that can be used to improve the optimal conditions for the preparation of a suitable source for consumption.

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Comparative characterization of strains of lactic acid bacteria isolated from Kazakhstan mare's milk and koumiss to create probiotic preparation

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ABSTRACT

The most widely used probiotics that benefit human and animal health are lactic acid bacteria (LAB) derived from milk and dairy products. Therefore, this study aimed to investigate the probiotic properties of LAB strains isolated from Kazakhstan mare's milk and koumiss (fermented mare's milk) samples. A total of 24 LAB strains were isolated to test their probiotic properties. Based on analysis of probiotic properties, the strains 3K, 7K, 9K, 10K and 11K were identified by 16S rDNA sequence analysis. According to PCR analysis, three strains (3K, 7K, 9K) were assigned to the species *Limosilactobacillus fermentum* and the remaining two strains (10K and 11K) were assigned to the species *Lacticaseibacillus paracasei*. In summary, the high biological potential of the strain *Lacticaseibacillus paracasei* 10K was identified as having probiotic property, which suggests its possible use as a promising candidate.

Keywords: mare's milk, koumiss, LAB, probiotic properties.

INTRODUCTION

In recent years, probiotics have been used extensively for the prevention of gastrointestinal disorders in human and animals **[1]**. In this sense, functional food supplements, including pro-, pre- and synbiotics, are gaining increasing attention as an environmentally sound strategy to improve health. Meanwhile, probiotic bacterial strains should have a set of characteristics that allow them to compete with pathogenic and conditionally pathogenic microorganisms **[2]**. *Lactobacilli* and *Bifidobacteria* are beneficial human and animal gut bacteria with therapeutic functions **[3]**.

Milk and fermented milk products contain large amounts of bacteria with probiotic properties, which positively affect the maintenance of the body's intestinal system **[4]**. Recently, mare's milk, widely represented in the diet of the population of Kazakhstan, is now being actively used as a product with healthy ingredients in a naturally digestible form. Koumiss is a sour milk drink made mainly from mare's milk by fermentation with a special starter **[5]**. Scientific studies revealed high koumiss activity in treating gastric and duodenal ulcers, chronic gastritis and enterocolitis **[6]**, **[7]**. In this regard, production of probiotics with strains derived from koumiss needs to be expanded as a highly therapeutic and dietary product, contributing to enhancing the human and animal's immune system. The main microbiota involved in making koumiss are lactic acid bacteria (LAB) **[8]**, **[9]**. For instance, *L. helveticus* NS8 was investigated as potential strain isolated from koumiss, which might benefit health **[10]**. Probiotic preparation with *Lactobacillus casei* Zhang (LcZ) derived from Chinese koumiss, has been proved to positively affect human intestinal microbiota **[11]**. Nevertheless, a few studies still exist on strains derived from Kazakhstan koumiss, their characterizations, and probiotic properties.

One of the first and important steps in finding and selecting a strain promising for use in the food industry is determining its taxonomic identity. Correct identification of the strain at the species level allows the researcher to understand its safety, origin, habitat and physiological characteristics of the isolated microorganism. Therefore, the study wasstudy aimed to isolate active strains of lactic acid bacteria from Kazakhstan mare's milk and koumiss samples and evaluate their probiotic properties.

Scientific Hypothesis

The hypothesis of the study is to select potential LAB isolated from mare's milk and koumiss for use as probiotics and to pay attention to properties such as acid and bile tolerance, antimicrobial effect, etc. that allow LAB strains to be considered as probiotic candidates.

MATERIAL AND METHODOLOGY

Samples

Samples of raw mare's milk (n = 6) and koumiss (n = 6) were obtained from mares aged four and a half years of Zhaby, Kazakh and Mugalzhar breeds. The farms are located in the foothills of Talgar district (Tuzdybastau and Panfilov villages) and Karasai district (Almaty region, Kazakhstan). The horses were kept under standard conditions, with the same management conditions. The horses were provided with clean water and fed pasture grass. The samples of mare's milk and koumiss were collected during horse lactation (September 2021) in the morning. The raw 1000 mL and fermented dairy products of each sample was taken into flasks and were immediately placed on ice, stored at 4 °C for further analysis. Sampling of milk and dairy products was carried out according to the procedure of GOST 26809.1-2014 **[12]**.

Chemicals

The De Man, Rogosa, and Sharpe (MRS) broth (Merck, Germany), was used for LAB isolation; gram staining was performed using a special Gram staining kit (Merck, Germany); different antibiotic disks (levomycin, neomycin, tetracycline, streptomycin and erythromycin) (Scientific Research Centre of Pharmacotherapy, St. Petersburg, Russia) were used for antibiotic susceptibility test.

Instruments

The morphological and cultural properties of the lactic acid bacteria were studied using a Micros MC-300 electron microscope (Austria). DNA concentrations were determined using the QubitTM dsdna HS Assay Kit (Life Technologies, Oregon, USA) on a fluorimeter, Qubit 2.0. PCR amplification was performed on a GeneAmp PCR System 9700 amplifier (Bio-Rad, USA).

Laboratory Methods

Laboratory researches were carried at the Kazakh National Agrarian Research University (Almaty, Kazakhstan). The isolation of lactic acid bacteria strains was done by culture characteristics and macroscopic analysis **[13]**, acid and bile resistance **[14]**, antibiotic resistance was done by disc diffusion method **[15]** and the agar diffusion method was employed for antimicrobial effects **[13]**, and molecular identification were investigated by 16S rRNA gene via PCR analysis **[16]**.

Description of the Experiment

Sample collection and isolation of LAB: Lactic acid bacteria from samples were isolated by inoculating 10 mL of each milk sample into De Man, Rogosa, and Sharpe (MRS) broth (Merck, Germany) and incubated at 37 °C for 24 h. The morphological and cultural properties of the lactic acid bacteria were studied using a Micros MC-300 electron microscope. Colonies were transferred to MRS agar and incubated in a refrigerator at 4°C for further study (GOST 33951-2016) **[13]**.

Resistance to acid and bile: Isolates were selected according to the characteristics of the cultures. Isolates forming colonies with formed off-white pinhead colonies characteristic of *Lactobacillus* spp. were selected. The tolerance of the cultures to different concentrations of acid and bile salt were tested. To determine the acid tolerance of the tested strains, hydrochloric acid (HCl) was added to MRS liquid medium seeded with the tested bacteria, setting the pH between 6 and 2; then cultured at 37 °C for 24 h. Determination of resistance to NaCl was carried out by inoculating cultures in liquid MRS medium with different salt contents (2, 4, 6, and 8%), in which the bacteria were cultured at 37 °C for 36 hours.

Resistance to bile was determined by adding daily culture to MRC broth containing 0.3, 0.4, and 0.5% bile concentrate. Bacterial growth was analysed by counting viable colonies after 2 and 4 hours of culturing, following inoculation in agar medium at 37 °C for 48 hours. For this purpose, 0.1 ml of a solution that contained a particular previously isolated strain was added to 10 ml of MRS broth, then inoculated for 24 hours at 37 °C **[14]**.

Antibiotic susceptibility test and antimicrobial effect of isolated bacterial cultures: The choice of antibiotics was based on the natural resistance of LAB and the different classes and mechanisms of action of

antibacterial drugs. To determine strains' sensitivity to antibiotics, standard disks impregnated with standard solutions of amphenicols – levomycin (30 μg), polyketide – tetracycline (30 μg), aminoglycoside antibiotics – streptomycin (30 μg) and neomycin (30 μg), macrolide – erythromycin (15 μg) was used. Milk agar was a nutrient medium for lactic acid bacteria **[15]**. One-day cultures grown at optimum temperature were used in the experiments in the form of cell suspension in the amount of 1 billion/ml, based on the calculation of 0.1 ml of suspension per one Petri dish. After seeding the dishes with the tested strain cultures, discs impregnated with antibiotic were placed on the surface of the nutrient medium. Cultivation was carried out for 72 hours at 37 °C. The sensitivity of lactic acid bacteria to antibiotics was determined by measuring the diameter of the growth suppression zone.

The agar diffusion method was used to determine the antagonistic activity of the bacteria **[13]**. The *Sarcina flava, Bacillus subtilis, Staphylococcus aureus, Escherichia coli (E.coli)* cultures are opportunistic bacteria. In the body, it is permanently present in the intestinal tract, therefore, they were used as indicator bacteria. *Salmonella Dublin* was selected as the causative agent of gastrointestinal infectious diseases. Test cultures were grown on media with the optimal composition for each species: agar wort mixed with MPA in a 1:1 ratio. 0.1 ml of suspension was added to the melted and cooled medium, mixed and poured into Petri dishes. Wells with a diameter of 10 mm were cut in the layer of nutrient medium, into which a liquid suspension of daily cultures of the studied lactic acid bacteria was added and placed in the thermostat at 37 °C for 24 hours. After 24 hours, the zones of growth suppression of the test cultures by lactic acid bacteria were measured. The strains that showed the strongest antagonistic properties by the maximum number of relevant indicators with wide zones of inhibition were selected for further studies.

Molecular identification of LAB strains: Genetic study of microbial isolates was based on nucleotide sequence analysis of the 16S rRNA gene. DNA extraction was performed according to the standard protocol (the PureLink Genomic DNA Kit, Promega, USA). Qualitative DNA assessment was performed by electrophoresis on 1% agarose gel.

Universal primers were used to determine the nucleotide sequence of 16S rRNA gene: forward 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse 806R (5' GGACTACCAGGGTATCTAAT-3') **[16]**. The PCR reaction was performed in a total volume of 20 μl. The PCR conditions were as follow as: stage 1 – 5 min at 95 °C – 1 cycle; stage 2 – 30 sec at 95 °C, 40 sec at 55 °C, 50 sec at 72 °C – 30 cycles; stage 3 – 10 min at 72 °C – 1 cycle.

To assess the efficiency of PCR, the amplification products were visualized on 1% agarose gel. The separation of gene fragments was performed using an automatic genetic analyzer. The obtained nucleotide sequence was compared with nucleotide sequences from international GenBank databases (NCBI: https://www.ncbi.nlm.nih.gov/genbank*).* Phylogenetic trees were constructed via MEGA 6 software. Alignment of nucleotide sequences was performed using ClustalW algorithm. The Neiighbor-Joining (NJ) method was used to construct phylogenetic trees.

Number of samples analyzed: We analyzed a total of 12 samples.

Number of repeated analyses: All measurements were performed two times.

Number of experiment replication: All experiments were performed in three replications.

Statistical Analysis

Data were presented as mean (±) standard deviation. SPSS version 25 (IBM Corporation, New York, USA) was used to perform all statistical analysis. The statistical comparison analysis was done using Student‘s t-test. Statistically significant data were considered when $p < 0.05$.

RESULTS AND DISCUSSION

The beneficial properties of koumiss are due to the unique composition of mare's milk, which, unlike cow's milk, is closer in composition to human milk **[17]**. Nowadays koumiss is used not only for treatment of pulmonary tuberculosis, but also gastrointestinal diseases, non-specific lung diseases, some diseases of cardiovascular and nervous systems **[18]**. The huge needs of the nascent food industry and health care institutions demanded increased production of mare's milk and koumiss. Therefore, in the present work, mare's milk and koumiss are chosen as research objects to fill the gaps in potential probiotic research.

In this study, a total of 24 bacterial isolates were isolated on MRS agar after 24 hours incubation at 37 °C. They were marked with the letters 'K' (koumiss) and 'M' (mare). Isolates 1M, 2M, 3M, 4M, 5M, 6M, 7M, 8M, 9M, 10M and 11M were isolated from four mare’s milk samples. Isolates 1K, 2K, 3K, 4K, 5K, 6K, 7K, 8K, 9K, 10K, 11K, 12K and 13K were isolated from six samples of koumiss. In a study by Jin et al. (2021), 114 strains of lactic acid bacteria were also isolated from raw mare's milk and their probiotic traits were tested **[19]**. The biochemical characteristic of all strains was examined. All strains were found to be facultative anaerobes and all

were Gram-positive, catalase- negative and immobile. 2M, 3M, 6M, 7M, 11M and 3K, 7K, 9K, 10K, 11K were bacilliform. 1M, 4M, 5M, 8M, 9M, 10M and 1K, 2K, 4K, 5K, 6K, 8K, 12K, 13K were identified as cocci forming bacteria.

Requirements for probiotic strains include resistance to low pH of gastric juice and bile, antagonism to opportunistic and pathogenic flora, etc. **[20]**, **[21]**. The high acidity of gastric juice is known to kill most bacteria and viruses, which prevents them from multiplying and spreading. Thus, the survival of lactic acid bacteria when exposed to human gastric juice is a critical factor **[22]**. Subsequently, all strains were evaluated for viability at different pH values (6, 4, and 2) for 24 hours of cultivation. Based on the obtained results (Table 1), strains were able to grow at pH 6 ($p \leq 0.05$). At pH 4, 11 strains grew, of which 4 were isolated from mare's milk samples and 7 from koumiss samples. However, 3 strains could not grow at pH 2 for 24 hours of cultivation. Only 8 strains (2M, 3M, 8M, 3K, 7K, 9K, 10K and 11K) were able to grow at pH 2 ($p \leq 0.05$). According to a study by Azat et al. survival at pH 3.0 is considered the optimal acid tolerance for probiotic strains **[23]**. The difference in results may be because the strains' acid regulatory mechanisms failed to maintain their intracellular pH and internal acidification decreased enzyme activity, damaging certain proteins and DNA, leading to death. However, the pH value (2.0) used in the current study to choose potentially probiotic strains is very selective and it guarantees the isolation of very acid-tolerant strains. There is a lack of studies reporting resistance of koumiss LAB strains to low pH values. Previous study on Mongolian koumiss observed that only two strains showed normal growth at pH 2.0 **[24]**. These data potentially indicate that the strains tested in our research are the most acid-tolerant and can be used as promising probiotic strains.

Bile enters the duodenal section of the small intestine, causing the death of a large number of bacteria, as cell membranes composed of lipids and fatty acids are very sensitive to destruction by bile acid salts. In this regard, the effectiveness of probiotic microorganisms depends on their resistance to bile acids **[25]**. Therefore, bile salt tolerance has often been used as the most important criterion for the selection of active strains suitable for use as probiotics **[26]**. All strains were also tolerant to 0.3% and 0.4% bile. Moreover, among the 8 strains that were acid tolerant strains such as 3M, 3K, 7K, 9K, 10K and 11K had better tolerance to 0.5% bile ($p \leq 0.05$) (Table 1). Strains of *Lactobacillus* spp. isolated from milk and curd were resistant and showed maximum growth at 0.8% bile salt concentration in the study by Kasimin et al. **[27]**.

Hydrochloric acid is produced by special parietal cells from the glands of the stomach. The main functions of hydrochloric acid are protein digestion, antibacterial action, etc. Sodium chloride (NaCl) is the main source of hydrochloric acid formation of gastric juice **[28]**. NaCl is an inhibiting substance that can inhibit the growth of certain types of bacteria. Some strains of lactic acid bacteria are resistant to NaCl, so it was important to test the tolerance of LAB to NaCl. All strains could tolerate concentrations of 2% NaCl, and only some strains were resistant to concentrations of 4% and 6% sodium chloride. Among all strains, those such as 2M, 3M, 3K, 7K, 9K, 10K, and 11K showed better tolerance compared to other strains ($p \leq 0.05$) (Table 1). Our results agreed with those in the research by Kasimin et al., where most strains isolated from milk and dairy products were not resistant to media containing more than 6.5% NaCl **[27]**.

Antimicrobial activity is one of the most important factors in selecting effective and novel probiotics **[29]**. The antimicrobial action of LAB is supported by the production of several substances, such as organic acids, hydrogen peroxide, low molecular weight antimicrobials and bacteriocins **[30]**. The results of the strains' antagonistic abilities are shown in Table 2. We can conclude that strains 7K, 10K, and 11K have the most pronounced antagonistic properties, and their use as probiotic strains is quite appropriate.

All strains intended for probiotic use must be investigated to establish the sensitivity to the appropriate range of antimicrobial agents relevant for humans or animals **[31]**. The antibiotic resistance of isolated strains is shown in Table 3. LAB that are studied in our analysis were relatively resistant to the following antibiotics: erythromycin and tetracycline, which disrupt protein synthesis but also the genome replication processes of microorganisms. Erythromycin actively penetrates through the cell membrane of bacteria and binds irreversibly to the subunits of bacterial ribosomes, thus inhibiting protein synthesis of the pathogen. Growth inhibition was observed with erythromycin in the following strains: 3M, 3K, 7K. Only three strains 7K, 10K and 11K showed significant resistance ($p \leq 0.05$). Notably, the 3M strain was the most sensitive to this antibiotic. A similar result was obtained by Guo et al. where 33 *Lactobacillus* strains were tested for antibiotic resistance **[32]**. All *Lactobacillus* spp. strains were found to be resistant to vancomycin but sensitive to erythromycin and gentamicin. Aryantini et al. investigated the safety and probiotic characteristic of *Lactobacillus* spp. isolated from fermented mare's milk, where strains were sensitive to ampicillin and streptomycin but resistant to erythromycin **[33]**. In summary, strains 7K, 9K, 10K, and 11K were the most resistant to the above antibiotics.

Table 1 Growth performance of isolated strains in milk hydrolysate containing bile and sodium chloride.

No	Strain notation	pH value			Bile, %			NaCl, %			
		6	4	2	0.3	0.4	0.5	2	4	6	8
1	1M	+	-	-	+	+	-	+	-	-	-
2	2M	+	+	+*	+	+	-	+	+	-	-
3	3M	+	+	+	+	+	+*	+	+	+	+*
4	4M	+	-	-	+	-	-	+	+	-	-
5	5M	+	+	-	+	+	-	+	+	-	-
6	6M	+	-	-	+	-	-	+	+	+*	-
7	7M	+	-	-	+	-	-	+	+	+	-
8	8M	+	+	+*	+	+	-	+	+	+	-
9	9M	+	-	-	+	+	-	+	-	-	-
10	10M	+	-	-	+	-	-	+	-	-	-
11	11M	+	-	-	+	-	-	+	+	-	-
12	1K	+	-	-	+	+	-	+	-	-	-
13	2K	+	-	-	+	+	-	+	-	-	-
14	3K	+	+*	+*	+	+	+*	+	+	+	+*
15	4K	+	-	-	+	+	-	+	+	-	-
16	5K	+	-	-	+	-	-	+	-	-	-
17	6K	+	+	-	+	-	-	+	-	-	-
18	7K	+	+	+*	+	+	+*	+	+	+	+*
19	8K	+	-	-	+	+	-	+	+	-	-
20	9K	+	+	+*	+	+	+*	+	+	+	+*
21	10K	+	+	+*	+	+	+*	+	+	+	+*
22	11K	+	+	+*	+	+	+*	+	+	+	+*
23	12K	+	-	-	+	+	-	+	-	-	-
24	13K	+	+	-	+	-	-	+	+	+	-

Note: «+» - growth; «-» - no growth; * - ($p \leq 0.05$).

Table 2 Antagonistic activity of lactic acid bacteria strains.

No	Strains	Culture test, (mm)				
		Sarcina flava	*Bacillus subtilis*	*Staphylococcus aureus*	*E. coli*	*Salmonella dublin*
1	2M	-	5.4 ±0.2	-	-	-
2	3M	-	9.7 ±0.1	-	10.1 ±1.2*	-
3	8M	-	-	-	8.7 ±0.3	-
4	3K	11.7 ±0.2	-	8.9 ±0.3*	-	-
5	7K	5.5 ±0.2*	3.7 ±1.1	4.1 ±0.2	5.8 ±0.4	-
6	9K	8.8 ±0.3	7.0 ±0.3	-	8.2 ±0.2	-
7	10K	11.7 ±0.1*	11.2 ±0.1*	12.2 ±0.1	15.3 ±0.1*	12.2 ±0.1*
8	11K	10.2 ±0.3*	10.3 ±0.1*	10.2 ±0.3	11.4 ±1.1*	11.7 ±0.5*

Note: Clear zones were measurd in mm. Results represent the mean ± standard deviation of three replicates. Strains 2M, 3M, 8M isolated from of horse milk, strains 3K, 7K, 9K, 10K and 11K isolated from koumiss; "-" – no activity; * - ($p \leq 0.05$).

Table 3 Study of antibiotic resistance of LAB strains.

No	Strains	Antibiotics, suppression zones (mm)				
		Levomycin 30 mc	Neomycin 30 mc	Tetracycline 30 mc	Streptomycin 30 mc	Erythromycin 15 mc
1	3M	14.3 ±0.6	16.8 ±0.2	25.8 ±0.1	41.6 ±0.2*	47.1 ±0.3
2	3K	12.2 ±0.1	18.3 ±0.1	22.4 ±0.3	24.1 ±0.2*	35.3 ±0.2
3	7K	R*	14.5 ±0.5	33.6 ±0.1	25.1 ±0.4	33.8 ±0.3
4	9K	11.8 ±0.05	16.5 ±0.3	R	26.3 ±0.05	28.7 ±0.3
5	10K	R*	7.9 ±0.1*	R*	17.2 ±0.6	15.3 ±0.4*
6	11K	R*	R	R	15.9 ±0.1*	16.1 ±0.1*

Note: The suppression zones were measured in mm. The results are the mean value ± standard deviation of the three replicates. R – stable; * - ($p \leq 0.05$).

In our study, genetic identification of five strains (3K, 7K, 9K, 10K, and 11K) was performed by direct nucleotide sequence determination of the 16S rRNA gene fragment, followed by comparison of nucleotide identity with sequences deposited in the international Gene Bank database (NCBI: https://www.ncbi.nlm.nih.gov/genbank), and construction of phylogenetic trees with nucleotide sequences of reference strains. The results of sample amplification are shown in Figure 1. The results of phylogenetic analysis of 16S rRNA gene sequences in the strains are presented on a phylogenetic tree. As it can be seen from the data presented, strains can be classified into two species, one of which is the species *Limosilactobacillus fermentum*, whose nucleotide sequences are characterized by 100% similarity (Figure 2-4). The second cluster includes a strain of *Lacticaseibacillus paracasei* whose 16S rRNA gene sequence similarity was 100% (Figure 5-6). To date, Wu et al. identified the both species in Inner Mongolian koumiss samples **[34]**. Furthermore, Pan et al. proved the cholesterol-reducing effect of the *Limosilactobacillus fermentum* SM-7 strain derived from koumiss in mice **[35]**.

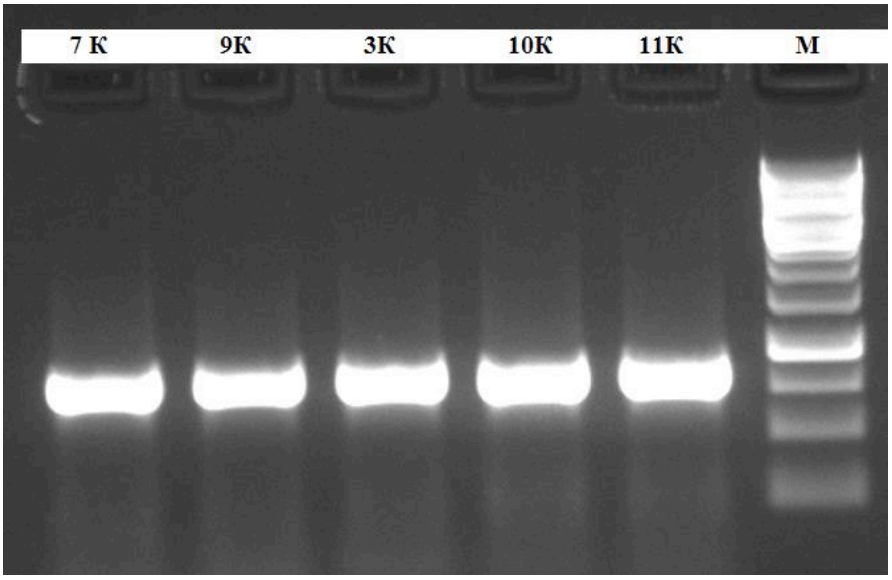

Figure 1 PCR electrophoresis pattern of the amplification products of the 16S rRNA fragment of the DNA gene. (M) 100bp Plus DNA molecular weight marker.

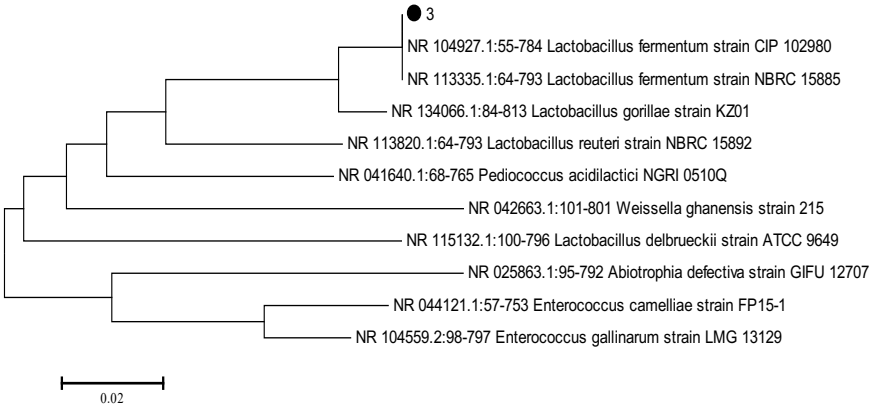

Figure 2 Phylogenetic tree based on analysis of 16S rRNA fragment structures showing the kinship of strains of the genus *Limosilactobacillus fermentum*.

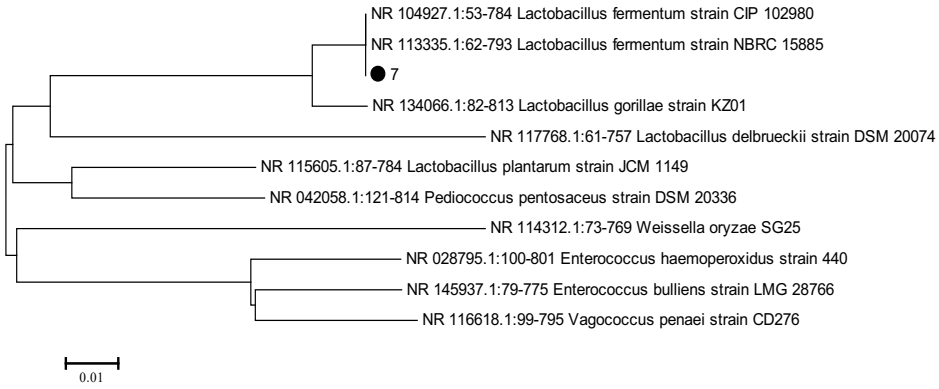

Figure 3 Phylogenetic tree based on analysis of 16S rRNA gene fragment structures showing the relationship between strains of the lactic acid bacteria genus *Limosilactobacillus fermentum*.

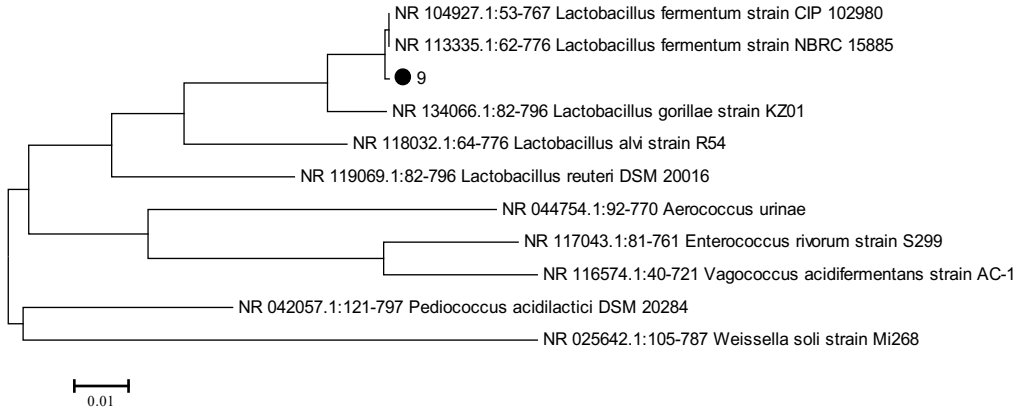

Figure 4 Phylogenetic tree based on the analysis of 16S rRNA fragment structures showing the relationship between strains of the lactic acid bacteria genus *Limosilactobacillus fermentum*.

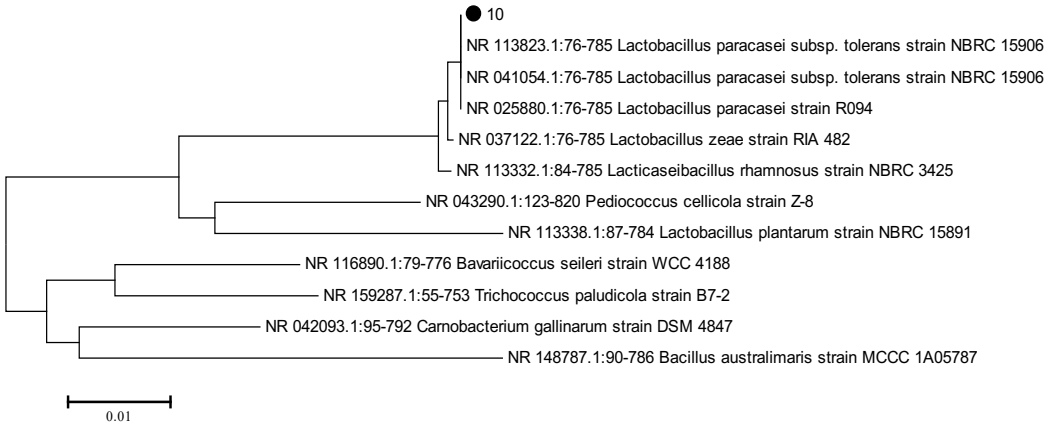

Figure 5 Phylogenetic tree based on the analysis of 16S rRNA fragment structures showing the relatedness of the lactic acid bacteria genus Lacticaseibacillus paracasei strains.

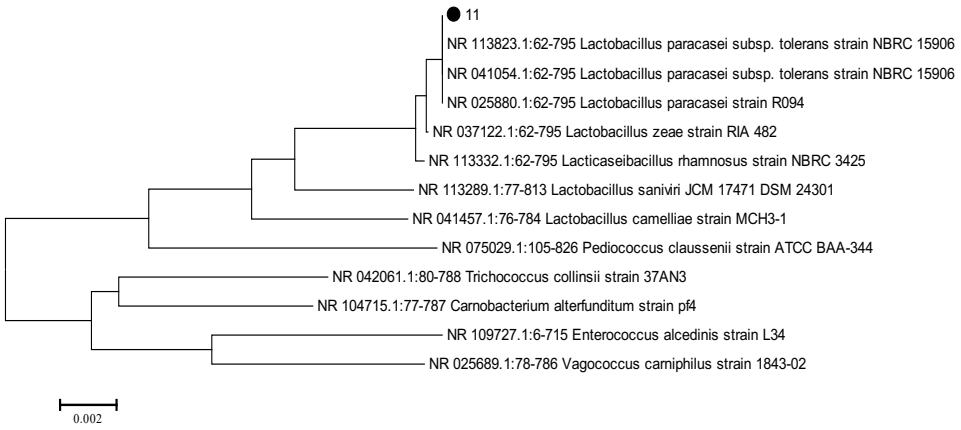

Figure 6 Phylogenetic tree based on 16S rRNA fragment structure analysis showing the relationship between strains of the genus *Lacticaseibacillus paracasei*.

CONCLUSION

The current research is the first step in studying beneficial Lactobacillus strains derived from Kazakhstan mare's milk and koumiss, which ensure further in-depth study. The results showed that the isolated novel LAB strains, especially *Lacticaseibacillus paracasei* 10K strain, possess several important probiotic properties such as bile and acid tolerance, antibiotic resistance and antimicrobial action. This makes it possible to recommend isolated strains of LAB as potential probiotics for applications in the food and pharmaceutical industries. However, *in vivo* studies of the quantitative and qualitative effects of strains on growth and immunity of organisms are needed.

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Improving the quality and the technology of processed cheeses

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ABSTRACT

This article investigates processed cheese's nutritional value and safety by adding vegetable additives (dry Spirulina powder). Processed cheese for lunch is taken as a basis for the formulation. As a control, we took cheese made according to classical technology. We used cheeses from cow's milk. We used combined raw materials in the developed technology: cow's and goat's milk cheeses. Spirulina was added to the formulation as an enrichment agent in the 1%, 2%, and 3% ratio, respectively. The sample with a 1% addition was found to be rational according to the results of the organoleptic evaluation. The formulation was optimised in further study by selecting 0.5%, 1.5% and 2%. A centre composite plot was used to add points around the pre-lagged optimum. A regression formula was obtained, and the melting salts and the dosage of the added enrichment agent were determined. Also, the share of cheese from goat's milk in the recipe of processed cheese was determined. The recipe was calculated on the principle of material balance. Experimental samples were examined for fatty acid and amino acid composition. The tables compare the best sample on organoleptic evaluation with the control. It was found that when 3% is added, the cheese acquires a dark green tinge. The colour is deep green when 2% is added; when 1% or less is added, the colour is salad. The dose of melting salts in the recipe was reduced to 2%; in the classic recipe, it was 3.9%. The protein of the experimental sample turned out to be closer to the ideal protein. PDCAAS is equal to 96.9, while in the control sample, PDCAAS is equal to 39.9. Also, when comparing the fatty acid composition, the thrombogenicity coefficient was lower in the experimental sample than in the control.

Keywords: quality, processed cheeses, thrombogenicity factor, utilisation factor, PDCAAS, fatty acid, amino acid, composition

INTRODUCTION

The science of processed cheese is developing rapidly, as evidenced by the rapid increase in articles on this subject in scientific databases. Trends in the development of processed cheese technology are different and act in different directions, such as improving organoleptic characteristics, developing functional processed cheeses, increasing shelf life, reducing the number of emulsifying salts, researching new types of emulsifying salts for different types of processed cheese **[1]**. Expanding the assortment range for processed functional cheeses is becoming increasingly widespread in studying processed cheese technology. For this purpose, the influence of additives increasing the nutritional and biological value on the consistency, shelf life and colour of processed cheeses is studied. The effect of algae such as Chlorella vulgaris was studied. The study revealed that adding 2% Chlorella vulgaris did not affect the overall acceptability of processed cheese, but it significantly increased the protein content and added hardness **[2]**. So, the effect of adding Spirulina platensis (Gomont) Geitler to the recipe of pasty processed cheese: the conclusion was that the addition changes the colour, taste and smell, but at the same time increases the nutritional value, it was found that the addition of 1% dry powder Spirulina platensis is acceptable as to increase the nutritional value and to maintain a pleasant colour and smell **[3]**. Positive results

were obtained in cultivating Spirulina fusiformis on a cost-effective medium **[4]**. The study of the influence of adding Spirulina Maxima **[5]** in the formulation of pasty processed cheeses showed that Spirulina Maxima has antioxidant properties and affects the shelf life of processed cheeses. At the same time, the colour becomes dark green at the addition of 3% Spirulina Maxima, so adding 1-2% is recommended for the colour and smell of processed cheeses. The effects of various vegetable additives were studied **[6]** in terms of physicochemical parameters, and changes and increases in the nutritional value of the final product were monitored. All this suggests that the effects of Spirulina on sliced processed cheeses and from non-traditional raw materials have not been studied. Changing the amount of added melting salts in processed cheese formulations or replacing traditional melting salts is also important considering the trend towards safe products. Replacement of salts on calcium chloride, technological aspects at reduction of the dosage of melting salts and the influence on the structure of processed cheese is not studied by many scientists **[7]**, nevertheless it is a significant aspect in manufacture of processed cheeses of functional purpose. Also, there are no articles about processed cheeses from combined raw materials in the scientific world. There are works about the technology of goat's milk cheeses, mainly soft varieties **[8]**, less often about hard ones.

In our case, we use hard cheese from cow's milk and fresh cheese from goat's milk, thereby laying down the question of melting salts used. The ripening time and melting duration are changed. This article aims to address these issues. Many authors study the influence of adding vegetable additives on the composition, but the changes in consistency are left aside. the study of amino acid and fatty acid composition is also one of the aspects of proving the functionality of food products. Processed cheeses are products accessible to a wide segment of the population. Thanks to this quality, processed cheese can be an excellent product for functional use. Also, the bioavailability of elements in the cheese composition should include phytonutrients with high digestibility. In Kazakhstan, the population has many problems due to the environmental situation. Nuclear explosions, nuclear waste, mining of heavy metals, oil, etc., affect the population's health. An important aspect is to cleanse the body from free radicals, radionuclides, heavy metals, toxins, etc. The number of people with cancer is growing yearly, and the disease worsens. The solution to the problem can be products that address at least one of the problems. A good solution may be the addition of Spirulina to processed cheese. The hypothesis is the possibility of combining goat's milk cheese in the recipe of processed cheese and enrichment with Spirulina. The objectives are: development of technology for the production of processed cheese from combined dairy raw materials and enriched with Spirulina; change of amino acid and fatty acid composition compared with processed cheeses made from cow's milk, made by traditional classical technology from local raw materials.

Scientific Hypothesis

Adding 1% Spirulina Maxima to processed cheese from goat's and cow's milk optimizes the composition of amino acids and fatty acids. We chose in the recipe of a new type of processed cheese a combination of vegetable filler – Spirulina Maxima, and soft cheese from goat's milk, as they have excellent functional therapeutic preventive, and antioxidant properties.

MATERIAL AND METHODOLOGY

Samples

Hard cheese from cow's milk and cheese from goat's milk were used to produce processed cheese from combined dairy raw materials, according to the technology proposed in **[9]**. The formulation was calculated by material balance; a mixture of melting salts tripolyphosphate and sodium citrate was used. The proportions of melting salts were selected using laboratory model samples in small volumes. We received reliable results.

The amino acid composition was determined by gas chromatographic method and by measuring the proportion of amino acids by capillary electrophoresis using the capillary electrophoresis system "Kapel", methodology M-04-38-2009.

The fatty acid composition of the substance was analysed by capillary gas chromatography on a Shimadzu instrument, DB-WAX column, length 30 m, inner diameter 0.25 mm. Total lipids were initially extracted using Rose-Gothlib, after which the oily substance was derivatised using MeOH/BF3 (methanol containing 14% boron trifluoride). Fatty acids were analysed as methyl esters. Temperature programme: 50 °C for 2 minutes, rise to 200 °C at a rate of 10 °C/min, rise to 218 °C at a rate of 2 °C/min, rise to 250 °C at a rate of 10 °C/min, hold at 250 °C for 10 minutes.

For the production of processed cheese, we purchased hard cheese from cow's milk and produced cheese from goat's milk with a maturation period of 10 days. Spirulina powder was purchased from Algos, Republic of Kazakhstan.

To assess the organoleptic properties of the processed cheeses, a profillogram, or sensory profile, was established. This involved a comprehensive sensory evaluation conducted by a panel of trained assessors. Each

assessor was presented with samples of the processed cheeses and evaluated based on predefined sensory attributes. These attributes encompassed taste, aroma, texture, and overall quality. Sensory attributes were selected based on their relevance to processed cheese quality. These typically included attributes like creaminess, saltiness, bitterness, and specific flavour notes. A structured, numerical assessment scale was employed to score each attribute. Assessors rated the intensity or quality of each attribute for each cheese sample.

The coefficient of concordance (W) was calculated to gauge the degree of agreement among the sensory assessors regarding the sensory attributes. This coefficient is essential in determining the reliability and consistency of the sensory evaluations.

The coefficient of concordance (W) is typically derived from the rankings or scores provided by the assessors. It quantifies the extent to which the assessors' evaluations align with each other. A higher W value suggests a higher level of agreement among the assessors.

The profillogram, which encapsulates the sensory attributes and their associated scores or rankings, visually represents the organoleptic characteristics of each cheese sample.

By examining the profillogram for each sample, it becomes possible to compare the sensory profiles of different cheeses. This aids in making informed decisions regarding product quality, formulation optimization, and potential adjustments.

Melting salts were purchased from Elegita Asia, Almaty, Republic of Kazakhstan. Samples were run according to the formulation presented below in Table 1.

Table 1 Formulation for the cheese "Almaly" with the mass fraction of fat in dry matter of the product 60% (per tonne in kg).

Name of raw material	Weight
Large rennet cheeses: with a mass fraction of dry matter of 60%, fat in dry matter 50%	400
Cheese from goat's milk with a mass fraction of fat of 40%	200.0
Concentrate of plant raw materials with a mass fraction of dry matter of 50%	10
Peasant butter with a mass fraction of dry matter 75%, fat 72.5%	156
Cottage cheese with a mass fraction of dry matter 27%, fat 9%	100
Mixture of sodium tripolyphosphate with sodium citric acid (food grade) with a mass fraction of dry matter 20%	40
Drinking water	104
TOTAL	1010
OUTPUT	1000

Chemicals

All chemicals purchased by „Laborpharma“, Almaty, the Republic of Kazakhstan, were of analytical grade quality.

Laboratory Methods

Organoleptic evaluation: In a closed method, the organoleptic indicators of dairy products were evaluated by profile method using questionnaires. When performing profile analysis, point scales were used to assess the intensity of individual attributes, sensation manifestations were consistently determined, and the results were graphically depicted in the form of a profillogram (profile); When determining organoleptic indicators, the consistency of experts' opinions was assessed by the coefficient of concordance (W). If W <0.6, the consistency of experts' opinions was considered poor, and the next round of surveys was conducted.

Methodology for determining colour characteristics: Characteristics Methodology for determining the colour characteristics of processed cheese samples were examined by CIELAB using a Konica Minolta colourimeter model CR-410, Japan, using the characteristics: colour brightness (L*), red colour components (a*), yellow colour components (b*). The method consists of capturing spectral curves on the reflected surfaces of a sample of processed cheese, with brightness in the visible area of 380-750 nm. The sample is spread out on a board at room temperature, with an area of 18x8x2 cm and a calorimeter, set in the leftmost row and ran, displaying data on a monitor. The process is repeated for the middle and the rightmost row. The data read on the monitor of the colourimeter is indicated as follows: L*=,a*=,b*= where L*–is brightness, a*– presence of red colour, b*– presence of yellow colour.

Amino acid scoring (AAS, AAS): the percentage ratio of AA of the protein under study to the content of the same AA in the "ideal" protein, in which the content of each NSAQ corresponds to the indicators determined by the adequacy scale for animal or human needs **[10]**.

Gas chromatograph GC-17A, Shimadzu (Japan): temperature mode: initial temperature 140C0; delay 1 min; increase 2C0/min to 230C0; delay 27 min; Capillary electrophoresis system "Kapel", RFLaboratory Methods.

Description of the Experiment

Sample preparation: Cheeses were melted according to the recipe, ready samples were stored sealed in disposable special cups weighing 50 g each. They were stored under refrigerated conditions at 4-6 °C.

Number of samples analyzed: We analyzed 4 samples.

Number of repeated analyses: All measurements of instrument readings were performed 3 times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was 3 times.

Design of the experiment: Experimental Design Selection:

Initial Planning: The process commenced with the initial planning stage, where the overall design of the experiment was determined. This included decisions regarding the selection of factors to be studied, their levels, and the experimental goals.

Sample Preparation:

Raw Material Selection: Careful selection of raw materials, including goat's milk cheese and other ingredients, was a fundamental step.

Replicates: Each experimental sample was prepared three times to ensure the reliability of the results.

Data Collection and Processing:

Data Gathering: All three samples for each formulation were examined, and the data were meticulously recorded.

Statistical Processing: Statistical analysis was conducted to determine standard deviations (± SD) and assess the significance of differences between samples and conditions. Statistical software, "STATISTICA 12.0," was employed for this purpose.

Composite Planning Implementation:

Rationale: The concept of composite planning was introduced to rationalize the experimentation process. It involved designing efficient experimental setups to evaluate multiple factors and their interactions with minimal experiments.

Strategic Experiment Selection: Specific experiment points within the design space were strategically chosen to efficiently collect data and make informed decisions regarding recipe adjustments.

Optimization of Formulation:

Formulation Adjustments: The data collected from the experiments were used to optimize the processed cheese formulation by introducing correction points into the experimental plan. A central composite plan was utilized for this purpose.

Surface Response Method Application:

Methodology: The surface response method was employed to identify optimal conditions in the refined model.

Results and Discussion:

Prerequisites and Justifications for Raw Material Selection: Discussion on the selection of raw materials, focusing on the benefits of using goat's milk cheese.

Effect of Goat Milk Raw Material: Examine the impact of different types of raw goat milk material on processed cheese production.

Production of Functional Foods: Exploration of the development of processed cheeses with functional additives for improved nutritional value.

Amino Acid and Fatty Acid Composition Analysis: Discussion on analysing amino acid and fatty acid compositions in the experimental samples.

Statistical Analysis

In our study, we employed several statistical methods to analyze the data. The following statistical tests and methods were used:

Analysis of Variance (ANOVA): ANOVA was utilized to assess the differences among multiple groups, particularly in comparing color attributes among different cheese samples. For example, we conducted ANOVA to determine if there were significant differences in color attributes such as brightness (L*), redness (a*), and greenness (b*) among the various cheese samples.

T-Tests: We performed t-tests to compare specific pairs of cheese samples for parameters such as amino acid composition and fatty acid composition. For instance, we used t-tests to compare the amino acid composition of

the experimental sample with 1% Spirulina to the control sample without Spirulina, assessing whether there were statistically significant differences in individual amino acids.

Regression Analysis: Regression analysis was applied to explore the relationships between variables, such as the dose of melting salts and the added vegetable filler, and various characteristics of the processed cheese. We used regression to determine the optimal dosage of these ingredients for achieving desired properties.

Descriptive Statistics: We calculated means and standard deviations to summarize data, particularly when presenting amino and fatty acid composition. These statistics were used to describe the central tendencies and variability of the data.

Hypothesis Testing: Hypothesis testing was employed to assess the significance of observed differences, as indicated by p-values. For instance, we used hypothesis testing to determine whether differences in amino acid composition between samples were statistically significant.

It is important to note that all statistical analyses were performed with a significance level of $p \leq 0.05$, indicating statistical significance when p-values were below this threshold. The choice of statistical methods and parameters tested was determined based on our study's specific objectives and hypotheses.

In this study, "the idea of composite planning" refers to an approach to statistical and experimental design used to rationalise and optimise experiments. Composite planning involves the creation of efficient experimental designs that allow the evaluation of multiple factors and their interactions in a relatively small number of experiments. This approach is particularly useful when dealing with complex systems and many variables.

This study's use of compositional planning aimed to minimise the number of experiments required to optimise a processed cheese formulation. Strategic selection of experiment points in the design space allows researchers to collect data and make informed decisions about recipe adjustments efficiently. This approach saves resources and speeds up the optimisation process. The software package "STATISTICA 12.0" was used for various aspects of data analysis in this study. Although it includes a wide range of statistical functions and tools, the following functions were used: for statistical and regression analysis of the experimental data. This made it possible to evaluate the relationship between the different variables, identify significant factors and determine their influence on the processed cheese formulation; for visual presentation of the data, including profilograms or sensory profiles, which helped to interpret sensory scores; to assess the significance of differences between samples and conditions, various statistical tests such as ANOVA (analysis of variation) or t-tests were performed. Each experimental sample for a particular formulation was prepared 3 times, all 3 samples were examined, and the figures were entered into the planning matrix. The data were then processed, and the formulation was optimised by adding correction points to the experimental plan, using a central-composite plan. All data in the tables are summarised as the mean of 3 trials for each sample ± standard deviation.

RESULTS AND DISCUSSION

Prerequisites and justifications for raw material selection: There are very few works on the production of processed cheeses from goat's milk, because of the high cost of goat's milk cheeses; nevertheless, to produce hypoallergenic products with a higher nutritional value, it is a priority to use fewer products from cow's milk in the technology of processed cheeses. Compared to cow's milk, goat's milk has a higher concentration of short- and medium-chain fatty acids and lipoprotein lipase associated with the fat phase **[11]**. A study on the possibility of producing processed cheese based on mature cheeses from goat's milk **[12]** showed that cheeses with maturation periods of 10, 20 and 40 days are suitable for melting; the final moisture content of the cheese should be 63.0 ±1.0% (p = 0.0008), with a melting temperature of 85.0 ±0.1 °C, within 9.0 ±0.5 min. The cheese was made by analogy. The phosphate salt used in this paper is JOHA (S10 2.5 per cent and HBS 0.3 per cent); sodium phosphate, and polyphosphate salts. In our case, we used a mixture of tripolyphosphate and sodium citrate. Despite this, there is contradictory information about the melting of goat's milk cheese, so **[13]** concluded that the most meltable (e.g. for pizza) goat's milk cheeses should have a moisture content of 48%. Thus, the composition and the type of raw materials play a role in producing processed cheese. The effect of the kind of goat milk raw material for processed cheese production on α and β-casein values was also studied **[10]**, it was found that cheeses (50% of cheese with a maturation period of 10 days, 25% of cheese with a maturation period of 20 days and 25% of cheese with a maturation period of 40 days) had higher α- and β-casein values. This α- and β-casein content is due to the high content of intact casein in cheese with a short maturation time. A technology of production of processed cheese from sheep's milk was also proposed **[14]**, as a result, when compared to processed cheese from cow's milk in terms of whiteness, the best indicators were for the cheese from sheep's milk: fatty acid composition was better, the content of casein was also higher, processed cheese from sheep's milk was also better in all physical indicators.

The production of functional foods has received an impetus to development with the increasing interest of the world's population in healthy lifestyles and quality nutrition. This connection included products with phytofillers

(medicinal herbs, phytosterols, algae, etc.) Dry powder of white cabbage and coriander was used in brine cheese **[15]**, wild onion was used in the production of processed cheese **[1]**, rice husk was used in the production of yoghurt **[16]**, amaranth in goat cheese production **[17]**. Thus, we can conclude that using combined raw materials for processed cheeses for functional purposes is still poorly studied, as well as the shelf life of such cheeses and the impact on the composition of the finished product technology and types of raw materials used in the recipe. Developing technology of processed cheeses from combined dairy raw materials, such as cheese from cow's milk and cheese from goat's milk, can be an interesting and promising project. In the development of this technology, the following auspices have been taken into account, and work has been carried out:

Study of the properties of raw materials: Before development began, research was carried out on the properties of cow's and goat's milk cheeses. The fat, protein, lactose and other components of each type of cheese were determined. This helped to understand what changes to the cheese melting and combining process might be required.

Recipe selection: The optimum ratio of raw materials from cow and goat milk was determined to obtain the desired characteristics of processed cheese **[18]**.

Melting and emulsification: Melting is a key step in creating processed cheese. Optimum temperature and time conditions have been developed for melting a mixture of cow's and goat's milk cheeses. Emulsification also plays an important role in creating a homogeneous cheese texture.

Tests and analyses: After developing the technology, exploratory experiments were carried out using the PFE 33 plan to evaluate the quality, texture and flavour. After processing the data obtained, adjustments were made, and the recipe was optimised **[19]**.

Furthermore, the cheeses prepared according to the optimised formulation were subjected to laboratory tests: amino acid and fatty acid composition were determined. During the search experiments, organoleptic parameters were determined, and melting salts were selected. Figure 1 shows the characteristics obtained with Chromometer CR410 **[20]**, colour indices of the experimental sample, which was the most acceptable.

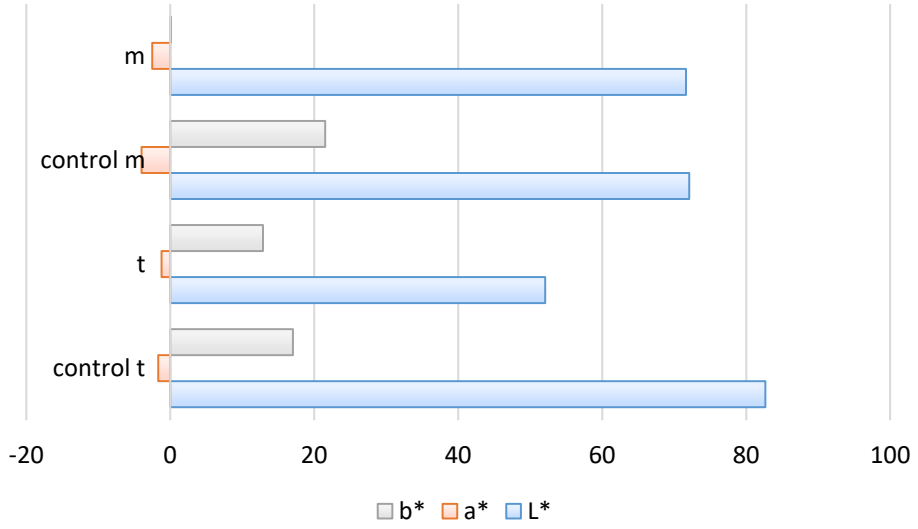

Figure 1 Colour indicators of the finished product.

According to the data presented in Figure 1, it can be seen that processed cheeses do not contain red colour and do not have dark colour (values on the scale a* with a minus sign); the lightest is processed cheese slice control cheese from cow's milk without the addition of vegetable ingredients, the next control m – is a cheese made according to the classical technology with a minimum expansion of vegetable filler, cheese M – is developed according to a new technology cheese prototype with a minimum (1%) addition of vegetable filler, cheese T – experimental sample with adding p, cheese T– experimental sample with adding vegetable filler. Experimental sample T, as seen from the figure, has a saturated green tint, and the colourimeter shows it as the least bright, corresponding to reality.

Figure 2 shows the results of experimental data processing on finding the optimum dose of melting salts and the dose of plant filler.

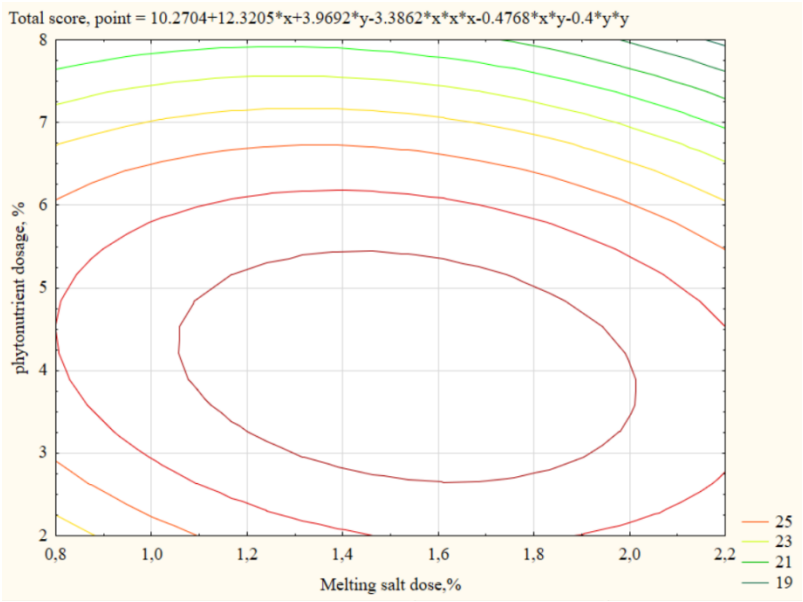

Figure 2 Results of the analysis on finding optimum doses of plant filler and melting salts application.

As can be seen from Figure 2, by adding goat cheese with a maturation period of 10 days and a vegetable filler to the formulation, it is possible to reduce the dose of melting salts to 1.5-2%, and the optimum is the dose of filler 4-5%, but since the analysis of colouring showed that the experimental sample M is close to the control sample, then judging from Figure 2 it is possible to choose the formulation with the addition of 1%, because the red zone is the zone of optimum. Among the various rheological characteristics, the most significant is the effective viscosity **[21]** Figure 3 shows the dependence of viscosity on the dose of melting salts and the added vegetable filler.

Thus, based on the analysis of colour and analysis of organoleptic parameters, it is rational to use melting salts 2%, and the dose of vegetable filler (Spirulina Maxima) – 1%.

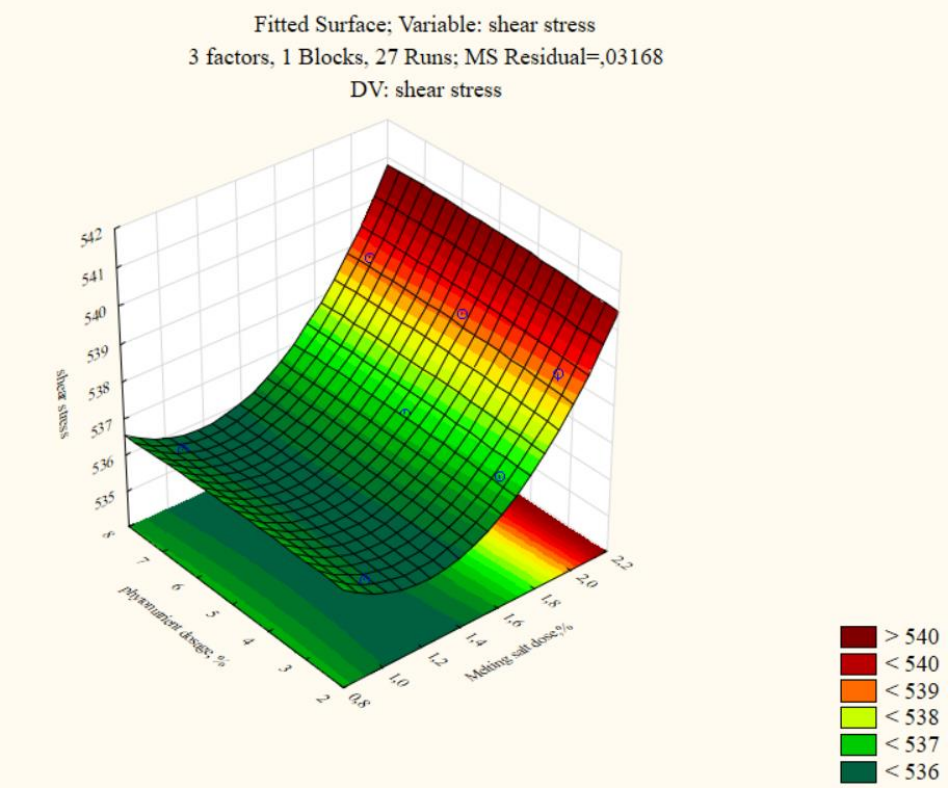

Figure 3 Variation of shear stress values as a function of melter salt dose and vegetable raw material dose.

Amino acid analysis composition: Animal products are undoubtedly one of the human diet's most concentrated sources of essential amino acids (AAs). However, their high price and the diseases associated with their excessive consumption have prompted the consumption of other alternative sources of proteins of animal origin, such as those from marine or aquatic species **[22]**. The amino acid composition of processed cheeses may vary depending on the formulation and composition of the original dairy raw material. However, processed cheeses generally contain various amino acids, the basic building blocks of protein. The amino acid profile of each cheese can vary depending on the type of milk (cow, goat, sheep, etc.), the lactation stage of the animal, milk processing methods, and even added ingredients and additives. The value of amino acid composition is discussed in many articles; for example, **[23]** states that the best indicator of protein quality is calculating the amino acid ratio from the limiting essential amino acid. Also, in 1991, on the initiative of FAO/WHO, the PDCAAS method of protein quality and digestibility was introduced **[24]**. The Protein Digestibility Adjusted Amino Acid Index (PDCAAS) is determined by comparing the amino acid profile of the food product in question with the standard amino acid profile, where 100 is the maximum possible score. This source describes the disadvantages of this method and suggests modification and refinement of the calculation. It is also suggested to study protein quality using the Amino Acid Score Difference Coefficient (ASDC) **[25]**.

"Ideal protein" is a protein with an amino acid composition perfectly balanced for the growth and development of a living organism. The closest to the ideal protein according to the amino acid profile is the base protein of the embryo: egg, or caviar. The main limiting factor is the amino acid lysine. Tables 2 and 3 show the proportions of amino acids in 1 g of "ideal" protein for the body –The perfect protein. Comparative analysis of amino acids in an ideal protein with amino acid data in control and experimental processed cheeses proved that in experimental cheeses, there are more essential amino acids than in control, and therefore a greater index of essential amino acids. The data suggest a high biological value of the developed processed cheeses using goat's milk in the formulation.

Tables 2 and 3 show the analysis of the amino acid composition of the experimental samples according to the recipe "M" processed cheese compared to the control sample from cow's milk and the control sample according to the developed technology without adding vegetable filler. To determine the quality of the experimental protein, we used the method of calculation of KRAS **[26]** and utilitarian coefficient **[27]**, the method of calculation of rationality coefficient **[28]**.

Table 2 Analysis of the amino acid composition of the experimental sample

Name of Essential Amino Acid	The perfect protein	Experimental Protein	Score
	mg/100g	mg/100g	%
isoleucine	40	40 ±0.25	100
leucine	70	140 ±0.31	200
lysine	55	180 ±0.64	327.27
methionine and cysteine	35	71 ±0.39	202.85
phenylalanine and tyrosine	60	120 ±0,8	200
tryptophan	10	10.2 ±0.52	102
threonine	40	98 ±0.25	245
valine	50	140 ±0.35	280
Index of essential amino acids	360	799.2 ±0.87	1.92
rationality coefficient			22.64 ±0.85
utilisation factor			61.12 ±1.01
Amino acid scoring difference coefficient			-54.9 ±1.93
PDCAAS			96.9 ±1.1

Note: Results are expressed as mean ± standard deviation; mean values with different top indices in a row differ significantly ($p \leq 0.05$).

A comparison of the two tables shows that the protein amount of the experimental sample is maximally near to ideal, and the utilitarian coefficient is higher than that of the control sample, which speaks in favour of the filler. The amino acid index with correction on digestibility is higher in the experimental sample. It is equal to 96.9 at 39.9 at processed cheese from combined dairy raw materials without adding vegetable filler, and 18.75 in processed cheese from cow's milk. Also, the coefficient of utilitarianism 61.1 against 13.72 of the control sample shows good protein digestibility of the experimental sample.

Table 3 Analysis of the amino acid composition of control sample m without the addition of a vegetable filler.

Name of Essential Amino Acid	The perfect protein	Experimental Protein	Score
	mg/100g	mg/100g	%
isoleucine	40	40 ±0.91	100
leucine	70	110 ±0.31	157.14
lysine	55	160 ±0.52	290.91
methionine and cysteine	35	64 ±0.31	182.85
phenylalanine and tyrosine	60	119.3 ±0.25	198.83
tryptophan	10	4.2 ±0.8	42
threonine	40	81 ±0.97	202.5
valine	50	130 ±1.00	260
Index of essential amino acids	360	708.5 ±1.97	1.569143 ±1.85
rationality coefficient			8.265833 ±0.98
utilisation factor			13.72 ±0.19
Amino acid scoring difference coefficient			-43.56 ±1.78
PDCAAS			39.9 ±1.08

Note: Results are expressed as mean ± standard deviation; mean values with different top indices in a row differ significantly ($p \leq 0.05$).

Study fatty acid composition: The study of the fatty acid composition of processed cheese is an important task to determine its nutritional value, quality and compliance with regulatory requirements. The fatty acid composition of processed cheese is determined by the type and content of fats included in the cheese product. The atherogenicity and thrombogenicity indexes indicate a diet's atherogenic and thrombogenic potential better than the PUFA/UFA ratio. These indices consider the different effects that individual fatty acids may have on human health. The thrombogenicity index value indicates the propensity to form blood clots in blood vessels **[29]**.

The comparison of fatty acid composition is shown in Table 3.

Table 4 Fatty acid composition of processed cheeses.

Fatty acids	Content, g.100g^{-1}	
	Control Sample	Experimental Sample
Saturated fatty acids		
Butyric acid (C4:0)	1.70	3.50
Caproic acid (C6:0)	2.60	2.57
Caprylic acid (C8:0)	3.40	1.43
Capric acid (C10:0)	3.40	3.09
Lauric acid (C12:0)	3.20	3.46
Myristic acid (C14:0)	8.80	10.21
Palmitic acid (C16:0)	21.50	30.10
Margaric acid (C17:0)	0.15	0.61
Stearic acid (C18:0)	3.60	1.05
Arachidic acid (C20:0)	0.05	0.21
Behenic acid (C22:0)		0.09
Total	48.40	66.23
Monounsaturated fatty acids		
Palmitoleic acid (C16:1)	1.25	1.58
Oleic acid (C18:1)	11.6	25.57
Gadoleic acid (C20:1)	0.12	0.25
Docosenoic acid (C22:1)	0	0.11
Total	12.97	27.51
Polyunsaturated fatty acids		
Hexadecadienoic acid (C16:2)	0	0.02
Linoleic acid (C18:2)	0.08	2.05
Linolenic acid (C18:3)	0.25	0.93
Eicosatrienoic acid (C20:3)	0	0.11
Docosadienoic acid (C22:2)	0	0.13

As can be seen from Table 3, the fatty acid composition of the experimental sample differs in the number of polyunsaturated fatty acids 6 times higher than in the control sample, the number of monounsaturated acids in the control sample 1.3 times, the amount of saturated fatty acids in the experimental sample is lower than in the control 1.15 times Thrombogenicity index in the control sample is 3.6, while in the experimental sample 2.15.

The study proved the feasibility of adding Spirulina, a dry powder, to the formulation of processed cheese. Some of the cyanobacteria produce toxins: microcystins. Microcystins can cause gastrointestinal disorders and, in the long term, liver cancer **[30]**, which places greater demands on the choice of manufacturer of Spirulina supplements. These toxic compounds are not produced by Spirulina itself **[31]**, **[32]**, but may appear due to contamination of Spirulina batches with other toxin-producing blue-green algae species. Also in the formulation M balanced fatty acid composition, the thrombogenicity index is 2.15. In purchased processed cheeses, this index reaches 4.57 **[32]**.

CONCLUSION

Throughout this study, a processed cheese formula was meticulously developed and fine-tuned, incorporating Spirulina Maxima at a precise dosage of 1%. Notably, this formula exhibits commendable attributes, particularly regarding color parameters and sensory qualities, rendering it highly promising for subsequent production phases. The introduction of Spirulina Maxima into the processed cheese formulation substantially enhanced its nutritional profile. The processed cheese variant fortified with 1% Spirulina showcased a nearly ideal protein composition, underscored by a remarkable protein digestibility rate of 96.9%. The Spirulina Maxima-enriched formulation demonstrated a superior fatty acid composition to the control sample. Significantly augmented levels of polyunsaturated fatty acids and reduced levels of saturated fatty acids were observed. This transformation culminated in lowered atherogenicity and thrombogenicity indices, hinting at potential health advantages. The production process for these processed cheeses was streamlined through judicious adjustments, including reducing melting salt dosage and incorporating goat's milk cheese. These refinements yielded not only improved chemical characteristics but also a reduction in production costs. The findings from this study illuminate a promising pathway for integrating Spirulina Maxima into functional food production. However, it is prudent to acknowledge that further research may be necessary to commercialise this product. Aspects requiring scrutiny encompass product stability, technological intricacies, and consumer assessments. In summation, this study serves as a compelling testament to the potential utility of Spirulina Maxima in the development of processed cheeses endowed with elevated nutritional profiles and conceivable health benefits. These outcomes, therefore, lay a robust foundation for the progression of subsequent research and the evolution of functional product manufacturing.

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Effects of pesticides on bee populations and safety of bee honey in Ukraine

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ABSTRACT

To prevent pest contamination of crops, they are treated with plant defense agents, the action of which is aimed at the destruction or development and reproduction control of hazardous organisms. But also these chemical agents cause pollution of environmental ecosystems. Furthermore, the use of pesticides on honey bees often leads to mass mortality of the bees and contamination of nectar and pollen. Honey, made by the bees of such nectar, may contain pesticide residues that are toxic to a bee brood and harm the viability and productivity of bee colonies. One hundred seventy-two samples of bee honey and 40 samples of dead bees were studied from different regions of Ukraine. Eight hundred thirty-seven bee colonies died from pesticide poisoning of the honey bees in 2021. The bees most died due to thiamethoxam (523 bee colonies), clothianidin 400 (bee colonies), and lambda-cyhalothrin (342 bee colonies). In 2022, the poisoning of the honey bees, from which 1,130 bee colonies died, was caused by seven insecticides. Lambda-cyhalothrin (653 bee colonies), thiamethoxam (352 bee colonies), imidacloprid (342 bee colonies), clothianidin (325 bee colonies), and acetamiprid (320 bee colonies) were most frequently detected. 11 insecticides, 11 fungicides and 2 each of acaricides and herbicides were found in the honey. There were 425 detection cases of insecticides, 285 fungicides, 8 acaricides, and 3 herbicides. In 2021-2022, 16 insecticides of the 3rd toxicity class were found in the dead bees.

Keywords: pesticide, honey bees, bee honey, safety, food product

INTRODUCTION

The increasing demand for food products stimulates the widespread use of pesticides when growing crops. Crops are treated with plant defense agents for the destruction of the development and reproduction control of hazardous organisms. But also these chemical agents cause pollution of environmental ecosystems. Furthermore, the use of pesticides on honey bees often leads to mass mortality of the bees and contamination of nectar and pollen **[1]**, **[2]**.

Honey bees, as the main pollinators of crops, play an important role in supporting an ecological balance and are considered the most important nontarget organisms exposed to pesticides' toxic effects **[3]**, **[4]**.

It should be noted that the bees are exposed to the effects of various environmental factors, but pesticide poisoning is one of the main decline reasons for the honey bee population **[5]**, **[6]**. In the future, beekeeping may be threatened by a considerable loss of the honey bee colonies since it may be difficult to restore apiaries after the loss of a large part of the bee colonies **[7]**, **[8]**.

In most countries, there are strict rules for the pesticides to be used in agricultural production, and the poisoning cases of honey bees are controlled by the applicable legislation **[9]**, **[10]**. However, bees' poisoning facts are recorded annually, which requires scientific research and the development of preventive measures for their pesticide poisoning **[11]**.

The poisoning facts of the bees with the pesticides, which were registered in different years, are known in Great Britain **[12]**, **[13]**. Furthermore, there is data on the poisoning of the bees during corn sowing in Germany because seeds were treated with plant defense agents **[14].** There are confirmed cases of pesticide poisoning of bees in Canada **[15]**. Therefore, the toxic effect risk of the pesticides on the bees should be always taken into account during their application **[16]**.

Suppose insecticides can cause the acute poisoning of the bees. In that case, it is necessary to point out that their effect was sometimes enhanced with the simultaneous combination of several active substances. Furthermore, pesticides are also found in environmental mixtures, so predicting their synergistic effects on the bees is difficult. The poisoning of the bees by highly toxic substances such as chlorpyrifos, deltamethrin, cypermethrin, and imidacloprid, as well as low-toxic ones such as prochloraz and thiacloprid, have been confirmed by studies **[17]**, **[18]**.

Some pesticides that are used to treat the crops against the pests mustn't kill any bees but make them vulnerable to mites and adverse environmental factors. Entomologists have also proven that the bees' memory and mental abilities deteriorate after being subjected to pesticides. Some pesticides cause epilepsy in the bees. The combined pesticides are the most dangerous for insects. It appeared those 4 days after the first contact with the pesticides, about 30% of the bees lost their ability to learn and began to undergo the remembering tests for flower smell. Previous studies have shown that glyphosate can affect the ability of the bees to learn and navigate in space **[19]**.

Honey made by the bees of the nectar of the plants, which are treated with pesticides, may contain toxic residues to the bee brood and harm the viability and productivity of the bee colonies. It should be noted that, according to the data of some scientists, honey can be a biological indicator of the use of pesticides on crops and their pollution of the environment **[20]**, **[21]**.

However, despite hundreds of approved pesticides being applied to the agricultural fields each year, only a small proportion of these organic compounds have been found in the honey and beeswax samples. This observation questioned the general suitability of bee products as an indicator for synthetic organic pesticides used when growing field crops **[22]**.

It was in studies revealed that the amount, frequency, and concentration of the pesticides in the bee honey were higher in the samples, which were collected from hives located in areas of intensive and high-tech agriculture. Insecticides that are the most dangerous for bees – neonicotinoids, organophosphorus compounds, herbicides, and fungicides were most often found in high concentrations **[23]**.

The pesticides of the neonicotinoid group require special attention due to their application almost worldwide. There is also growing concern about their negative effects as evidence accumulates of their impact on bee health and resistance. Scientists conducted tests on the distribution of these analytes in honey in many countries. As a result, their remains were found in most parts of the tested samples. Even though neonicotinoids were contained at levels considered safe for human consumption, a significant distribution of these pesticides in the bee habitat was established **[24]**. So, for example, during 2015-2017, honey studies in Poland revealed the remains of 21 pesticides. Acetamiprid and thiacloprid, quantified in 77 % of the samples, were most frequently detected **[25]**.

Assessing the risks to human health, Israeli scientists found that at least two pesticides were present in the samples of the examined bee products. Neonicotinoids and 2,4-dichlorophenoxyacetic acid were found in the honey samples, and more lipophilic pesticides were predominantly found in the beeswax **[26]**.

The pesticide distribution in the hive is a rather complex process mainly due to the interaction and food transfer between the colony members. That is why the presence of certain pesticides, as well as their concentration, are substantially different between the nectar, pollen, and other beekeeping products **[27]**.

The pesticide residues were also studied in the winter honey. Only eight residues were found: coumaphos, fluvalinate, boscalid, dimethoate, atrazine, bentazone, dichlorobenzene, and thymol. The honey from brood combs most often contained pesticide residues **[28]**, **[29]**.

As far as is known, bee honey belongs to ready-made food products and does not need to be cooked. That is why the toxicants in it enter the human body without impediment. This, in turn, reduces its nutritional and medicinal value **[30]**.

Indirect ecological and economic losses, as a result of the use of pesticides, are related to the pollution of underground and surface water; destruction of beneficial microorganisms, insects, natural predators, and wild birds, poisoning of animals, contamination of products, and impact on human health. Furthermore, the pesticides combined with xenobiotics lead to a permanent global population decline of the honey bees-pollinators, and loss of crops and plant products, which well may trigger a food security crisis. In addition, an account must be taken of the costs of public funds for controlling the pesticide circulation in the environment and food products. Thus, it may be concluded that if the total environmental, social, and economic costs for the pesticides to be used could be measured as a whole, the profitability of the pesticides to be used would be substantially lower **[31]**.

The studies to improve the diagnostics methods for bee poisoning are still ongoing despite significant analytics progress in the last few years. It is quite a complicated task since the determination of the pesticide residuals (often equal to sublethal doses) and the simultaneous presence of a wide compound range with various physicochemical properties in such a complex matrix as the honey and the body of the honey bee, is a serious problem for modern laboratory practice and requires the use of the highly sensitive and selective methods. In that context, new sample preparation approaches are also becoming topical **[32]**, **[33]**.

QuEChERS is a universal sample preparation method characterized by specificity, selectivity, accuracy, sensitivity, low cost, and adequate speed. It is suitable for determining the pesticide content in less-understood beekeeping matrices such as royal jelly and propolis **[34]**, **[35]**.

With due regard to these scientific facts, as well as the fact that honey is a widely-used product, monitoring its safety, among other controlling the pesticide content in this product, is required to be continued. This, in turn, aims to ensure consumer safety and determine the pesticide exposure risks to the health of the pollinators, other nontarget organisms, the ecosystem, and their potential consequences for human health.

Scientific Hypothesis

The number of plant protection products used in Ukrainian agriculture is increasing. The conducted research, including the diagnosis of bee poisoning, is aimed at obtaining data on the list of pesticides and their residual content in honey to substantiate the need for their further monitoring and control therefore The impact of pesticides on the bee population has a significant impact on the safety of honey in Ukraine, as a result of changes in the level of production and changes in the quality of products, which can have various ecosystem consequences for the plant world, since bees are important pollinators of plants. The results of the conducted studies justify how the use of pesticides can affect the level of honey production due to the decline of bee populations and the quality of honey due to pesticide contamination.

MATERIAL AND METHODOLOGY

Samples

In 2021, during the honey flow period, the bee honey samples were taken from the private apiaries in the amount of 156 samples from 21 regions of Ukraine (Table 1). Furthermore, during 2021 and 2022 the samples of the dead bees and honey in the combs were obtained to conduct diagnostic studies for determining the pesticide poisoning of the bee. During the study period, 172 samples of bee honey and 40 samples of dead bees were taken from different regions of Ukraine.

Today, beekeeping is a developed industry in Ukraine. It ranks first in honey production and export in Europe **[37]**.

Most samples were taken from the Central regions of Ukraine, i.e. Vinnytsia, Poltava, Kirovohrad, Cherkasy, and Dnipropetrovsk regions, where a significant number of the apiaries are located, which provide a larger volume of the produced honey in comparison with other regions. Thus, 17,070 tons of honey were obtained in this region according to statistical data for 2021 **[36]**.

The beekeepers from 23 districts participated in the sampling. In the Vinnytsia region, the samples were taken from 8 districts, in Dnipropetrovsk – 6, Poltava – 5, Kirovohrad – 3, and Cherkasy – 1.

For the tests, 16 samples were used – from the Vinnytsia region, 13 each from the Kirovohrad and Poltava regions, 9 from the Dnipropetrovsk region, and 9 from the Cherkasy region. Their percentage ratio to the total number of honey samples was 10.3; 8.3, 8.3; 5.8, and 5.1%.

The Northern part of Ukraine is represented by Zhytomyr, Kyiv, Chernihiv, Sumy, Volyn and Rivne regions. 14,614 tons of honey, which is 2,456 tons less than in the central part, were obtained in this region in 2021 **[37]**.

The samples were taken from the apiaries in 16 districts of the following regions: Zhytomyr– 1 district, Kyiv – 4, Chernihiv – 2, Sumy – 6, Volyn – 2, Rivne – 1.

From the Zhytomyr region, 3.8 % of the total number of honey samples were taken for analysis. From Kyiv – 6.4%, Chernihiv – 1.9%, Sumy – 5.1%, Volyn – 2.6%, Rivne – 3.2%. The total number of the taken samples by region was 6, 10, 3, 8, 4, and 5 samples, respectively.

A significant number of the apiaries of Odesa, Mykolaiv, Kherson, and Zaporizhzhia regions, which produced 14,106 tons of bee honey in 2021, which is 508 tons less than in the Northern part of the country, operate in the Southern part of Ukraine **[38]**.

Table 1 Number of taken bee honey samples by regions of Ukraine.

Region	District	Number of samples	Number of taken samples by region	Percentage of the total amount, %
Volyn	Volodymyr-Volynskyi	1	4	2.6
	Manevytskyi	3		
Kirovohrad	Novomyrhorodskyi	2	13	8.3
	Kropyvnytskyi	7		
	Novoukrainskyi	4		
Vinnytsia	Murovanokurylovetskyi	2	16	10.3
	Kalynivskyi	1		
	Orativskyi	1		
	Barskyi	1		
	Tyvrivskyi	2		
	Vinnytskyi	1		
	Apostolivskyi	2		
	Yuryivskyi	2		
Dnipropetrovsk	Kryvorizkyi	1	9	5.8
	Dnipropetrovskyi	2		
	Tomakivskyi	2		
	Zhytomyrskyi	6		
Zhytomyr	Mukachivskyi	1	6	3.8
	Khustskyi	1		
Zakarpattia	Uzhhorodskyi	1	3	1.9
	Kamyansko-Dniprovskyi	3		
Zaporizhzhia	Obukhivskyi	1	3	1.9
	Bilotserkivskyi	4		
Kyiv	Bohuslavskyi	3	10	6.4
	Brovarskyi	2		
	Khmelnytskyi	3		
	Kamianets-Podilskyi	3		
Khmelnytskyi	Shepetivskyi	3	9	5.8
	Mykolaivskyi	6		
	Yelanetskyi	2		
Mykolaiv	Bereznehuvatskyi	1	10	6.4
	Voznesenskyi	1		
	Podilskyi	2		
	Lymanskyi	1		
Odesa	Mykolaivskyi	1	6	3.8
	Odeskyi	2		
	Hadyatskyi	1		
	Poltavskyi	6		
Poltava	Myrhorodskyi	1	13	8.3
	Kremenchutskyi	4		
	Lubenskyi	1		
	Hoshchanskyi	5		
Rivne	Konotopskyi	2	5	3.2
	Sumskyi	1		
Sumy	Romenskyi	1	8	5.1
	Okhtyrskyi	1		
	Trostyanetskyi	1		
	Shostkynskyi	2		

The honey from the beekeeping of 8 districts were included in the study for the pesticide residues, in particular, from 4 districts of Odesa and 4 districts of Mykolaiv region, 3 districts from Kherson region, and 1 district from Zaporizhzhia region.

6 samples of the honey, or 3.8 % of the total samples, were taken from the Odesa region, 10 samples (6.4%) – from Mykolaiv, and 3 samples each (1.9%) – from Kherson and Zaporizhzhia.

The western part of Ukraine is represented by the apiaries of Lviv, Zakarpattia, Chernivtsi, and Ternopil regions, mostly breeding honey bees. The total honey it produces is much lower than in other regions. An exception is the Khmelnytskyi region, where a fairly large amount of honey is produced – 5,437 tons out of 10,059 tons **[38]**, obtained in the region as a whole in 2021. Therefore, the largest honey samples were taken from the beekeeping of 3 districts of the Khmelnytskyi region in the amount of 9 samples, corresponding to 5.8%. Out of 5 districts of Lviv it is 7 (4.5%), 2 districts of Ternopil – 5 (3.2%), 3 districts of Zakarpattia – 3 (1.9%), and 1 district of Chernivtsi region – 1.3% (2 samples).

In 2021, 3,181 tons of honey were pumped out in the Kharkiv region in the Eastern part of Ukraine **[38]**. For the tests, 12 samples of the bee honey were received, the relative amount of which was 7.7%, from 5 districts of Kharkiv region.

Chemicals

All chemicals were of analytical grade and were purchased from Sigma-Aldrich, i.e. solvents - acetonitrile, methanol and deionized water with LC-MS grade (Chromasolv, 99.9%); reagents – ammonium formate, magnesium sulfate, sodium chloride, sodium citrate dihydrate, sodium hydrocitrate 1,5-hydrate with purity 99%, A.C.S. reagent; sorbents: a mixture of primary and secondary amines (PSA), cat. number 52738-U and octadecyl modified silica gel (C18), cat. number 97727-U.

Animals, Plants, and Biological Materials

Laboratory and farm animals were not used directly during the studies.

Instruments

Liquid triple-quadrupole tandem mass spectrometer (Waters Xevo TQ-XS, USA).

Gas triple quadrupole mass spectrometer (Thermo TSQ 9000, USA).

Laboratory Methods

The internal method, which was used for the studies, was developed with the use of the QUECHERS sample preparation approach and the methods of liquid mass spectrometry (UPLC-MS/MS) and gas mass spectrometry (GC-MS/MS) **[39]**.

Description of the Experiment

Sample preparation: The selected material was prepared for the study, and 10 g was taken from each sample.

Number of samples analyzed: 172 samples of bee honey and 40 samples of dead bees were taken during the study period.

Number of repeated analyses: All measurements were performed 3 times.

Number of experiment replications: The number of replicates of each experiment to determine one value was 5 times.

Design of the experiment: The pesticide residues were determined after their appropriate extraction from the sample with a solvent, purification of extracts with the use of dispersion solid-phase extraction, and identification of analytes by retention time and the ratio of the mass of corresponding ions to their charge, quantitative determination – by the external standard method in terms of the peak area according to the method **[39]**.

Statistical Analysis

Statistical analysis of the results of experimental studies was performed in five replicates using standard methods of research of organoleptic, physical, physicochemical, microbiological, and other indicators. The obtained results of experimental research are processed using modern analytical integrated systems Microsoft Excel 2016 and Statistica 13.3. Adequacy of decision-making was carried out according to the criteria of Fisher, Cochran, and Student.

RESULTS AND DISCUSSION

Most scientists focus on the determination of the pesticide residues in honey products, but much less attention has been paid to the pesticide contamination of the honey bees and their deaths.

Most scientists in their scientific works, focus on the determination of pesticide residues in honey products, but much less attention is paid to pesticide contamination of honey bees and their death. Several scientific works are devoted to similar experimental and theoretical studies, in particular:

- research of pesticide residues in sunflower honey **[40]**, **[41]**;
- research of pesticide residues in rapeseed honey **[42]**, **[43]**;
- study of pesticide residues in alfalfa honey **[44]**, **[45]**;
- research on pesticide residues in acacia honey **[46]**, **[47]**;
- research of pesticide residues in buckwheat honey **[48]**, **[49]**.

We investigated the distribution area of the pesticide poisoning of honey bees by the regions of Ukraine for 2021–2022 (Table 2).

Table 2 Distribution area of pesticide poisoning of honey bees.

Region	District	Number of poisoning cases	Detected active substances of the 1st toxicity class	Number of dead bee colonies
		2021		
Vinnytsia	Vinnytskyi	1	Permethrin	10
	Zhmerynskyi	3	Thiamethoxam	300
Khmelnytskyi	Khmelnytskyi	1	Lambda-Cyhalothrin	42
Ivano-Frankivsk	Ivano-Frankivskyi	2	Imidacloprid	59
Poltava	Poltavskyi	2	Thiamethoxam, clothianidin, lambda-Cyhalothrin	223
Rivne	Rivnenskyi	1	Imidacloprid	26
Sumy	Shostkynskiy	2	Clothianidin	100
Total:		16		760
		2022		
Kirovohrad	Novoukrainskyi	2	tau-fluvalinate*	20
Vinnytsia	Vinnytskyi	2	Clothianidin, lambda-Cyhalothrin	177
	Khmilnytskyi	1		18
Dnipropetrovsk	Dniprovskyi	1	lambda-Cyhalothrin	10
Kyiv	Obukhivskyi	1	Thiamethoxam, clothianidin	10
	Bilotserkivskyi	1	lambda-Cyhalothrin	15
Khmelnytskyi	Khmelnytskyi	2	alpha-Cypermethrin	33
Odesa	Berezivskyi	2	Acetamiprid*	320
	Odeskyi	1	lambda-Cyhalothrin	46
Poltava	Poltavskyi	6	Imidacloprid, lambda-Cyhalothrin, thiamethoxam	342
Rivne	Dubenskyi	2	Clothianidin	74
Ternopil	Ternopilskyi	1	lambda-Cyhalothrin, clothianidin	45
Cherkasy	Zolotoniskyi	2	Cypermethrin, chlorpyrifos	20
Total:		24		1130

Note: Acetamiprid and tau-fluvalinate belong to the third toxicity class for the bees.

Kiljanek et al. focused their studies on bee poisoning with pesticides. Thus, out of 70 samples of the bees, which were suspected of chemical toxicosis, 57 samples, containing the pesticides and their metabolites, were found **[50]**.

In 2021, cases of pesticide poisoning of the honey bees were found in 7 regions of Ukraine: Vinnytsia, Khmelnytskyi, Ivano-Frankivsk, Poltava, Rivne, Sumy, and Kharkiv. A total of 837 bee colonies died. The large majority of the poisoning was registered in the central regions, where the largest number of bee colonies in Ukraine is located. Thus, 310 bee colonies died in the Vinnytsia region, their share of the total number was 37.0%. A significant part of the bee colonies also died in the Poltava region – 223, which corresponds to 26.6% of the total number. The fewest cases of the poisoning were found in the Rivne region, namely 26 dead bee colonies, i.e. 3.1%. It is necessary to point out that this area belongs to those with a small number of bee colonies in Ukraine **[8]**, **[18]**.

The bees died due to insecticides such as lambda-cyhalothrin, thiamethoxam, imidacloprid, clothianidin, and permethrin (Table 2, Figure 1). They all belong to the 1st toxicity class for the bees. lambda-Cyhalothrin was used alone and in combination with other insecticides and led to the death of 342 bee colonies in our country's Western, Eastern, and Central parts. It is necessary to point out that the bee poisoning, caused by imidacloprid, led to the death of 31 bee colonies in the North and South West of Ukraine. Permethrin and thiamethoxam in the Central region killed 10 and 523 bee colonies, respectively. Clothianidin was found in the samples of the dead bees in the North, Center, and West of the country. The total number of bee colonies that died as a result of the toxic effect of clothianidin is quite high and reaches 400. However, it's worth mentioning that the obtained data may not fully reflect the real situation in Ukraine, since not all facts of the bee poisoning have been confirmed by studies **[3]**, **[4]**.

If the facts of the pesticide poisoning of the honey bees are analyzed for 2022, an increase of 8 cases in comparison with the previous year was found, that is, their number reached 24. As a result, 1,130 bee colonies died, which is 293 colonies more than in 2021. The number of dead bee colonies prevailed in the Odesa region and amounted to 366. Most cases of pesticide poisoning of the bees were observed in the Poltava region, i.e. the number of dead bee colonies was 342.

In 2022 the honey bees were poisoned with 7 insecticides of Ist toxicity class, that is, lambda-cyhalothrin, thiamethoxam, imidacloprid, clothianidin, alpha-cypermethrin, cypermethrin, chlorpyrifos (Table 2, Figure 2). Lambda-cyhalothrin, widely used as a plant defense agent throughout Ukraine in 2021 and 2022, was most often found. 653 bee colonies in 6 regions died due to this pesticide poisoning. The use of insecticides with the active substance - clothianidin, which led to the death of 325 bee colonies in Ukraine, is quite popular **[9]**, **[10]**.

Similar to the previous year, thiamethoxam was found in 2 samples of dead bees from the Central region of our country. It caused the death of 352 bee colonies. Similar in quantitative meaning was the bee death due to imidacloprid, which amounted to 342 bee colonies. The dead bees due to alpha-cypermethrin have been found in 2 regions of Ukraine, resulting in the death of 78 bee colonies. Cypermethrin, chlorpyrifos, acetamiprid, and tau-fluvalinate were the less common poisoning causes, where 1 case was recorded, respectively. Among the mentioned pesticides, acetamiprid and tau-fluvalinate belong to the 3rd toxicity class for bees.

Acetamiprid led to the death of 320 bee colonies. Even though it is considered to be slightly toxic, in synergy with other pesticides it can cause the heavy mortality of the bees, which is confirmed by the studies of other scientists **[39]**, **[40]**.

16 insecticides of the 3rd toxicity class were found in the studied samples of the dead bees for 2021-2022 (Table 3). These include: acetamiprid, cyproconazole, tebuconazole, azoxystrobin, permethrin, promethrin, carbendazim, prothioconazole, propiconazole, difenoconazole, epoxyconazole, pyraclostrobin, picoxystrobin, tau-fluvalinate, hexythiazox, pyridaben.

Analyzing the study analyses for 2021, it should be noted that most of the samples of the dead bee contained clothianidin residues. It was found in 11 poisoning cases out of 16, which is 68.75%.

The insecticide lambda-cyhalothrin was registered quite often in the bodies of the dead bees: 6 cases out of 16, or 37.5%. The same number of the studied samples of dead bees contained tebuconazole. Thiamethoxam was present in 25%, and imidacloprid, cyproconazole, azoxystrobin, and promethrin – in 18.75% of the death cases of the honey bees. Other analytes, which we identified, caused fewer bee poisonings.

In 2022, a similar trend was observed regarding the causes of the pesticide poisoning of honey bees. Most samples of the dead bees contained lambda-cyhalothrin – 14 out of 24 analyzed in total, which corresponds to 58.33%. Such analytes as clothianidin, tebuconazole, and azoxystrobin were found in 41.67% of the dead honey bees' studied samples, corresponding to 10 cases of toxicosis caused by these insecticides. Thiamethoxam and cyproconazole were present in 6 dead bee samples, corresponding to 25% of their total amount. Cypermethrin was found in 5 samples (20.83%), and imidacloprid in 4 samples (16.67%) of the dead bees. The rest of the pesticides, that we studied, were less frequently detected in the samples of the dead bees.

The pesticide content in the bodies of the dead bees for 2021-2022 is presented in Table 3.

Table 3 Pesticide content in bodies of dead bees (2021-2022).

List of detected pesticides	Number of conducted studies	Number of detected cases	Concentration, µg/kg	Toxicity class for bees
2021				
Clothianidin	16	11	1.6-4.0	1
lambda-Cyhalothrin		6	0.8-52.6	1
Tebuconazole		6	0.5-5.1	3
Thiamethoxam		4	1.0-28.3	1
Imidacloprid		3	194.2-1166.0	1
Cyproconazole		3	4.5-1463.8	3
Permethrin		3	0.9-2.8	3
Azoxystrobin		3	10.4-375.6	3
Acetamiprid		2	5.1-63.1	3
Thiacloprid		1	1.0	1
Permethrin		1	8074.6	3
2022				
lambda-Cyhalothrin	24	14	17.7-458.8	1
Clothianidin		10	1.8-34.8	1
Azoxystrobin		10	1.6-217.1	3
Tebuconazole		10	1.0-21.8	3
Thiamethoxam		6	1.7-270.3	1
Cyproconazole		6	0.38-110.8	3
Cypermethrin		5	7.0-234.2	1
Imidacloprid		4	2.2-9.0	1
Difenoconazole		3	1.4-4.7	3
Acetamiprid		2	11.9-97.4	3
Propiconazole		2	74.7-92.7	3
Epoxyconazole		2	43.2-56.3	3
alpha-Cypermethrin		2	28.6-35.0	1
tau-Fluvalinate		2	776.1-10328.8	3
Chlorpyrifos		1	19.7	1
Carbendazim		1	67.1	3
Prothioconazole		1	102.9	3
Pyraclostrobin		1	10.2	3
Picoxystrobin		1	0.48	3
Hexythiazox		1	1.36	3
Pyridaben		1	8.03	3

The study results of the bee honey samples, and the pesticide residues found in them are shown in Table 4. A total of 172 honey samples were studied, 156 of which were taken from the apiaries in different regions of Ukraine to detect the pesticide residues, and 16 honey samples that were taken from the combs were obtained together with the dead bees to establish the fact of the pesticide poisoning of the bees. As a result of the tests, 11 insecticides, 11 fungicides, and 2 acaricides and herbicides were found. There were 425 detection cases of insecticides, 285 fungicides, 8 acaricides, and 3 herbicides **[19]**, **[30]**.

The combined use of the different classes of pesticides (insecticides, herbicides, fungicides) causes deep concern among scientists worldwide **[15]**.

As we can see from the test results of the dead bees and bee honey, insecticides of the neonicotinoid group were quite often detected. Such data were also obtained by scientists from other countries **[22]**, **[23]**, **[24]**.

Thus, the analysis of the pesticides in the bee honey, obtained in the Western regions of Mexico, showed the presence of 14 pesticides in different concentrations in 63% of the studied samples. The pesticides most frequently

found in higher concentrations were insecticides (neonicotinoids, then organophosphates), herbicides, and fungicides.

Table 4 Pesticide residues in bee honey.

Name of the active substance	Production classification	Number of detected cases	Concentration, µg/kg	Method detection limit (LOD), µg/kg
Clothianidin	Insecticides	55	0.1-12.4	0.1
Imidacloprid	Insecticides	74	0.1-43.7	0.1
Thiacloprid	Insecticides	89	0.2-190.8	0.1
Acetamiprid	Insecticides	96	0.1-510.6	0.1
Thiamethoxam	Insecticides	38	0.3-9.5	0.1
Chlorpyrifos	Insecticides	12	0.2-1.1	0.1
Dimethoate	Insecticides	22	0.1-4.5	0.1
Cypermethrin	Insecticides	4	0.8-2.2	0.1
alpha-Cypermethrin	Insecticides	5	0.6-3.4	0.1
lambda-Cyhalothrin	Insecticides	28	0.4-42.1	0.1
Tau-fluvalinate	Insecticides	2	29.2-36.9	0.1
Carbendazim	Fungicides	12	1.5-29.3	0.1
Flutriafol	Fungicides	76	0.2-4.8	0.1
Cyproconazole	Fungicides	81	0.1-112.2	0.1
Tebuconazole	Fungicides	63	0.5-2.9	0.1
Prothioconazole	Fungicides	1	102.9	0.1
Propiconazole	Fungicides	6	0.5-3.8	0.1
Difenoconazole	Fungicides	5	0.1-0.8	0.1
Epoxyconazole	Fungicides	9	0.3-1.4	0.1
Azoxystrobin	Fungicides	16	0.2-298.5	0.05
Pyraclostrobin	Fungicides	8	1.8-9.2	0.05
Picoxystrobin	Fungicides	8	2.6-16.4	0.05
Hexythiazox	Acaricides	4	1.0-3.8	0.1
Pyridaben	Acaricides	4	0.8-1.6	0.1
Promethrin	Herbicides	1	3.7	0.1
Metribuzin	Herbicides	2	2.5-6.4	0.1

These study results underline the need for continued monitoring of the pollutant substances in this product to determine the risks of pesticide exposure to the health of the pollinator, particularly the honey bees, ecosystems, and their potential consequences for human health and other nontarget organisms **[21]**, **[31]**.

Table 5 Pesticide contamination depending on species composition of bee honey.

Type of honey	Number of samples	% of total amount
poly floral	132	76.7
sunflower	15	8.7
rapeseed	12	7.0
buckwheat	7	4.1
acacia	3	1.7
honeydew	2	1.2
white	1	0.6

Among the studied honey samples in which the pesticide residues were found, the highest percentage was the bee poly floral honey – 76.7%, sunflower, and rapeseed honey – 8.7 and 7.0%, respectively. The smallest honey samples contaminated with pesticides were found in buckwheat honey – 4.1%, acacia – 1.7%, honeydew honey – 1.2%, and white honey – 0.6% (Table 5). This regularity is related to the flowering seasonality of these honey plants and the treatment of cultivated honey plants with the plant defense agents, compared with wild-growing plants. This is especially observed in the case of monofloral types of honey, such as acacia and white, which could be contaminated with pesticides in protective forest strips near the crops of the cultivated plants.

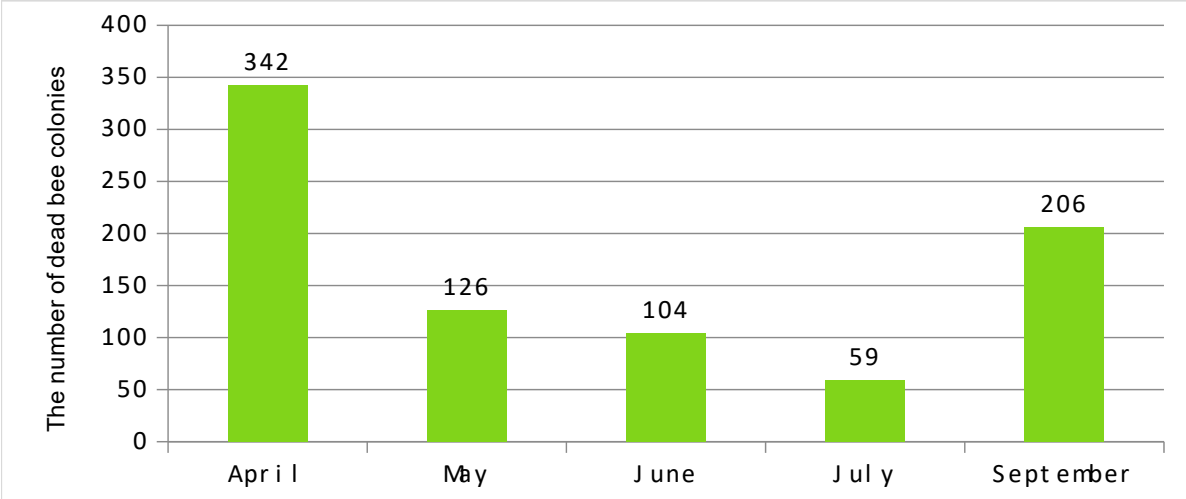

Figure 1 Number of dead bee colonies for 2021 by months.

If we consider the bee death to be seasonality, the largest number died in April and September 2021. From May to June 2021, the death of the bee colonies due to pesticide poisoning was approximately at the same level, while the lowest percentage of bee death was observed in July (Figure 1).

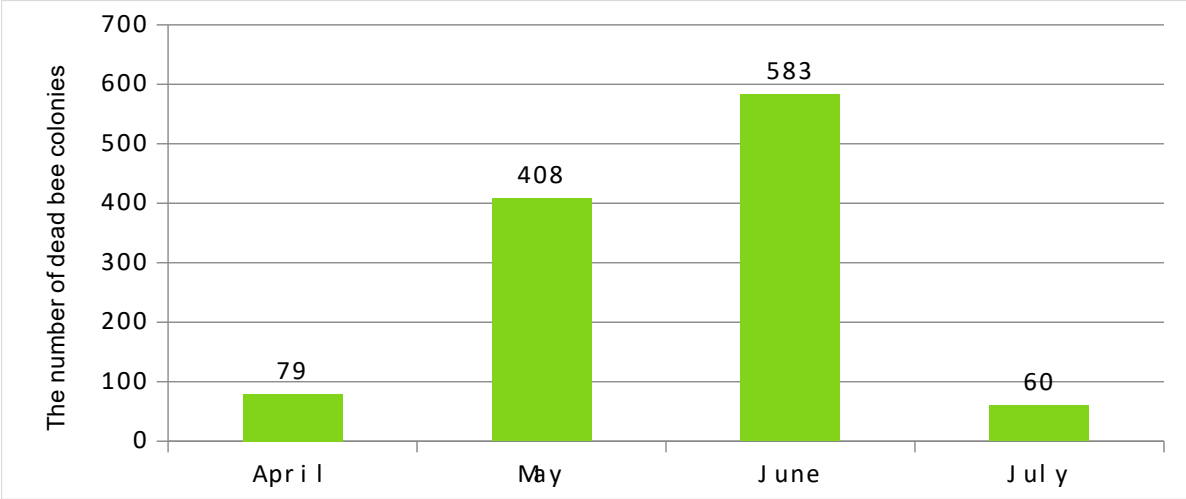

Figure 2 Number of dead bee colonies for 2022 by months.

In 2022, the highest mortality of the bee colonies was observed from May to June (Figure 2), while it was minimal in April and July, and in September, no bee samples with suspected pesticide poisoning were received in the laboratory. It relates to the weather conditions, which in the spring of 2022 were characterized by a large precipitation amount, and the adverse weather conditions in the fall, which did not contribute to the bee flight.

CONCLUSION

The use of pesticides for crops to be treated causes the poisoning of the bees during the period of the honey flow. It has been established that the use of pesticides on the territory of Ukraine causes significant death of the bee colonies and contamination of the honey. In 2021 and 2022, 837 and 1130 bee colonies died due to pesticide poisoning, respectively. The largest number of dead bee colonies in Ukraine was caused by 5 pesticides such as thiamethoxam, clothianidin, lambda-cyhalothrin, imidacloprid, and acetamiprid. The honey, which was made by the bees, contained 11 insecticides, 11 fungicides, and 2 acaricides and herbicides. It was proven that insecticides, fungicides, acaricides, and herbicides accumulated in the honey, and 16 insecticides of the 3rd toxicity class were found in the dead bees. The pesticide accumulation in bee honey depends on its species' origin and is related to the seasonal flight activity of the bees. With due regard to the wide use of plant defense agents in agricultural production, the safety risks for food products, including bee honey, are constant. Taking into consideration the fact that the bees are important subjects for the ecological balance, for the use and registration of agricultural chemicals in any country it is necessary to carry out the normative evaluation of the danger to these insects, as well as to develop the effective preventive measures for their poisoning of the bees.

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The use of protein-carbohydrate composition of okara, chickpea flour and whey protein in the technology of minced meat cutlets

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ABSTRACT

Kazakhstan's market for producing minced meat semi-finished products is not sufficiently developed. At the same time, the demand for products of the “economy” segment is growing. Providing balanced recipes for semi-finished meat products, with a rational combination of raw materials of animal and vegetable origin, is a significant problem. Chopped meat cutlets with high nutritional and low energy value have been developed, which are not inferior in functional and technological properties and sensory characteristics to traditional products. Pork and wheat bread were excluded from the recipes, with a replacement for lamb or broiler chicken meat in combination with a protein-carbohydrate composition (PCC) of the composition: soy minced okara – chickpea flour – whey protein concentrate (WPC 80) in a ratio of 9:5:10, at 1:3 hydration. The rational share of the introduction of PCC into the recipe of cutlets was 25% for minced beef – the meat of broiler chickens and 20% for minced beef – lamb. The studied samples of PCC, control minced meat with pork and bread, and two modified minced meat recipes for cutlets have similar values of the mass ratio of water fractions at three stages of dehydration during heat treatment. PCC particles are evenly distributed between the muscle fibers in minced meat. It has been shown that the developed PCC can serve as a substitute for minced meat not only in terms of the balance of the amino acid composition of the total protein but also in terms of the percentage of moisture with different forms of communication with the product, influencing the microstructure and consistency of raw semi-finished products of the combined composition, the consistency and juiciness of fried cutlets. According to the developed recipes, the mass fraction of protein in cutlets increased from 13.8 to 19.1-19.8%; fat decreased from 12.6 to 9.5-9.7%.

Keywords: semi-finished meat product, functional and technological properties, beef, nutritional value

INTRODUCTION

Kazakhstan's market to produce minced meat semi-finished products needs to be sufficiently developed, although at present, in the Republic of Kazakhstan, it is possible to note a significant increase in the volume of consumption of frozen and chilled semi-finished products. First, this is due to the employment of the population and the accelerated dynamics of modern life. At the same time, the demand for "economy" segment products is growing to a greater extent **[1]**.

Providing balanced recipes for semi-finished meat products, with a rational combination of raw materials of animal and vegetable origin, is an important problem. The reasons for the inclusion of vegetable raw materials in the recipes of meat products include WHO recommendations on the advisability of reducing the share of red meat and products of its processing as a risk factor for oncological diseases of the digestive system and a decrease in immunity in the context of the COVID-19 pandemic **[2]**, **[3]**, positive the influence of plant materials in combination with animals on metabolic processes in the human body **[4]**, a rich set of nutrients and biologically

active compounds in the composition of various types of plant materials: chia and quinoa seeds **[5]**, bioactivated seeds of legumes – chickpeas, lupins, mung beans **[2]**, lupine flour, amaranth cake **[4]**, cedar cake **[6]**, pumpkin seeds **[7]**.

A promising type of secondary raw material for the complex enrichment of meat products with dietary fibres, macro- and microelements, vitamins, and isoflavones that act as natural antioxidants is soy okara, especially against the background of the amino acid profile of soy protein, which contains all the essential amino acids **[8]**. When choosing components for the development of a protein-carbohydrate composition, we considered data on the amino acid composition of chickpea flour **[9]**, as well as the possibility of enriching products and diets using whey processing products, in particular, in the form of whey protein concentrate WPC-80 **[10]**.

We have developed a protein-carbohydrate composition of the composition: minced soy okara – chickpea flour – whey protein concentrate (WPC 80) in a ratio of 9:5:10 for use in the production of chopped semi-finished meat products in the form of a hydrated mixture **[11]**. The specific index of amino-acid content balance (IACB) was used to optimise the PCC composition. IACB was designated (U_A) and was calculated using the formula **[12]**:

$$U_A = \sqrt[n]{\prod_{j=1}^{n}\left(\frac{A_j}{A_{ej}}\right)}$$

Where:

A_j is the weight ratio of the j^{th} *amino-acid* in the product, mg%; A_{ej} is the weight ratio of the j^{th} *amino-acid* equal to the required daily intake of this amino-acid, mg%; n is the number of essential amino acids in the product.

According to the calculated data, PCC and various types of meat and vegetable raw materials according to ISAS can be arranged in the following descending row: WPC- 80 >PCC >minced beef >minced lamb >minced chicken> chickpea flour> okara (Figure 1).

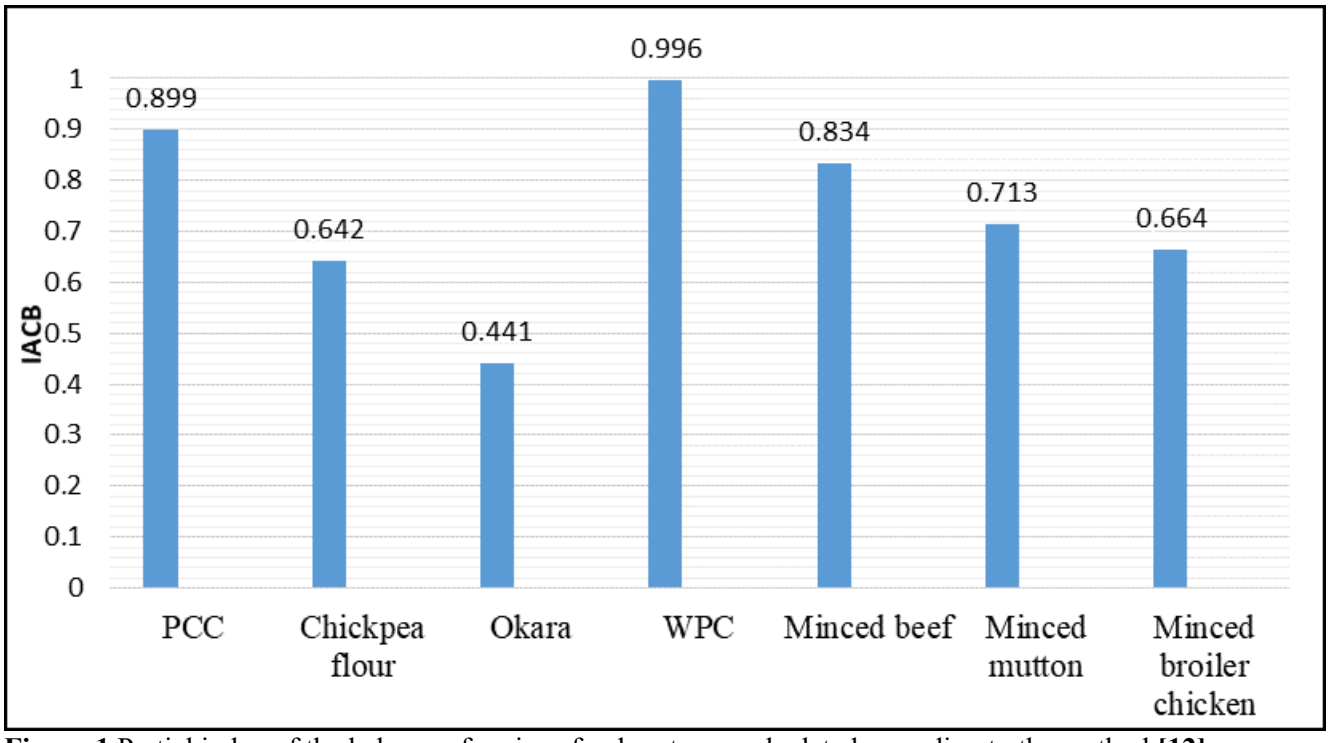


Figure 1 Partial index of the balance of various food systems, calculated according to the method **[12]**.

However, for the technology of semi-finished meat products, important: criteria for the applicability of PCC as prescription components is the ability to bind and retain moisture and fat during heat treatment, both alone and in combination with meat components. For the Republic of Kazakhstan, in addition to beef, the relevant types of

meat raw materials for inclusion in the recipes of semi-finished products are lamb and broiler chicken meat, including in combination with beef.

The work aims to develop minced meat cutlets with high nutritional and reduced energy value, not inferior in functional and technological properties and sensory characteristics to traditional products of this assortment group, without the use of pork and the traditional carbohydrate component – wheat bread, due to PCC with soy okara, chickpea flour, whey protein concentrate.

Work tasks:

- study of the influence of PCC on the functional and technological properties of minced meat for semi-finished products;
- development of modified recipes for semi-finished meat products using PCC based on minced meat composition: beef - broiler chicken meat; beef – lamb;
- comparative assessment of hydration characteristics and microstructure of the developed minced meat for cutlets;
- assessment of cutlets' nutritional and energy value obtained according to modified recipes.

Scientific hypothesis

We assume that the PCC of the composition of soy minced okara – chickpea flour – whey protein concentrate (WPC 80) in a ratio of 9:5:10 can serve as a substitute for minced meat not only in terms of the balance of the amino acid composition of the total protein but also in terms of the ratio of free and bound moisture, the effect on the microstructure and consistency of raw semi-finished products combined composition. We expect a reduction in the mass fraction of fat while maintaining the high juiciness and tenderness of the cutlets after heat processing.

MATERIAL AND METHODOLOGY

Samples

Protein-carbohydrate composition (PCC). For the manufacture of PCC, the following raw materials were used: soy minced okara (Standards of organization 81952917-001-2013); whole grain chickpea flour (Standards of organization 12396977-004-2015); whey protein concentrate WPC-80 (Standards of organization All-Russian Research Institute of Butter-and-Cheese Making VNIIMS 045-2019).

Minced meat for cutlets and ready-to-eat fried cutlets. For the manufacture of cutlets, the following raw materials were used: pork cutlet meat (ECE/TRADE/369:2006); beef cutlet meat (ECE/TRADE/326:2004); lamb cutlet meat; broiler chicken meat (E/]ECE/TRADE/355).

The following ingredients were used as components of cutlet recipes: wheat bread, fresh onion according to [E/ECE/]TRADE/WP.7/GE.1/2001/10 |; edible salt according to (CODEX STAN 150-1985); ground black pepper according to (ISO 959-1:1998;) drinking water according to (ISO 19458:2006). The recipes for samples of semi-finished products are presented in Table 1.

Chemicals

Technical formalin GOST 1625-89, grade FM, the highest grade (Chemical Industrial Reagent LLP, Shymkent, Kazakhstan).

Ethyl alcohol rectified from food raw materials (manufacturer: "DOSFARM LLP", Kazakhstan).

Homogenized paraffin medium HISTOMIX (manufacturer: BioVitrum, Russia).

Hematoxylin regression and eosin alcohol staining kit "MEDIX" (manufacturer: Russia). Fir balsam (manufacturer: Russia).

Instruments

The amount of free and bound moisture in the samples was determined by a STA 449 F3 Jupiter synchronous thermal analysis instrument with a sample holder (DSC/TG) type S in an aluminium crucible with a pierced lid (an empty aluminium crucible with a perforated lid was used as a reference); nitrogen class 5.0 (active gas flow rate 50 ml/min, protective gas flow rate 20 ml/min). Software: NETZSCHProteus – Thermal Analysis. Heating program: heating from 25 °C to 200 °C at 2 °C/min. The results were presented in the form of TG and DTG curves and processed according to the method **[13]** to obtain data on the kinetics of dehydration.

Samples were cut on microtome «Hospitex diagnostics». The obtained preparations were studied using a Biolam P1U4 microscope under 3.2-40 objective lenses with an overall magnification of 400×.

Laboratory Methods

Laboratory studies of raw materials were carried out based on JSC "Almaty Technological University" (Almaty, Kazakhstan) and in the laboratory of the Center for Collective Use "Control and Management of Energy Efficient Projects" of the Voronezh State University of Engineering Technologies.

The functional and technological properties of minced meat were determined by standard methods **[14]**.

Moisture-binding capacity (MB C) was determined by pressing a minced meat sample under a load of 1 kg and then calculating the difference in masses before and after pressing and the area of the wet spot, determined by a planimeter according to the Grau and Hamm method. In the modification of Volovinsky and Kelman and expressed in % of the total mass of moisture in the product.

Table 1 Recipes of control and experimental samples of chopped semi-finished products.

Raw materials	The norm for cutlets with a share of replacing meat raw materials with PCC						
	Contr. [15]	**Exp. No. 1 15% PCC**	**Exp. No. 2 20% PCC**	**Exp. No. 3 25% PCC**	**Exp. No. 4 15% PCC**	**Exp. No. 5 20% PCC**	**Exp. No. 6 25% PCC**
Unsalted raw materials, kg/per 100kg							
Meat cutlet beef with the content of connective and adipose tissue 15%	54.0	51	46	41	51	46	41
Cutlet pork meat with a mass fraction of adipose tissue 30%	10.0	-	-	-	-	-	-
Cutlet lamb meat	-	-	-	-	10	10	10
Meat of broiler chickens of manual deboning	-	10	10	10	-	-	-
Wheat bread	12.0	-	-	-	-	-	-
Breadcrumbs	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Fresh peeled chopped onion	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Drinking water	18.0	18.0	18.0	18.0	18.0	18.0	18.0
PCC	-	15	20	25	15	20	25
Total	100	100	100	100	100	100	100
Materials and spices, g/100kg							
Food salt	900	900	900	900	900	900	900
Ground allspice	100	100	100	100	100	100	100

Note: Contr. – Control, Exp. – Experiment, PCC – protein-carbohydrate composition.

The samples' free and bound moisture amount was determined by differential scanning calorimetry (DSC) and thermogravimetry (TG). DSC is based on recording the thermal effects of transformations occurring in the test sample under conditions of programmed temperature exposure. Thermogravimetry makes it possible to establish the changes occurring in the product, including the loss of mass while increasing the temperature **[13]**. Software: NETZSCHProteus – Thermal Analysis. Heating program: heating from 25 °C to 200 °C at 2 °C/min. The data obtained were presented in the form of TG and DTG curves.

The microstructure of raw minced meat was determined by standard histological methods **[14]**, **[16]**. Minced meat samples were fixed in 10% neutral buffered (pH 7.0) formalin solution to determine microstructural features, followed by dehydration in alcohols and pouring into Histomix homogenized paraffin medium. To analyze the microstructure of the samples, sections were prepared with a thickness of 2-3 μm according to the generally accepted method, followed by staining with hematoxylin-eosin.

An organoleptic evaluation of minced meat semi-finished products was carried out. It was carried out on a 9-point hedonic scale, per ISO 8586-1 (1993) and ISO 8586-2 (2008), evaluated by a commission of 15 people. The commission included the staff of the department and students of the Almaty Technological University.

The mass fraction of moisture, protein, fat, carbohydrates, and ash in finished semi-finished products was determined according to standard methods **[14]**, and the energy value was determined by calculation.

Description of the experiment

Sample preparation: For the preparation of PCC, soy minced okara, chickpea flour, and whey protein concentrate WPC-80 were weighed, dosed in a ratio of 9:5:10, mixed to a homogeneous mass and hydrated in a ratio of 1:3 for 30 minutes at a temperature of 12 ±2 °C.

For the preparation of minced meat, chilled pork cutlet meat with a mass fraction of adipose tissue of 30%, beef cutlet meat with a mass fraction of connective and adipose tissue of 15%, lamb cutlet meat with a mass fraction of adipose tissue of 10%, meat of hand-boned broiler chickens were used. Meat raw materials were ground on a spinning top with a diameter of 2-3 mm grid holes. The raw meat was dosed according to the recipe (see Table 1), and prepared hydrated non-meat components were introduced: wheat bread in the case of a control sample, hydrated PCC in an amount of 15, 20 or 25% in the case of prototypes. Salt was used in dry form after sieving. Sliced bread was soaked in cold water and then crushed on a spinning top with a diameter of 2-3 mm grid holes. Breadcrumbs were previously sifted. Forming and breading of cutlets were carried out manually. The formed cutlets were fried until they reached culinary readiness.

Number of samples analyzed: One PCC sample, one control sample and six experimental samples of minced meat for cutlets with PCC in a dosage of 15 to 25%, one control sample of fried cutlets of culinary readiness and six experimental samples were analyzed

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: In the first stage, the functional technological properties of the control and test samples of minced meat for cutlets were determined, and the appearance of the semi-finished products before and after heat treatment and the consistency of the finished cutlets were evaluated. For the second stage, two modified recipes of semi-finished products were chosen: one with the replacement of pork and bread with broiler chicken meat and PCC, and the second with a replacement with lamb and PCC. For four samples (PCC, one control and two modified recipes of semi-finished products), the dependence of the conversion of PCC and cutlet masses on temperature was determined; the kinetics of dehydration of these samples, the mass ratio of water fractions according to the stages of dehydration. Then, the features of the microstructure of raw minced meat, the features of the chemical composition, the energy value, and the organoleptic characteristics of cutlets after heat treatment were determined.

Statistical Analysis

Statistical processing was performed in Microsoft Excel 2016 and Statistica 12.0 (USA). The accuracy of the obtained experimental data was determined by the Student's t-test with a confidence probability of no more than 0.05 with the number of parallel determinations of at least 3.

RESULTS AND DISCUSSION

In the technology of meat semi-finished products with additives of plant and animal origin, which are protein-carbohydrate complexes, an important role is played by the water-binding capacity, moisture-retaining capacity and fat-retaining capacity of minced meat **[17]**. Table 2 presents the results of determining the WBC, MRC and FRC of the control and experimental samples of minced meat. Full recipes for control and test samples of minced meat are listed in Table 1.

Table 2 Influence of the PCC dosage on the functional and technological properties of minced meat.

Sample	Mass fraction PCC, %	WBC, %	MRC, %	FRC, %
Control	0	60.81 ±0.29	85.18 ±0.38	50.47 ±0.22
Exp. 1	15	63.52 ±0.28	87.14 ±0.38	52.20 ±0.21
Exp. 2	20	62.23 ±0.24	86.55 ±0.37	51.65 ±0.23
Exp. 3	25	65.05 ±0.25	90.93 ±0.39	54.96 ±0.20
Exp. 4	15	60.24 ±0.26	85.88 ±0.38	51.62 ±0.23
Exp. 5	20	65.15 ±0.23	88.51 ±0.37	55.13 ±0.21
Exp. 6	25	61.18 ±0.29	82.05 ±0.39	49.79 ±0.20

Note: Exp. – Experiment, PCC – protein-carbohydrate composition, WBC – water-binding capacity, MRC – moisture-retaining capacity, FRC – fat-retaining capacity

Water-holding capacity is characterised by water adsorption with the participation of hydrophilic amino acid residues, and fat-retaining capacity is characterized by fat adsorption due to hydrophobic residues. At low humidity, hydrophilic groups, interacting with water molecules, form a monomolecular layer **[17]**. In this regard, it is advisable to use PCC in a pre-hydrated form **[18]**. Okara, which we used as a PCC component, has a great potential for forming water-absorbing, water-binding, and fat-retaining properties of various food systems **[19]**. Polysaccharides represented by cellulose, hemicellulose, and lignin play an important role in forming a complex of functional and technological properties of soy okara **[20]**. This factor plays an important role in ensuring the dietary properties of products with its use, including for gero-dietary nutrition and the expansion of meat-based products, which is relevant for the Republic of Kazakhstan **[21]**, **[22]**. In addition, the okara carbohydrate complex can be positioned as dietary fibers that positively affect meat systems' functional and technological properties **[23]**. The protein-carbohydrate complex of chickpea flour also contributes to forming the functional and technological properties of PCC and minced meat with its use **[4]**. The effectiveness of using chickpea flour instead of beef to improve the consumer and protective properties of minced meat semi-finished products has been confirmed **[24]**. At the same time, the optimal dose of the inclusion of chickpea flour in the recipe of minced meat semi-finished products from beef indicated in **[24]** is consistent with the dosage of chickpea flour added to the recipes of similar semi-finished products when combining beef with poultry meat or lamb. Protein's high water retention capacity in meat products increases yield, extends shelf life, and improves texture **[17]**. An additional effect in forming the functional and technological properties of PCC and minced meat with its use is achieved due to WPC-80, which consists mainly of whey proteins **[25]**. The study of the influence of PCC on the functional and technological properties of recipe compositions of cutlets showed that the best indicators of functional and technological properties are recipe No. 3 (beef + poultry meat + 25% PCC) and recipe No. 5 (beef + lamb + 20% PCC).

Introducing a protein-carbohydrate composition into minced meat leads to the stabilization of meat coagulation structures, as was shown by combining a meat food system with buckwheat **[26]**. A strong, elastic, heat-resistant membrane is formed that protects fat globules and does not lead to any change even when heated **[27]**. A further increase in the amount of the protein-carbohydrate composition to 25% leads to a slight decrease in the values of the WBC, MRC, and FRC indicators relative to the maximum, and therefore, an increase in the mass fraction of PCC to increase these indicators is inappropriate **[28]**. As a result, we can conclude that creating a recipe for minced semi-finished meat products using a protein-carbohydrate composition of 20% makes it possible to obtain a product with high functional and technological properties **[29]**. In addition to functional and technological properties, the organoleptic characteristics of semi-finished products were evaluated: appearance before and after heat treatment, as well as consistency, since the organoleptic assessment of the consistency of cutlets is an important criterion when choosing the ratio of components in the recipe **[30]**. Sensory evaluation plays an important role in the consumer's acceptance of PCC in semi-finished meat products, other meat products, as well as vegetable analogues of meat **[31]**. The consistency of cutlets was assessed according to a previously developed scoring scale, taking into account the recommendations **[32]**. The profilograms of the consistency of the samples corresponding to the scoring are shown in Figure 2. As shown in Figure 2, prototypes No. 2 and No. 6 received the lowest scores in terms of crumbling, elasticity and density. The ratings of prototypes No. 3 and No. 5 almost completely coincide with the ratings of the control sample. Such consistency indicators received the highest marks for these samples as density, water content, elasticity, plasticity, and dimensional stability **[33]**. It has been established that the inclusion of PCC in minced meat in an amount of 20-25% for different samples makes it possible to obtain semi-finished products with high organoleptic characteristics due to the high water-holding capacity of PCC, which includes soy minced okara. The products are juicy, retain their shape well, and are characterized by a non-crushed texture **[33]**.

For a comparative assessment of hydration characteristics during heating, four samples were selected at the second stage of research: PCC at hydration 1:3; a control sample of minced meat, and modified recipes for cutlets No. 3 and No. 5. Samples No. 3 and No. 5 were selected based on the totality of the assessment of functional and technological properties and profilograms of the consistency of the samples presented in Figure 3.

Controlled hydration processes play an important role in the technology of food products from raw materials of animal origin **[34]**. It is equally important to know the regularities of the processes of dehydration of raw materials of animal origin and products of their processing under conditions of programmed heating since this allows simulation of key technological processes and simulation of their parameters that affect both the consumer properties of the products produced and the technical and economic indicators of production **[35]**. Thermogravimetry makes it possible to establish the changes occurring in the product, including the loss of mass while increasing the temperature **[36]**.

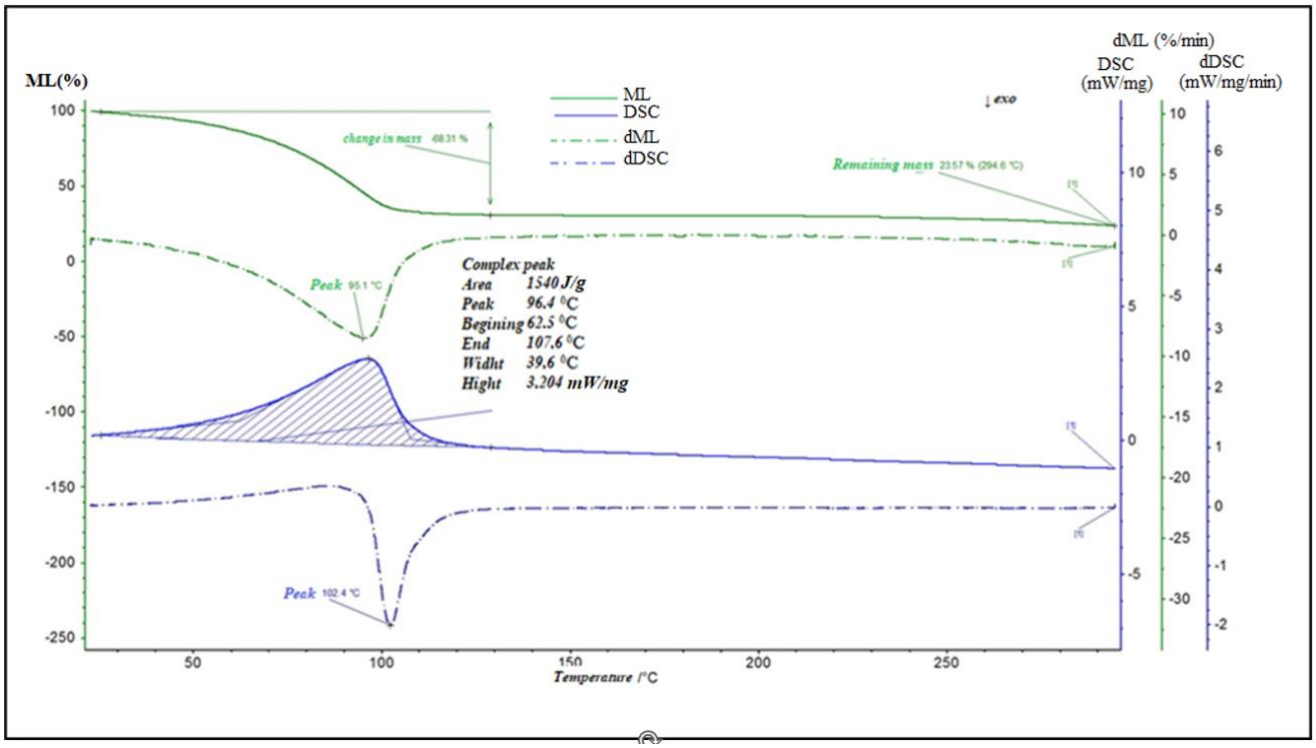
a

b

Figure 2 Profilograms of the consistency of the control and experimental samples. Note: composition cutlets: a – beef + poultry + PCC; b – beef + lamb + PCC; PCC – protein-carbohydrate composition.

Concentrated mostly in tissue cells, water is in a free and bound state. The mass fraction of water, or rather the ratio of its free and bound forms, is one of the most important characteristics of the product, affecting the structure, consistency and microbiological parameters **[37]**.

The primary information obtained from the synchronous thermal analysis device of the STA 449 F3 Jupiter model is presented in the form of curves of mass loss (ML), the rate of mass loss (dML), differential scanning calorimetry thermoanalytical curve (DSC) i.e. heat flow curve and the rate of change of heat flow curve (dDSC) in Figure 3 and Figure 4.



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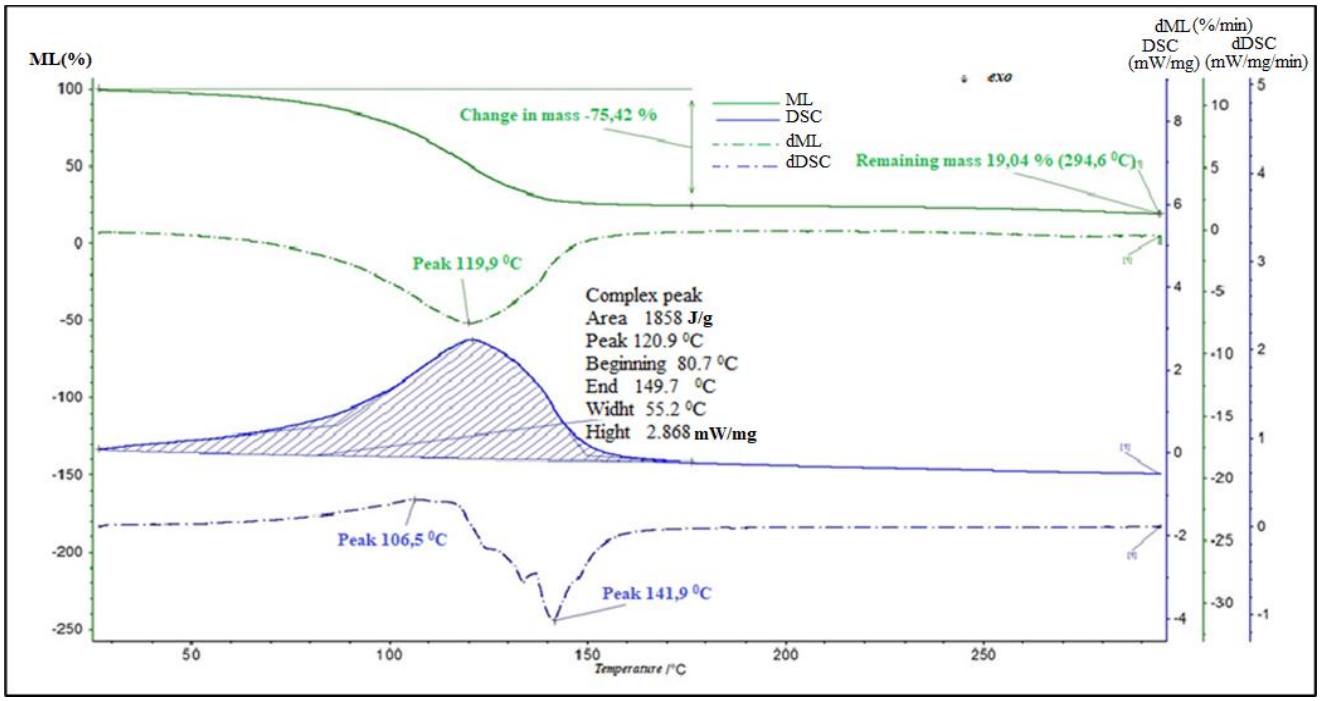


b

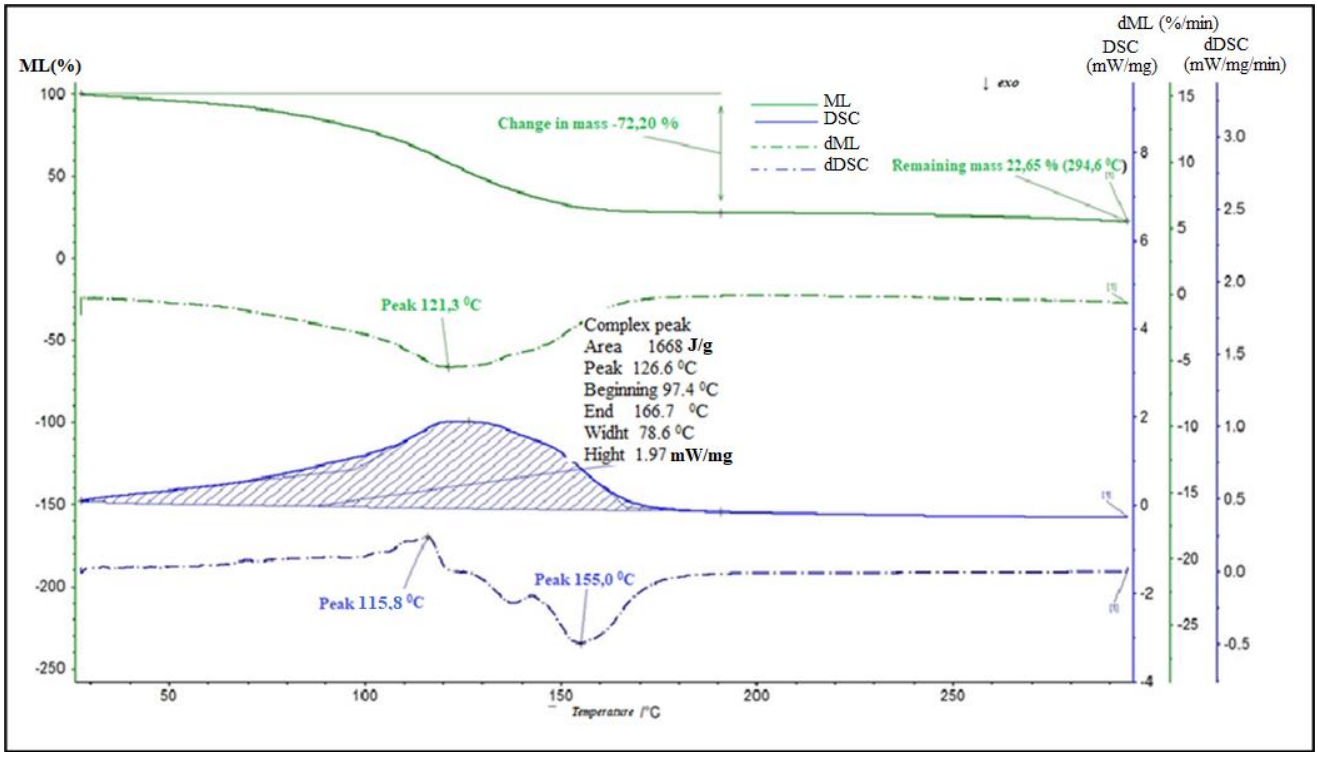
Figure 3 Thermograms for samples: a – PCC; b – control (pork cutlets). Note: PCC – protein-carbohydrate composition; ML – mass loss; dML – rate of mass loss; DSC – Differential scanning calorimetry; dDSC – rate of change of heat flow.

The result of the temperature effect exerted on the samples was a monotonous decrease in their mass, a significant loss observed from a temperature of 30 °C and ended at 120 °C. Further temperature effect does not have a significant effect on the weight of the samples.

Following the DSC method for biological objects **[13]**, to quantify kinetically unequal molecules according to the obtained curves, the plot of mass change is converted into the dependence of the degree of substance conversion α on temperature (Figure 6).

The TG curve obtained in α-T coordinates has an S-shaped form, reflecting the complex nature of the interaction of water and dry substances of the samples and suggests differences in the rate of dehydration in

different sections of the curve. For a more visual range of dehydration temperatures, a graphical dependence (-logα) on the value of 1000/T is plotted (Figure 7).



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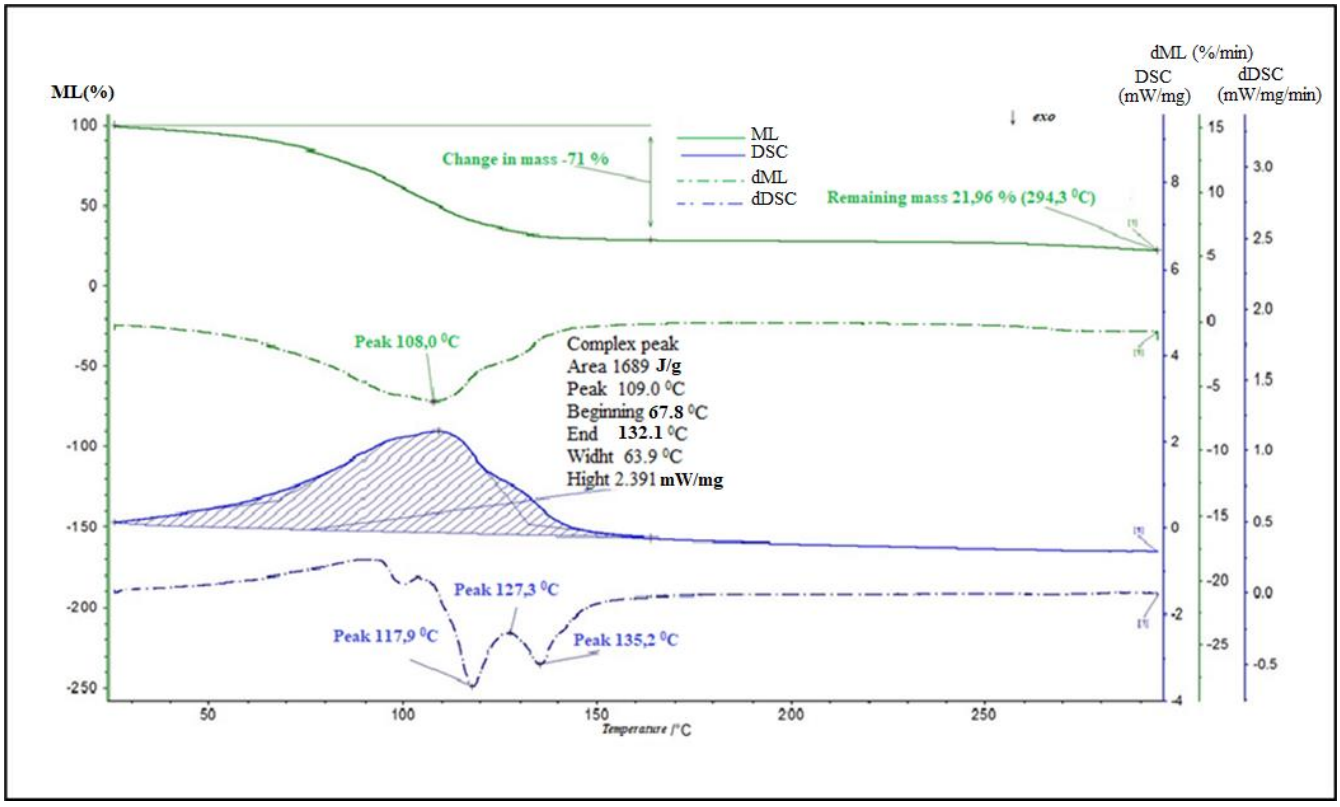

b

Figure 4 Thermograms for samples: a – Expt.3; b – Expt.5. Note: Expt - experiment; ML – mass loss; dML – rate of mass loss; DSC – differential scanning calorimetry (heat flow); dDSC – rate of change of heat flow.

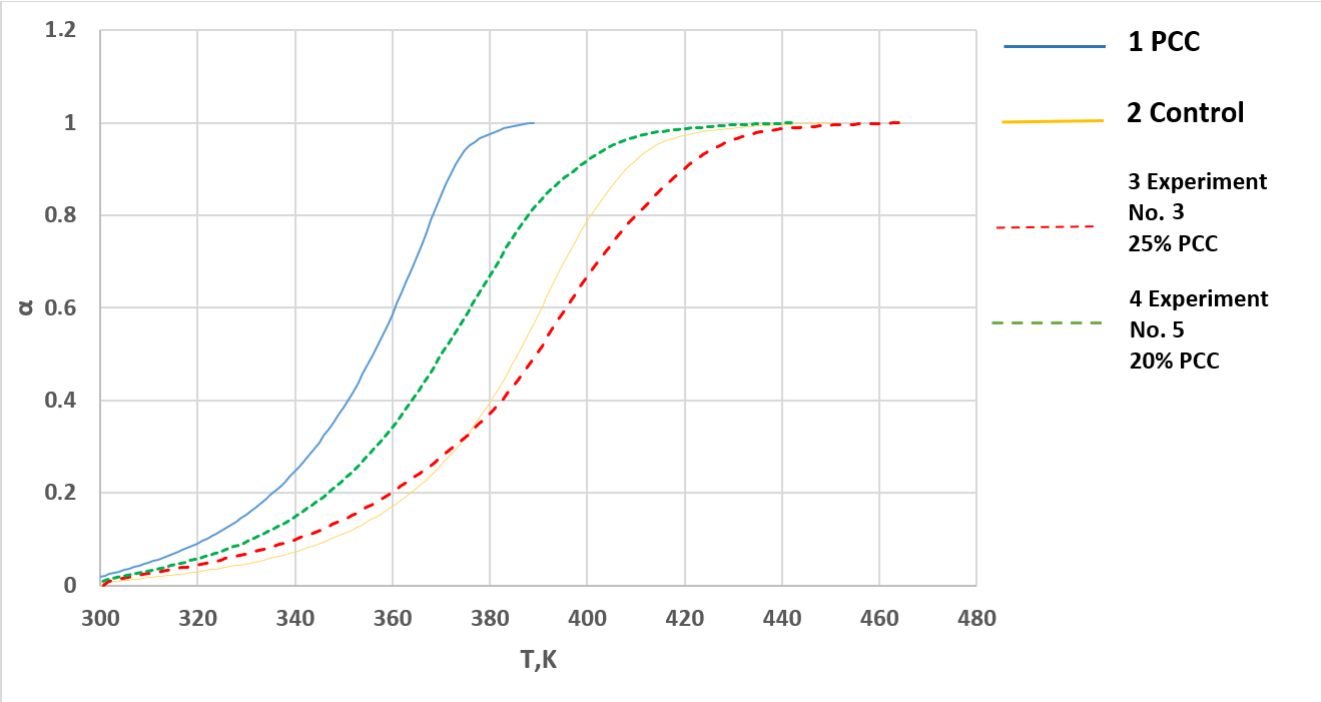

Figure 5 Dependence of substance transformation (α) on temperature (T). Note: PCC – protein-carbohydrate composition.

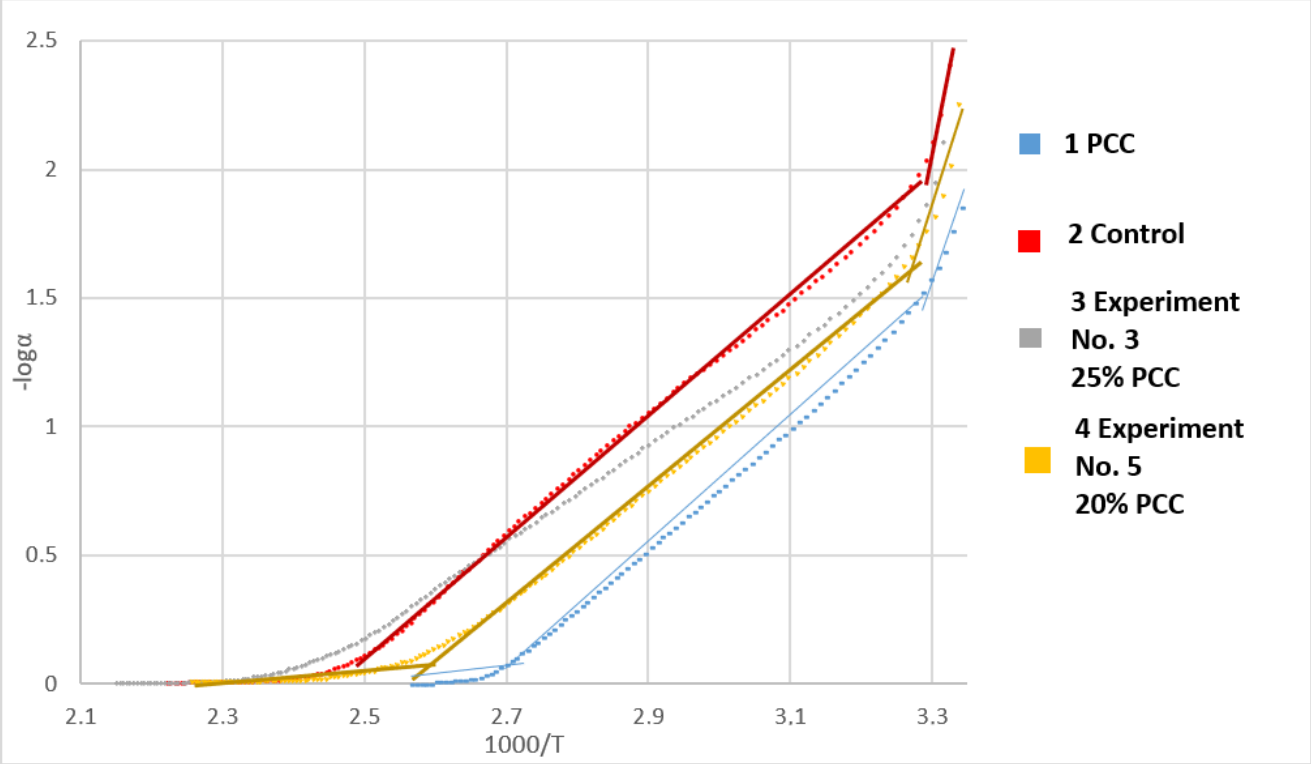

Figure 6 Dependence (-log α) on the value of 1000/T during heating of the studied samples. Note: PCC – protein-carbohydrate composition; α - range of the degree of substance conversion.

In the first section of the curve, heating and release of the first water fraction occurs – water contained in voids and capillaries. In the second section, the removal of the second water fraction begins, and when the first and second fractions are removed, a single pattern is observed.

The moisture removal rate from samples is proportional to the increase in temperature. In the third section, the dehydration rate decreases regardless of the increase in temperature. This demonstrates a significant difference in the binding energy of the third water fraction from the first and second. The removal of the first and second water

fractions from samples 1 and 4 (PCC) and the recipe modified with minced lamb is carried out at a lower temperature effect; this is due to the looser spatial structure of the samples.

Table 3 Dehydration kinetics of samples.

Sample	Dehydration stage	Δ T, K	Δ t, °C	Δ α, %	Mass fraction of removed water, %
PCC	I	295-304	22-31	0-3	2.9
	II	304-367	31-94	3-76.2	73.5
	III	367-389	94-116	76.2-100	23.6
Control	I	295-305	22-32	0-1.2	1.3
	II	305-399	32-126	1.2-78.2	76.9
	III	399-450	126-177	78.2-100	21.8
Experiment No. 3	I	300-307	27-34	0-2.2	2.5
	II	307-410	34-137	2.2-80	78.4
	III	410-465	137-192	80-100	24.1
Experiment No. 5	I	298-305	25-32	0-2.2	2.4
	II	305-385	32-112	2.2-76.4	74
	III	385-442	112-169	76.4-100	23.6

Note: PCC – protein-carbohydrate composition; Δ T – temperature range in degrees Kelvin; Δ t – temperature change interval in degrees Celsius; Δ α – substance conversion range

Data on the kinetics of dehydration of the studied samples are presented in Table 3. The information available in the literature and the methods used for evaluation suggest that the first and second water fractions correspond to physically and mechanically bound moisture, which has a low binding energy with the sample, and osmotically bound moisture, respectively **[38]**. In this regard, the evaporation of these fractions is quite active. The third water fraction corresponds to adsorption-bound moisture, as a result of which the partial removal of this fraction proceeds slowly. The presence of simple sugars and disaccharides in the composition of PCC allows, similarly to lactose in the case of UV concentrates of cheese whey, to increase the hydration of proteins through non-covalent interaction with water and protein molecules by hydrogen bonds **[39]**.

Thus, the studied samples (PCC, minced meat semi-finished products with poultry meat, minced meat semi-finished products with lamb) are characterized by close values of the mass ratio of water fractions at three stages of dehydration (Figure 7).

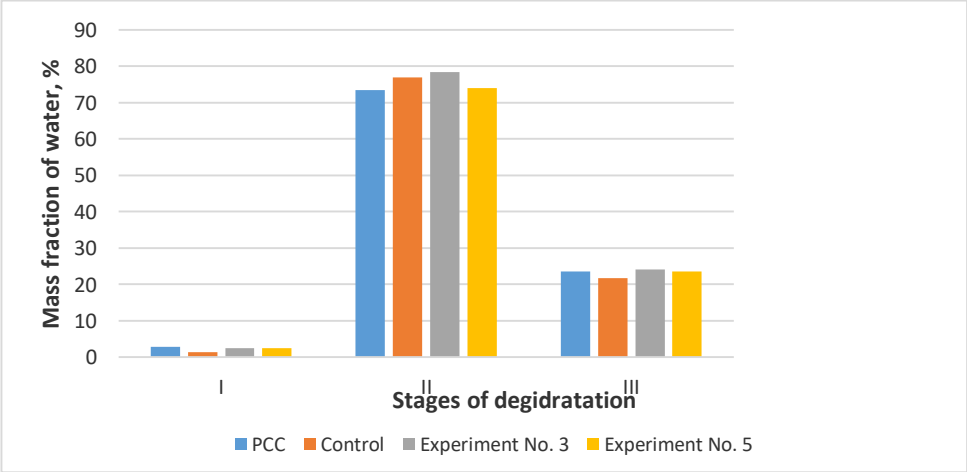

Figure 7 Distribution of removed water by dehydration stages during heating of samples of PCC and minced meat. Note: PCC – protein-carbohydrate composition.

The highest level of energy characteristics of the connection of water fractions with biopolymers of tissue structures of semi-finished products has a sample of chopped meat semi-finished products No. 3 with a recipe modified with poultry meat and PCC. In terms of the mass content of the third fraction, samples No. 3 and 4, prepared according to modified recipes, are superior to the control sample.

At the next stage of the work, a comparative assessment of the microstructure of the combined meat and vegetable minced meat was carried out using protein-carbohydrate raw materials as an objective criterion for assessing the quality of the combined minced meat.

In the control sample of minced meat (Figure 8), scattered muscle particles of various sizes are observed, and connective tissue in different fields of view is visible. The found areas of muscle tissue are fragmented in places, nuclei and transverse striation are absent **[40]**. In some areas, muscle fibers merge into a continuous conglomerate. In the connective tissue, collagen fibers are loosened, partially fragmented.

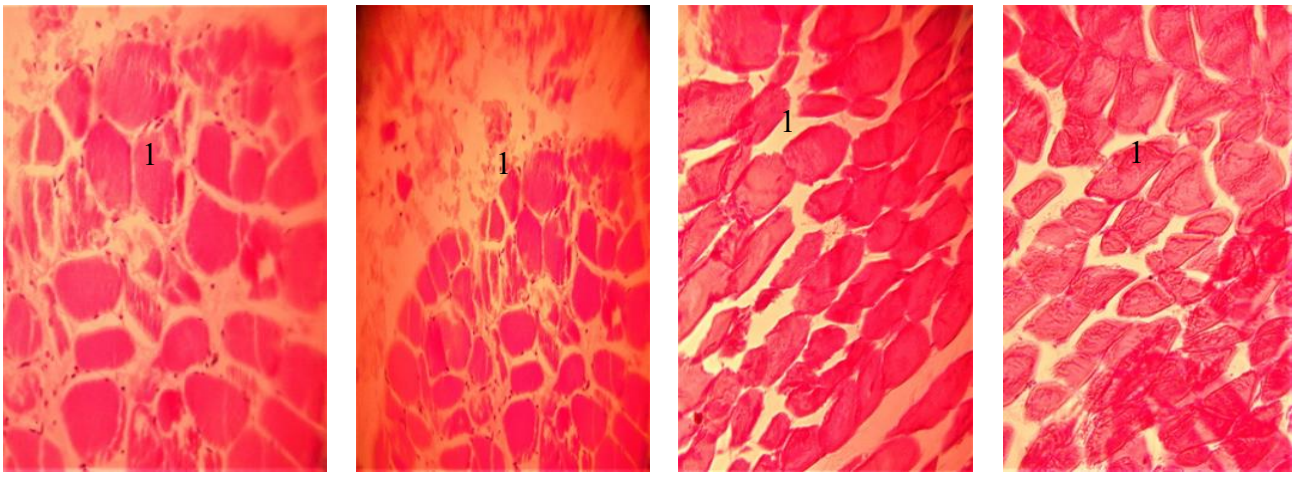

Figure 8 Minced meat (control) histoarchitectonics in different fields of view: 1 – muscle tissue; increased ×400.

The presence of PCC is determined during the visual assessment of histological preparations of minced meat according to modified recipes (Figures 9-10). Okara particles in PCC consist of rounded cells stained in shades of dark pink, surrounded by a narrow, even non-staining lumen.

The particles of the protein-carbohydrate component are evenly distributed between the muscle fibers in minced meat, similarly to the particles of cedar cake **[6]**. The results are consistent with the data of a microscopic study of improved chopped semi-finished meat products through white lupine flour and elecampane root powder **[41]**.

The microstructural characteristics of minced meat systems correlate with other indicators, for example, hydration properties together with the corresponding forms of moisture bonding, which makes it possible to predict the functional and technological properties and behavior of minced meat under conditions corresponding to the technological processing modes in the production of minced meat semi-finished products.

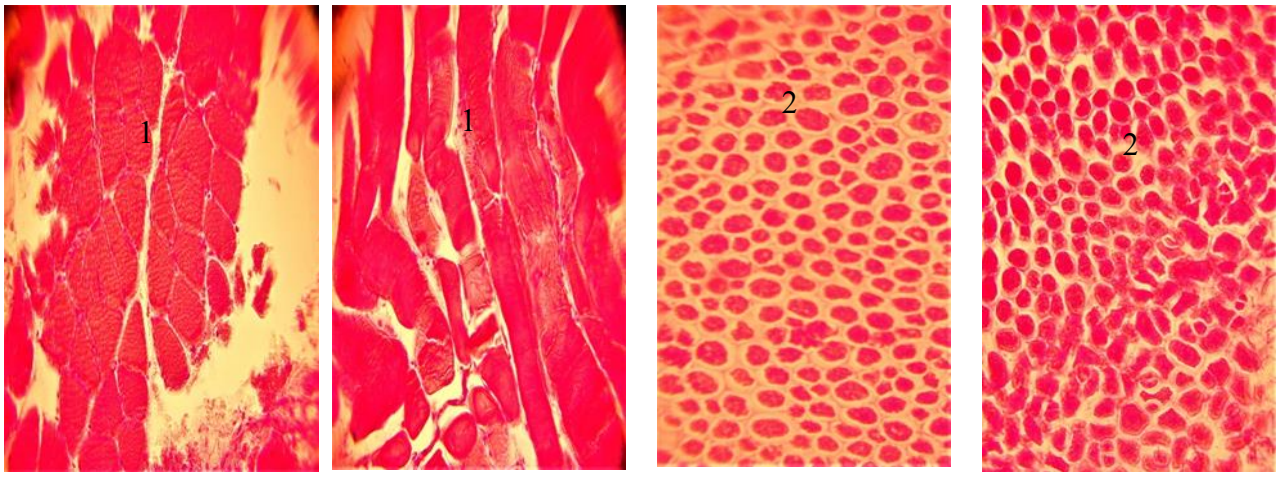

Figure 9 Minced meat histoarchitectonics (beef + lamb + 20% PCC): 1 – muscle tissue, 2 – vegetable protein-carbohydrate component; increased ×400.

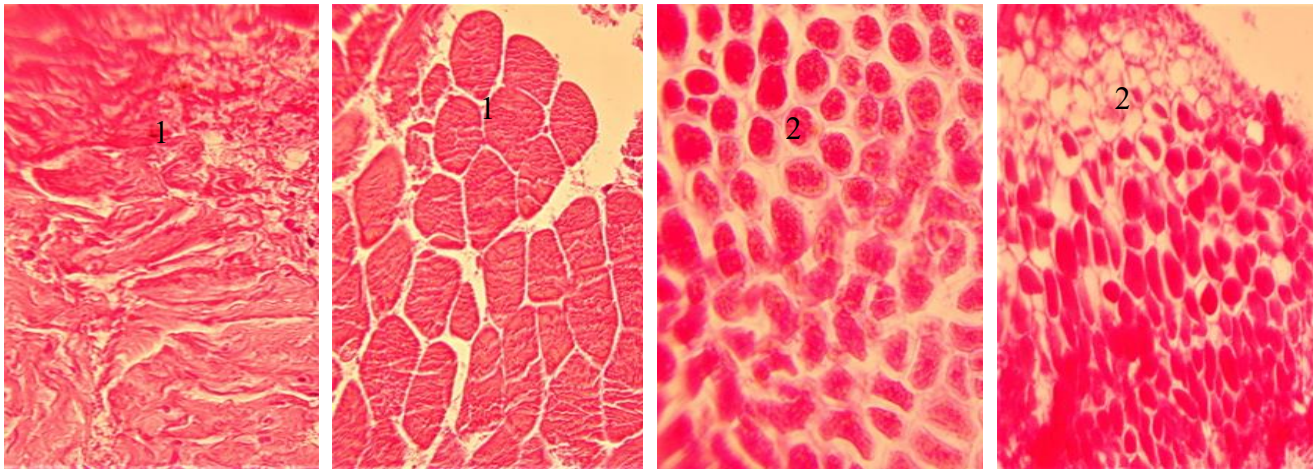

Figure 10 Minced meat histoarchitectonics (beef + broiler meat + 25% PCC): 1 – muscle tissue, 2 – vegetable protein-carbohydrate component; increased ×400.

Products correspond to the traditional organoleptic indicators for chopped cutlets: the shape is rounded-flattened, the surface is evenly covered with breadcrumbs, without torn and broken edges. Minced meat is evenly mixed without visible inclusions of PCC components. Prototypes of cutlets retain moisture well during thermal processing. A comparative assessment of indicators of nutritional and energy value of PCC, as well as semi-finished products obtained according to the control and modified recipes, is presented in Table 4.

Table 4 Indicators of nutritional and energy value of PCC and meat minced semi-finished products.

Sample	Mass fraction, %					Energy value of 100 g of product	
	fat	protein	carbohydrates	water	ash	kcal	kJ
PCC	4.2	38.9	5.5	50.4	0.97	209.3	876.5
Control	12.6	13.8	7.5	66.1	1.5	179.9	753.3
Experiment No. 3	9.5	19.8	2.8	66.92	0.98	175.13	733.29
Experiment No. 5	9.7	19.1	2.5	67.5	1.2	187.84	786.50

Note: PCC – protein-carbohydrate composition.

The sample in experience No. 3 was more juicy than the control sample. The sample in experiment No. 5 also had excellent juiciness, while the experimental samples had a delicate texture and were distinguished by a reduced mass fraction of fat compared to the control: 5,3% less fat in experiment No. 5 and 6% less fat in experiment No. 3.

The results can be used in developing recipe-component solutions for food modules concerning the technology of minced meat semi-finished products, which ensure the preservation of water fractions in the composition of meat products during heat treatment. The expected technical and economic effect is associated with an increase in the degree of use of vegetable raw materials in the production of chopped semi-finished meat products in the main production, an expansion of the range of enriched food products due to natural raw materials, and an increase in the sustainability of the raw material base of meat processing enterprises.

CONCLUSION

It has been established that the protein-carbohydrate composition of the composition: soy minced okara - chickpea flour - whey protein concentrate (WPC 80) in a ratio of 9:5:10, with hydration 1:3, has a positive effect on the functional and technological properties of minced meat for chopped semi-finished products. Modified recipes for cutlets have been developed with the composition, kg per 100 kg: kg/100 kg: beef cutlet meat – 41; meat of hand-boned broiler chickens – 10; PCC – 25; cutlet beef meat – 46; minced lamb – 10; PCC – 20. It has been shown that the developed PCC can serve as a substitute for minced meat not only in terms of the balance of the amino acid composition of the total protein but also in terms of the percentage of moisture with different forms of communication with the product, influencing the microstructure and consistency of raw semi-finished products of the combined composition, the consistency and juiciness of fried cutlets. According to the developed recipes, the mass fraction of protein in cutlets increased from 13.8 to 19.1-19.8%; fat decreased from 12.6 to 9.5-9.7%, with a corresponding decrease in the energy value of products. The development of food modules using protein-carbohydrate compositions as raw materials with a high degree of resource security and potential availability to

deterministic groups of consumers needing correction of food rations by amino acid composition, dietary fibers, etc. has a perspective.

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

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Monitoring the spread of leptospirosis agent as one of the reasons of low-quality milk

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ABSTRACT

On the global scale of the zoonoses problem, leptospirosis is among the five diseases that pose the greatest threat to humankind today. Leptospirosis is a worldwide zoonotic disease caused by pathogenic *Leptospira species*. In general, leptospirosis has been registered in more than 150 species of mammals. There are about 300 serovars of *Leptospira* spp. Serovar *Hardjo* is one of the most common causes of leptospirosis among cattle globally. In cows, the infection can be completely asymptomatic or cause abortions, stillbirths, infertility, and mastitis. The study's relevance is determined by the negative impact on the economy – productivity loss, and high cost of medical-preventive activities. Leptospirosis also affects humans. In this regard, the present study aimed to determine the prevalence of antibodies to *Leptospira interrogans* serovar *Hardjo* in tank milk samples from cows selected from farms in different regions of Ukraine. The method of indirect enzyme-multiplied immunoassay was used for this problem to be investigated. We have investigated 114 tank samples from 66 Ukrainian farms, of which 63.2% were positive, and 36.8% negative. It was established that antibodies to the causative agent of leptospirosis were recorded in different regions of Ukraine. It has been established that the largest number of positive samples was from Kyiv and Cherkasy regions. Our study results complement the study results of other authors and indicate the circulation of this causative agent among the cows in Ukraine, as well as being of practical value for diagnosing and controlling leptospirosis among the cattle.

Keywords: cattle, *Leptospira Hardjo*, antibodies, diagnosis, enzyme-multiplied immunoassay

INTRODUCTION

On the global scale of the zoonoses problem, according to the criteria of socio-economic rating, leptospirosis belongs to the five diseases that pose the greatest threat to humankind today. At least one million clinical disease cases of leptospirosis are annually observed among human and a crude mortality rate range from 5% to 15%. Leptospirosis affects a broad host range, including the cattle, sheep, goats and wild animals. The prevalence of serovar *Hardjo* among cattle in foreign countries is 72% – in England, 34.7% – in Ireland, 11% – in Spain, 42% – in the USA, and so on. The circulation of *L. interrogans* serovar *Hardjo* among cattle is observed in Ukraine in the range of 25.8-60.0% **[1]**, **[2]**.

Leptospirosis is a worldwide zoonotic disease **[3]** caused by infection with pathogenic *Leptospira species*. In general, leptospirosis has been registered in more than 150 species of mammals, but the infectious agent can also be detected in other classes of animals (reptiles, amphibians, etc.) **[4]**. There are approximately 300 serovars of *Leptospira* spp. **[5]** which are divided into 28 groups **[6]**. Leptospirosis among cattle can be caused by different serovars depending on the region and the host. Serovar *Hardjo* is one of the most common causes of leptospirosis among cattle globally.

It includes two species: *Leptospira interrogans* serovar *Hardjo* (prajinto) and *Leptospira interrogans* serovar *Hardjo* (bovis), although there are genetic and epidemiological differences between the two species; both species

are indistinguishable by serological testing **[7]**. Currently, cattle host this serovar, which secretes leptospires with urine **[8]** and secretions from the genital tract **[9]**.

In the cattle, the infection causes significant economic losses and can be completely asymptomatic, or it can be the cause of abortions, stillborn calves, female infertility, reduced milk productivity, mastitis, birth of weak calves, embryonic mortality, as well as high cost of medical-preventive activities **[10]**, **[11]**, which is due to the use of antimicrobial substances, which, in turn, reduces the quality of dairy products **[12]**.

Factors that foster the spread of the disease are many rodents, dogs and other wild animals, contaminated water and soil sources. The disease is also common in humans **[13]**. Symptoms of leptospirosis in humans are fever, myalgia, headache, renal failure, and pulmonary bleeding **[14]**, **[15]**. Leptospires enter the organism through mucous membranes or skin failures and spread through blood **[16]**.

Diseased animals can release the causative agent periodically or regularly for months, years, or throughout their lives. People who work in slaughterhouses, farms, meat processing plants, and veterinarians have the highest risk of disease incidence with leptospirosis **[17]**. As a rule, humans become infected through direct contact with infected animals that release the microorganism with their urine, or through indirect contact with contaminated water or soil. It is also reported about a possible transmission of leptospirosis through the consumption of raw milk obtained from infected cows **[18]**, **[19]**.

In recent years, leptospirosis in milk tank samples has yet to be studied in Ukraine. The present study aimed to investigate the prevalence of antibodies to *Leptospira interrogans* serovar *Hardjo* in tank samples of milk taken from different farms and regions of Ukraine.

In Ukraine, data on the spread of leptospirosis in tank samples of cow's milk are not systematic, and in some cases, they are absent at all, which indicates the relevance of this issue.

Scientific Hypothesis

The spread of leptospirosis in tank samples of milk in the studied farms may be significant, which will allow these farms to assess the risks of its spread and develop effective elimination measures to obtain high-quality and safe dairy products.

MATERIAL AND METHODOLOGY

Samples

Tank milk samples from cows, which were sent for the study to the serology laboratory of LLC “Veterinary Diagnostics Center” from different regions of Ukraine, were in sterile test tubes.

Chemicals

Dilution buffer Prionics Lelystard B.V (Netherlands);
Washing fluid Prionics Lelystard B.V (Netherlands);
Conjugate Prionics Lelystard B.V (Netherlands;
Chromogen (TMV) substrate Prionics Lelystard B.V (Netherlands);
Stop solution Prionics Lelystard B.V (Netherlands), (realtor UkrzooVetPostach, Kyiv, Ukraine).

Animals, Plants and Biological Materials

The animals were of different breeds (*Holstein*, *Ukrainian black* and *white*), age, duration of lactation and productivity. Information on clinical conditions, vaccination, treatment, herd size, diet, maintenance, watering, milking system, and breeding was absent. There needed to be more information on vaccination, herd size, diet, maintenance, watering, milking system, and breeding.

Instruments

Immunoenzyme analyzer Tecan Sunrise (Austria), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Tablet Microtest Plate 96 Well, F (Germany), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Dry air thermostat MicRomed (China), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).
Sartorius pipette dispenser (Germany), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).
Eppendorf pipette dispenser (Germany), (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).
Laboratory utensils, (realtor (Khimlaborreaktyv) Limited Liability Company, Ukraine).

Laboratory Methods

The presence of antibodies to *Leptospira interrogans* serovar *Hardjo* in tank samples of milk was determined by indirect EIA, with the use of commercial test system PrioCHECK *L. Hardjo* Ab Plate Kit (Thermo Fisher Scientific, Applied Biosystems, Lelystad, The Netherlands) **[20]**. EIA uses antigens to capture and quantify the amount of target antibodies present in a milk sample. The test result is the colour reaction measured by the reader in terms of optical density values. Optical densities provide a numerical-non-quantitative determination of the amount of antibodies to *L. Hardjo* in the studied sample. The final numerical result is a standardized percent positivity (PP) relative to a fixed reference sample (PP = optical density of test sample/optical density of reference sample). Recommended Prionics interpretation for PP from tank milk: PP <40% – negative for *L. Hardjo* specific antibodies, 40% PP – 60% – questionable result, and PP> 60% – positive result.

Description of the Experiment

Sample preparation: Tank milk was used for the experiment. Sampling was carried out directly on the farms, from coolers-tanks into 100 ml plastic tubes with screw caps after mixing. The samples were delivered to the laboratory at a temperature of 4-8 °C within 12 hours from their sampling. Subsequently, they were unpacked and homogenized, and 100 mcl of milk was taken from each sample for the experiment.

Number of samples analyzed: 114 tank samples of milk were analyzed.

Number of repeated analyses: The retry number of each experiment for one value to be determined was 5 times.

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: First, we chose the farms that specialize in cattle breeding and have a dairy production direction, to select the samples from each farm individually. A team of 5 researchers conducted all studies on the investigated farms from December 2022 to September 2023. Maintaining conditions and milking procedures were assessed and documented in a standardized data collection form. When the cows were clinically examined, an anamnesis was taken, and the milk was entered into the tank; the samples were taken into sterile tubes after mixing the milk in the tank to ensure homogeneity. The samples were delivered to the laboratory at a temperature of 4-8 °C within 12 hours from selection. At the next stage, we conducted individual experiments to determine the presence of antibodies to *Leptospira interrogans serovar Hardjo*.

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.

RESULTS AND DISCUSSION

According to the data of **[21]** the prevalence of *L. Hardjo* in tank samples of milk studied was 34.59 and 73% among unvaccinated herds in Ireland during 2018-2020, respectively. According to the data of **[22]** the prevalence of *L. Hardjo* in milk tank samples was 86% among unvaccinated herds in Ireland.

Studies in Brazil show that in 77 samples out of 208 animals had antibodies to leptospirosis **[23]**; other authors **[24]** indicate that the prevalence of antibodies to leptospirosis in Brazil is 52%.

For example, a study of 109 herds in Japan showed that 71 herds were positive for *Leptospira Hardjo*, and the prevalence at the herd level was 65.1% **[25]**, which coincides with our studies. According to some sources **[26],** the seropositivity of animals at the herd level was 4.8% in Nepal.

These are just a few names of scientists actively working in research related to *Leptospira interrogans*. The directions of their research may differ depending on their specialization and interests.

Studies of 45 farms in India show that specific antibodies to *Leptospira Hardjo* were 27.76% **[27]**, **[28]**. In the Netherlands, studies carried out during 2017-2021 indicate that *L. Hardjo* infections were detected in 120 dairy herds **[29]**. The authors' studies **[30]** in Egypt showed that 39.33% of the 236 studied animals had antibodies to leptospirosis. A total of 48 randomly selected cattle herds were studied in Algeria between 2015 and 2019. The prevalence of serovar *Leptospira interrogans Hardjo* was 31.25% **[31]**. The authors' study from Ethiopia **[21]** from 2019 to 2020 showed that out of 77 dairy farms selected for the study, 57 were marked as positive for *L. Hardjo*. In Pakistan, the prevalence of antibodies to leptospirosis among the cattle was 56.25% **[32]**. The studies conducted in Tanzania showed the prevalence of serovar *Leptospira Hardjo* at 13% **[33]**. Researchers **[34]** determined that the prevalence of leptospirosis in Manabí, Ecuador, at the herd level is 98.18%.

During the study period, to determine specific antibodies to *Leptospira interrogans serovar Hardjo*, the tank samples of milk from 16 regions of Ukraine were sent to the serology laboratory of LLC "Veterinary Diagnostics".

No samples were sent from 8 regions (Odesa, Volyn, Luhansk, Chernivtsi, Zakarpattia, Lviv, Zaporizhzhia, Rivne). The largest farms were studied from Kyiv, Cherkasy, Sumy, Khmelnytskyi, Poltava, Chernihiv, and Zhytomyr regions (Figure 1).

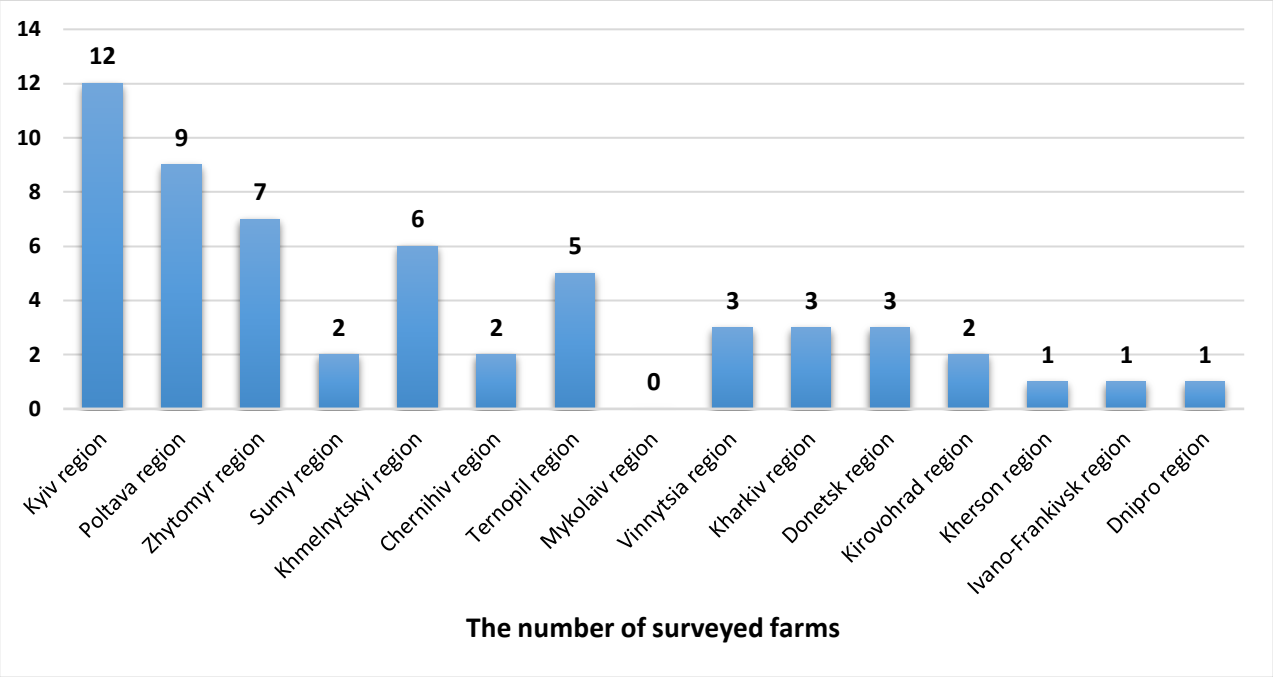

Figure 1 Number of studied farms in regions of Ukraine for *Leptospira interrogans serovar Hardjo*.

A total of 114 tank samples of milk were studied (Figure 2).

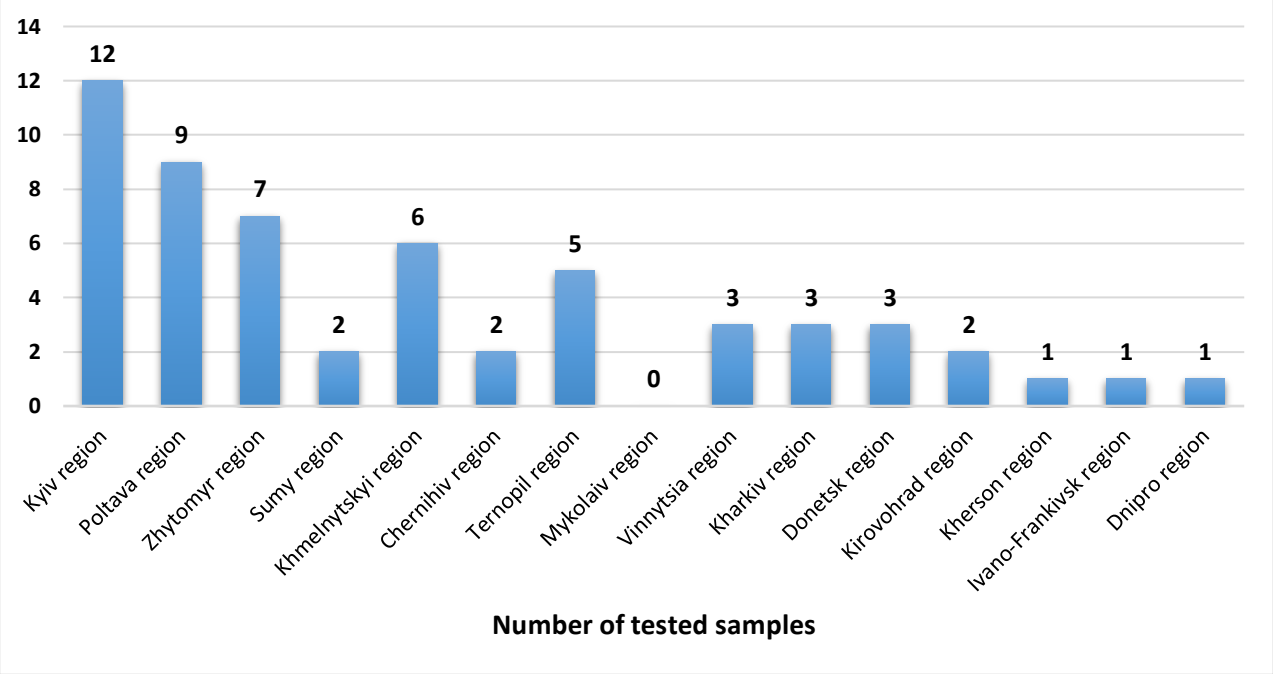

Figure 2 Number of studied milk tank samples in Regions of Ukraine for *Leptospira interrogans serovar Hardjo*.

From 66 farms in Ukraine, 72 samples were positive (Figure 3), which was 63.2%. In turn, 42 samples were negative (Figure 4), which is 36.8%.

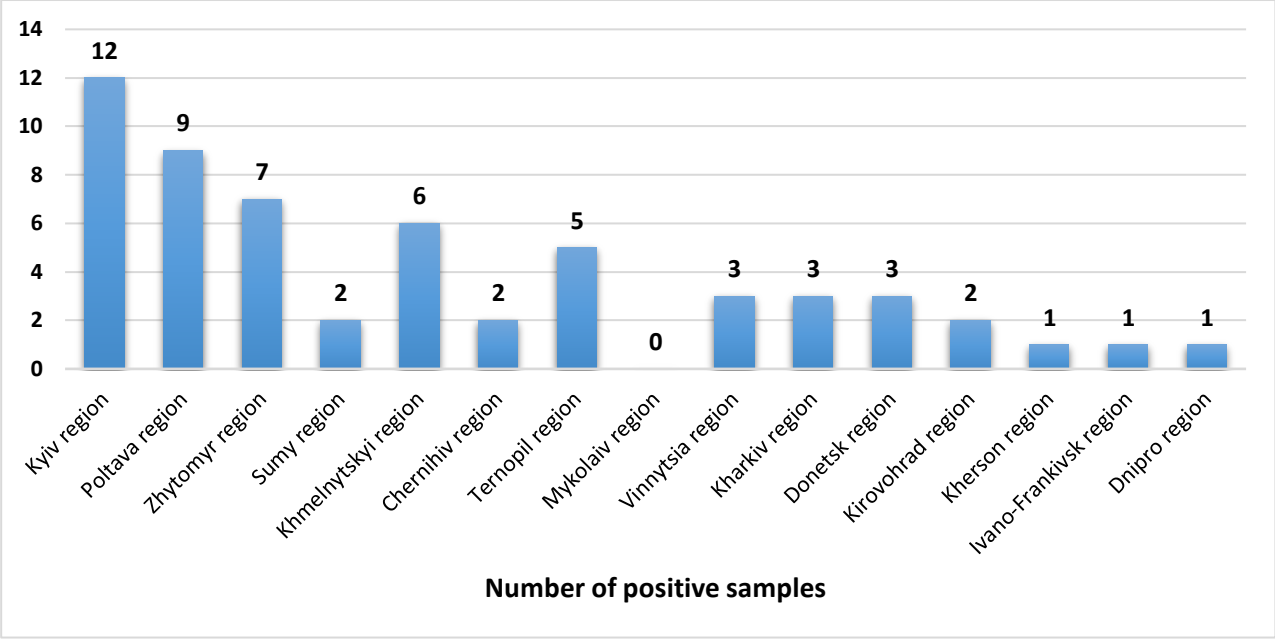

Figure 3 Number of positive milk tank samples in Regions of Ukraine for *Leptospira interrogans serovar Hardjo*.

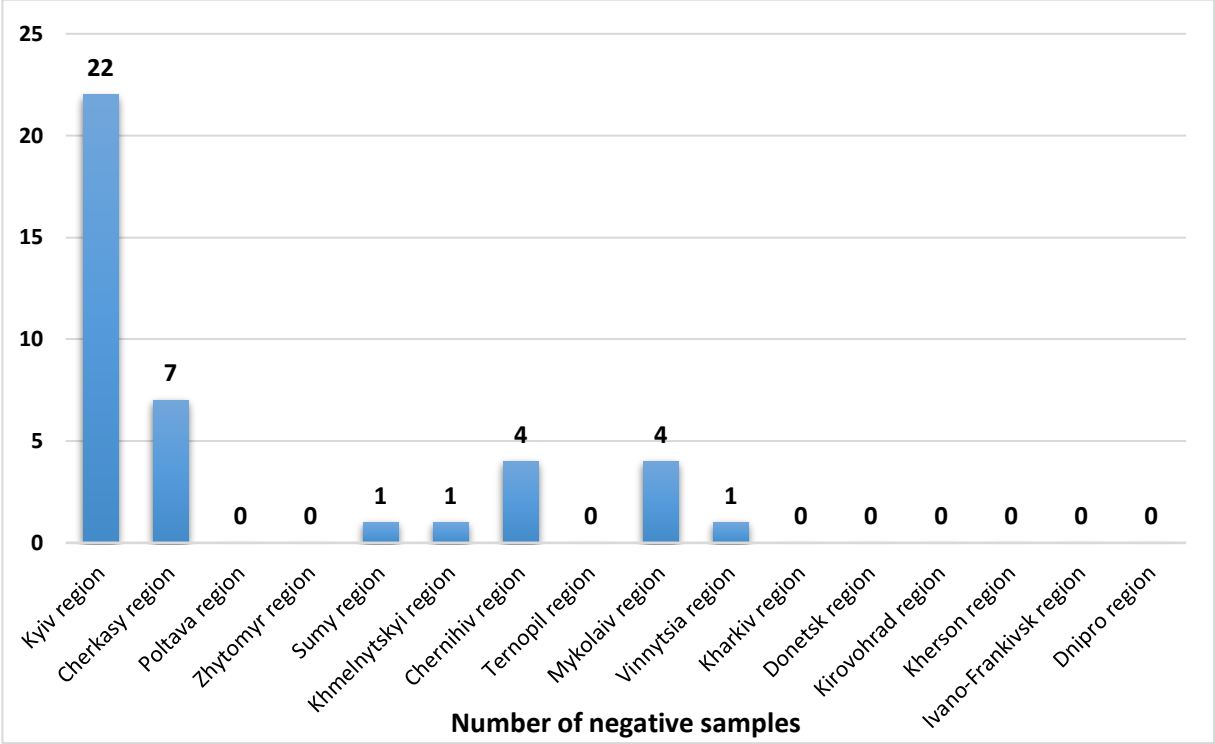

Figure 4 Number of negative tank samples of milk in Regions of Ukraine for *Leptospira interrogans serovar Hardjo*.

A detailed analysis of the circulation data of *Leptospira interrogans* serovar *Hardjo* in farms and regions of Ukraine is presented in (Table 1).

Our studies showed that 12 farms (34 tank milk samples) from Kyiv region were examined from 2017 to 2020, which is 29.8%, specific antibodies to *Leptospira interrogans* serovar *Hardjo* were detected in 12 samples (16.6%), 22 samples (52.4%) were negative. From Cherkasy region, 11 farms (22 tank milk samples) were examined, (19.3%), positive reactions were found in 15 samples (20.8%), and negative – In 7 samples (16.7%).

From Poltava – 5 farms (9 tank milk samples) were examined (7.9%), and all 9 samples were positive (12.5%). From the Chernihiv region – 5 farms (6 tank milk samples) were examined (5.3%), 2 samples (2.8%) were positive, and 4 samples (9.5%) were negative. From the Sumy region, 5 farms (6 tank milk samples) were examined (5.3%), 2 samples (2.8%) were positive, and 4 samples (9.5%) were negative. From the Khmelnytskyi region, 5 farms (6 tank milk samples) were examined (5.3%), and all 6 samples (8.3%) turned out to be positive.

From the Vinnytsia region, 4 farms (4 tank milk samples) were examined (3.5%), 3 samples (4.2%) were positive, and 1 sample (2.4%) was negative. From the Zhytomyr region, 3 farms (7 tank milk samples) were examined (6.1%), and all 7 samples (9.7%) were positive. From Kharkiv and Donetsk regions, 3 farms (3 tank milk samples from each region) were examined from each region (2.6%), and all 3 studied samples (4.2%) were positive. From the Ternopil region, 2 farms (5 tank milk samples) were examined (4.4%), and all 5 samples (6.9%) were positive.

Table 1 Circulation of *Leptospira interrogans* serovar *Hardjo* in regions of Ukraine.

It. No.	Oblast (region)	Number of studied farms	Number of studied samples	% of the total number	Positive	%	Negative	%
1	Kyiv	12	34	29.8	12	16.6	22	52.4
2	Cherkasy	11	22	19.3	15	20.8	7	16.7
3	Mykolaiv	4	4	3.5	0	0	4	9.5
4	Vinnytsia	4	4	3.5	3	4.2	1	2.4
5	Sumy	5	6	5.3	2	2.8	4	9.5
6	Donetsk	3	3	2.6	3	4.2	0	0
7	Ternopil	2	5	4.4	5	6.9	0	0
8	Kherson	1	1	0.9	1	1.4	0	0
9	Khmelnytskyi	5	6	5.3	6	8.3	0	0
10	Ivano-Frankivsk	1	1	0.9	1	1.4	0	0
11	Dnipro	1	1	0.9	1	1.4	0	0
12	Kharkiv	3	3	2.6	3	4.2	0	0
13	Poltava	5	9	7.9	9	12.5	0	0
14	Chernihiv	5	6	5.3	2	2.8	4	9.5
15	Zhytomyr	3	7	6.1	7	9.7	0	0
16	Kirovohrad	1	2	1.7	2	2.8	0	0
	Total	66	114	100	72	100	42	100

From the Kirovohrad region, 1 farm (2 tank milk samples) was examined (1.7%), and all 2 samples (2.8%) turned out to be positive. 1 farm was examined from Dnipro, Kherson, and Ivano-Frankivsk regions, 1 tank milk sample of which is (0.9%) per farm, and in all the examined samples, which is 1.4% of the total number of positive samples, it was found specific antibodies to *Leptospira interrogans serovar Hardjo*. During this period, 4 farms (4 tank milk samples) were examined from the Mykolaiv region (3.5%), and all 4 studied samples turned out to be negative, which is 9.5% of the total number of negative tank milk samples.

According to **[31]** the prevalence of *L. Hardjo* in 2009 in tank milk samples in Ireland among non-vaccinated herds studied was 34, 59 and 73%, **[32]** reported during 2018-2020, the prevalence of *L. Hardjo* in tank in milk samples in Ireland, the average of non-vaccinated herds was 86% – Indicating an increase in the percentage of outbreaks over a certain period.

Studies in Brazil show that in 77 samples out of 208, animals had antibodies to lepto-spira **[33]**, other authors **[36]** indicate that the prevalence of antibodies to leptospirosis in Brazil is at the level of 52%.

For example, a study of 109 herds in Japan showed that 71 herds were positive for Leptospira Hardjo, and the prevalence at the herd level was 65.1% **[36]**, which coincides with our research. Some sources **[34]** report that in Nepal, the seropositivity of animals at the herd level was 4.8%.

Studies of 45 farms in India show that specific antibodies to *Leptospira Hardjo* were 27.76% **[35]**. In the Netherlands, research conducted during 2017-2021 indicates that *L. Hardjo* infections were detected in 120 dairy herds **[37]**. The authors' research **[38]** in Egypt demonstrated that 39.33% of the 236 studied animals had antibodies against leptospirosis. A total of 48 randomly selected cattle herds were studied in Algeria between 2015 and 2019, the prevalence of serovar *Leptospira interrogans Hardjo* was 31.25% **[39]**. The study's results by the authors from Ethiopia **[43]** from 2019 to 2020 show that out of 77 dairy farms selected for the study, 57 were marked as positive for *L. Hardjo*. In Pakistan, the prevalence of antibodies against leptospirosis in cattle is 56.25% **[40]**. Studies conducted in Tanzania demonstrate serovar *Leptospira Hardjo's* prevalence at 13% **[41]**. Researchers **[42]** established the prevalence of leptospirosis in Manabi, Ecuador, at the herd level to be 98.18%.

CONCLUSION

According to the data of the serology laboratory of LLC "Veterinary Diagnostics Center", specific antibodies to *Leptospira interrogans* serovar *Hardjo* were detected in 63.2% of the studied tank milk samples. The largest number of positive detection results of antibodies to *Leptospira interrogans* serovar *Hardjo* in the tank milk samples was noted in Cherkasy 15 (20.8%), Kyiv 12 (16.6%), Poltava 9 (12.5%), Zhytomyr 7 (9.7 %) and Khmelnytskyi 6 (8.3%) regions. In general, infection with *Leptospira interrogans* serovar *Hardjo* was found in almost all studied regions of Ukraine, except the Mykolaiv region. Infection with *Leptospira interrogans* serovar *Hardjo* was detected in almost all regions of Ukraine, except for the Mykolayiv region. Information about the expansion of leptospirosis of the great horned thinness is not complete, but it is possible to use Vicoristan to assess the prediction of the risks of its expansion and develop an effective program for the control of its disease. The prospects for further investigations include in-depth monitoring, which will lead to the continued availability of many milk streams directly from the dairies of Ukraine, which specialize in processing the milk of animals, as well as expanding the range of surveillance to identify new infections.

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Fatty acids, their proportions, ratios, and relations in the selected muscles of the thigh and roast beef

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ABSTRACT

The study aimed to examine, compare, and statistically evaluate the quality of the beef thigh and roast beef muscle in terms of the fatty acids profile concerning human health. *Musculus semimembranosus* and *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef were used for analysis to evaluate the fatty acid profile. Chemical analysis of the thigh and roast beef muscle samples was performed using Fourier transform infrared (FTIR) spectroscopy. The measured data were statistically processed according to descriptive characteristics, analysis of variance, and differences were tested using Scheffe´s test at $\alpha = 0.05$. The SAS program package, version 8.2, was used to evaluate the results statistically. A statistically significant difference ($p \leq 0.05$) was recorded in the dry matter proportion between m. quadriceps femoris and m. longissimus dorsi. A statistically significant difference was found in the intramuscular fat proportion, polyunsaturated fatty acid proportion, the ratio of polyunsaturated fatty acids to saturated fatty acids, the ratio of polyunsaturated fatty acids to monounsaturated fatty acids, as well as between *m. semimembranosus* and *m. longissimus dorsi* and between *m. quadriceps femoris* and *m. longissimus dorsi*. Strong, statistically significant ($p \leq 0.01$, $p \leq 0.001$) correlations were found mainly between intramuscular fat and polyunsaturated fatty acids, between intramuscular fat and the ratio of the polyunsaturated fatty acids to saturated fatty acids, between intramuscular fat and the ratio of polyunsaturated fatty acids to monounsaturated fatty acids. In conclusion, it was stated that the muscles of the thigh and roast beef of the young cattle are characterized by statistically significant differences in the proportion of fatty acids. The ratio of polyunsaturated fatty acids to saturated fatty acids meets the recommended values concerning maintaining the health of the food consumer. Still, the ratio of the n-6 to n-3 polyunsaturated fatty acids poses a risk concerning cardiovascular diseases.

Keywords: beef muscle, fatty acid, proportion, ratio, correlation

INTRODUCTION

In general, meat consumption varies by region due to specific eating habits, levels of financial income, and product availability **[1]**. In recent years, beef consumption has decreased and the reasons that explain consumer changes are due to the presence of various factors, such as the replacement of red meat with white meat, which occurs either for nutritional, health or economic reasons, environmental reasons, respectively. Traditionally, the relative price of beef compared to other types of meat was considered a factor that could explain the lower demand. Other factors are being promoted, such as lifestyle, food safety, and a new understanding of consumer interests in the environment or animal welfare, sustainability, and food processing **[2]**, **[3]**, **[4]**, **[5]**.

The aim of our study was to examine, compare and evaluate the quality of selected muscles of the beef thigh and roast beef in terms of fatty acid profile in relation to human health.

Scientific Hypothesis

1. *Musculus semimembranosus* and *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef affect the intramuscular fat proportion.
2. *Musculus semimembranosus* and *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef affect the saturated fatty acid proportion.
3. *Musculus semimembranosus* and *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef affect the monounsaturated fatty acid proportion.
4. *Musculus semimembranosus* and *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef affect the polyunsaturated fatty acids proportion.
5. *Musculus semimembranosus* and *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef affect the ratio of the polyunsaturated fatty acids to saturated fatty acids.

MATERIAL AND METHODOLOGY

Samples

Two muscles, *quadriceps femoris* and *semimembranosus,* of the beef thigh and one muscle *longissimus dorsi,* of the roast beef were used for fatty acid analysis.

Animals, Plants and Biological Materials

Sampling for research was carried out on a cattle farm at the foot of the Levočské vrchy (Levoca mountains) in Slovakia. Young Limousine male cattle of 9 months of age weighing 300 kg were used for the research.

Thigh and roast beef from young cattle were sampled and bred in a herd with another 24 pieces of cattle in the free-range system. The young cattle in the fattening ward received a stable feed ration of corn, alfalfa silage, barley straw, and meadow hay, supplemented with a supplementary core mixture during sampling. Thigh and roast beef were transported from the farm to the Institute of Animal Husbandry (Department of Veterinary Disciplines) of the Faculty of Agrobiology and Food Resources, the Slovak University of Agriculture in Nitra, where the muscles were anatomically separated.

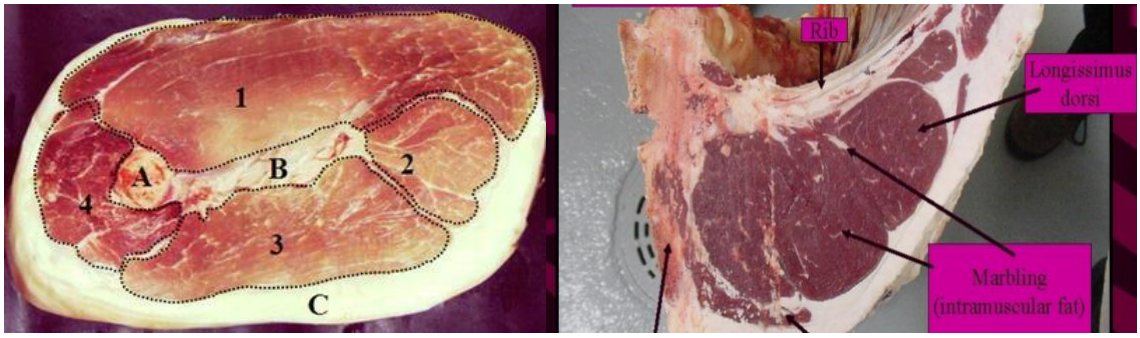

Figure 1 Beef thigh and roast beef muscles used in research. Note: Thigh muscle in cross-section: 1. *musculus semimembranosus*; 2. *m. semitendinosus*; 3. *m. biceps femoris*; 4. *m. rectus femoris* a *m. vastus medialis* (*m. quadriceps femoris*) **[6]**, Roast beef in cross-section: rib; *m. longissimus dorsi*, marbling (intramuscular fat) **[7]**.

Instruments

Scales, type Kern 440-49N with an accuracy of d = 0.01 g, a laboratory mixer, type Grindomix 200 and a laboratory instrument Nicolet 6700 FTIR spectrometer, Thermo Nicolet Corp., Madison, WI were used in the research.

Laboratory Methods

Chemical analysis of beef thigh and roast beef samples was performed according to the Fourier Transform Infrared (FTIR) spectroscopy method in the chemical laboratory of the Institute of Animal Husbandry (Department of Special Zootechnics), Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra.

Infrared spectroscopy includes the infrared portion of the electromagnetic spectrum. The principle of the infrared spectroscopy method is the use of molecules with specific energy content. This energy content corresponds to the frequencies at which the molecules rotate or vibrate **[8]**. Fourier transform infrared spectroscopy is suitable for qualitative and quantitatively determining ingredient content in foods, including meat, regarding information on the functional group in the infrared spectrum **[9]**. Thigh muscle and roast meat samples were analyzed for dry matter, fat, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, n-6 polyunsaturated fatty acids, and n-3 polyunsaturated fatty acids. FTIR spectra were recorded by scanning 4 cm^{-1} at 4000 – 650 cm^{-1}, marked near the middle infrared area. The analytical output was the infrared spectrum

illustrated as a function of energy dependence. This energy dependence was expressed as percent transmittance or absorbance units at the wavelength of the incident radiation.

Description of the Experiment

Sample preparation: The individual muscles were divided into 6 equal parts, the so-called partial samples, a total of 18 pieces, which were used to prepare the basic samples. Each sample was prepared according to the official method **[10]**, which is recognized by the Codex-AOAC, particularly for meat and meat products. A laboratory mixer was used, type Grindomix 200. The principle of the method is to mix and homogenize the sample thoroughly. 50 g, which is the analytical sample, was taken and weighed from each basic sample. The analytical sample was quantitatively transferred to a ground glass flask and prepared for chemical analysis.

Number of samples analyzed: 18

Number of repeated analyses: 2

Number of experiment replication: 1

Statistical Analysis

The initial data obtained from the chemical analysis were mathematically calculated for the ratios: polyunsaturated fatty acids to saturated fatty acids, polyunsaturated fatty acids to monounsaturated fatty acids, monounsaturated fatty acids to saturated fatty acids, and n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids. The obtained data were statistically evaluated according to the indicators of descriptive characteristics, i.e. $\bar{x}$ – arithmetic mean and SD – standard deviation, the result of which is information on the accuracy of the measurement. Analysis of variance (ANOVA) was used to compare groups, i.e. the assumption of agreement of variance was verified by the F test (F). A statistical comparison of differences was made between thigh muscles and roast beef using Scheffe´s test. For the results, the p-value of the respective achieved statistical significance was evaluated at the selected level of significance $\alpha = 0.05$. The linear relationship between the two variables was tested according to the Pearson correlation coefficient (r). The values of (r) are set between +1 and -1, and a value of 0 means no linear relation between the data in the file. According to Cohen **[11]**, the value (r) between the two variables means less than 0.1 is trivial dependence, 0.1 to 0.3 is weak dependence, 0.3 to 0.5 is medium dependence, and more than 0.5 is strong dependence. The result of the correlation relationship (r) between the two variables was statistically tested at a significance level of $\alpha = 0.05$, $\alpha = 0.01$, and $\alpha = 0.001$. The SAS program package, version 8.2, was used to evaluate the results statistically. The basic file in each statistical file of thigh muscles and roast beef muscles represented 6 statistical units, 18 ($n \leq 30$).

RESULTS AND DISCUSSION

Dry matter in the selected beef thigh and roast beef muscles

Average proportion and statistical evaluation of the dry matter proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi of the* roast beef is shown in Table 1.

Table 1 Average proportion and statistical evaluation of the dry matter proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *longissimus dorsi* of the roast beef.

Group	n	$\bar{x}$ ±SD, %	Scheffe´s test, α = 0.05	
			Beef thigh – *m. quadriceps femoris*	Roast beef – *m. longissimus dorsi*
Beef thigh – *m. semimembranosus*	6	27.62 ±0.84	$p > 0.05$	$p > 0.05$
Beef thigh – *m. quadriceps femoris*	6	28.36 ±0.26	$p \leq 0.05$	
Roast beef – *m. longissimus dorsi*	6	27.19 ±0.42		
Analysis of variance		F (6.67, $p \leq 0.01$)		

Note: m. – muscle, n – multiplicity, SD – standard deviation, $p > 0.05$ – no statistically significant difference, $p \leq 0.01$, $p \leq 0.05$ – a statistically significant difference.

Previous studies have also shown that muscle fiber composition is one of the variable muscle growth characteristics that affect meat quality, especially in terms of meat palatability, including affecting taste **[12]**, meat color, pH, water-binding ability, tenderness, and nutritional value of meat **[13]**, components of connective tissue and intramuscular fat **[14]**. Muscle properties can be improved by the efficiency of nutrition and feeding and thus improve the economic value of livestock **[12]**. Wegner et al. **[15]** argue that for beef farms, a proper understanding

of muscle characteristics is important to produce meat in maximum quantity and quality. Muscle mass can be maximized through the number and size of muscle fibers and the transformation of muscle fibers.

The dry matter proportion was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The result of the comparison shows that the dry matter proportion of the thigh muscles and the roast beef muscle is different in the groups statistically significant F (6.67 $^{++}$, $p \leq 0.01$). The muscles of the thighs and roast beef affected the dry matter proportion.

The dry matter in the *musculus semimembranosus* of the thigh reached an average proportion of 27.62% and in *m. quadriceps femoris* of the thigh slightly higher, 28.36%. The average dry matter proportion was the lowest in *m. longissimus dorsi* of the roast beef from all examined muscles, i.e. 27.19%. The difference in dry matter proportion between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant ($p > 0.05$). Also, the difference in dry matter proportion was not statistically significant ($p > 0.05$) between *m. semimembranosus* of the thigh and *m. longissimus dorsi* of the roast beef. But the difference between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef was statistically significant ($p \leq 0.05$). Statistical evaluation of the dry matter proportion results in the thigh muscles and roast beef muscle based on the standard deviation revealed that the largest fluctuation of the measured values was at *m. semimembranosus* of the thigh and the lowest at *m. quadriceps femoris* of the thigh (SD = 0.84 vs. SD = 0.26).

Fresh beef contains 65 to 80% moisture (20 to 35% dry matter) **[16]**. Our dry matter proportion of the beef thigh and roast beef muscles is also within the stated values. Water in beef exists in three forms; free water, immobilized water, and bound water **[17]**.

Intramuscular fat in the selected beef thigh and roast beef muscles

Average proportion and statistical evaluation of the intramuscular fat proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi of the* roast beef is shown in Table 2.

The intramuscular fat proportion was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, on the basis of which the null hypothesis H_0 was rejected. The result of the comparison shows that the intramuscular fat proportion in the thigh and roast beef muscles is different in the groups statistically significant F (22.12 $^{+++}$, $p \leq 0.001$). The muscles of the thighs and roast beef affected the intramuscular fat proportion.

Table 2 Average proportion and statistical evaluation of the intramuscular fat proportion in the *musculus semimembranosus* and in the *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x}$ ±SD, %	Scheffe´s test, $\alpha = 0.05$	
			Beef thigh – *m. quadriceps femoris*	**Roast beef – *m. longissimus dorsi***
Beef thigh – *m. semimembranosus*	6	0.48 ±0.05	$p > 0.05$	$p \leq 0.05$
Beef thigh – *m. quadriceps femoris*	6	0.48 ±0.11	$p \leq 0.05$	
Roast beef – *m. longissimus dorsi*	6	0.83 ±0.14		
Analysis of variance		F (22.12, $p \leq 0.001$)		

Note: m. – muscle, n – multiplicity, SD – standard deviation, $p > 0.05$ – no statistically significant difference, $p \leq 0.001$, $p \leq 0.05$ – a statistically significant difference.

The intramuscular fat proportion in the *m. semimembranosus* of the thigh reached the average proportion of 0.48% and in *m. quadriceps femoris* of the thigh also 0.48%. The average proportion of intramuscular fat was higher in *m. longissimus dorsi* of the roast beef, of all observed muscles, i.e. 0.83%. The difference in the proportion of intramuscular fat between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant ($p > 0.05$). But the difference in the proportion of intramuscular fat was statistically significant ($p \leq 0.05$) between *m. semimembranosus* of the thigh and *m. longissimus dorsi* of the roast beef. Also the difference in the proportion of intramuscular fat between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef was statistically significant ($p \leq 0.05$).

Statistical evaluation of the results of the proportion of intramuscular fat in the thigh and roast beef muscles based on the standard deviation revealed that the measured values fluctuated at *m. thigh semimembranosus* of the thigh SD = 0.05, at *m. quadriceps femoris* of the thigh SD = 0.11, versus *m. longissimus dorsi* of the roast beef SD = 0.14.

The storage of fat in the carcass of bovine animals and the composition of fatty acids in meat play an important role in the variation of dietary properties **[18]**.

Consumers increasingly prefer tasty, juicy, and tender beef. They increasingly seek lower-fat options, believing that such meat is healthier. Intramuscular fat has an important effect on meat palatability due to its specific contribution to influencing juiciness, taste, and tenderness **[19]**. Intramuscular fat storage appears to be regulated by various factors as opposed to those that regulate fat storage in adipose tissue, such as subcutaneous, and metabolic differences between them. Intramuscular adipocytes have higher activity of hexokinase and phosphofructokinase enzymes. Subcutaneous adipose tissue exhibits higher levels of lipogenic enzymes, such as NADP-malate dehydrogenase, phosphogluconate-6-dehydrogenase, and glucose-6-phosphate dehydrogenase, which play important functional roles in lipid metabolism **[20]**.

Saturated fatty acids in the selected beef thigh and roast beef muscles

Average proportion and statistical evaluation of the saturated fatty acids proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef is shown in Table 3.

The saturated fatty acid proportion from the proportion of the total fatty acids of the intramuscular fat in the thigh and roast beef muscles was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The comparison result shows that the saturated fatty acid proportion from the total proportion of fatty acids of intramuscular fat in the thigh and roast beef muscles is different in the groups statistically not significant F (0.41^{-}, p >0.05). The thighs and roast beef muscles did not affect the saturated fatty acid proportion.

Table 3 Average proportion and statistical evaluation of the saturated fatty acids proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x}$ ±SD, %	Scheffe´s test, α = 0.05	
			Beef thigh – *m. quadriceps femoris*	**Roast beef – *m. longissimus dorsi***
Beef thigh – *m. semimembranosus*	6	36.30 ±2.72	p >0.05	p >0.05
Beef thigh – *m. quadriceps femoris*	6	37.12 ±0.71	p >0.05	
Roast beef – *m. longissimus dorsi*	6	39.95 ±0.75		
Analysis of variance		F (0.41, p >0.05)		

Note: m. – muscle, n – multiplicity, SD – standard deviation, p >0.05 – no statistically significant difference.

The saturated fatty acid proportion from the total proportion of the fatty acids of intramuscular fat in the *m. semimembranosus* of the thigh reached the average value of 36.30%, and in *m. quadriceps femoris* of the thigh slightly higher, 37.12%. The average proportion of saturated fatty acids out of the total fatty acid proportion of intramuscular fat in *m. longissimus dorsi* of the roast beef was found to be 36.95%.

The difference in the proportion of saturated fatty acids from the total proportion of fatty acids of intramuscular fat between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant (p >0.05). Also, the difference in the proportion of saturated fatty acids from the total fatty acid proportion of intramuscular fat between *m. semimembranosus* and *m. longissimus dorsi* of the was not statistically significant (p >0.05). But not even the difference between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef was not statistically significant (p >0.05).

Statistical evaluation of the results of the saturated fatty acids proportion from the total proportion of fatty acids of intramuscular fat in the thigh and roast beef muscles based on the standard deviation revealed that the largest fluctuation of the measured values was at *m. semimembranosus* of the thigh and fairly balanced at *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef (SD = 2.72 vs. SD = 0.71 and SD = 0.75).

Vahmani et al. **[21]** state that intramuscular fat in bovine carcass muscle is proportionally composed of an average of 45-48% saturated fatty acids. These values are higher than our results in the intramuscular fat of the examined thigh and roast beef muscles.

Saturated fatty acids have historically been considered undesirable in the human diet **[22]**. The main saturated fatty acids in ruminant meat, which include cattle, are myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0). Some saturated fatty acids (lauric acid, myristic acid, and palmitic acid) have been shown to increase cholesterol by their properties, which are an indicator of the risk of coronary heart disease **[23]**.

In general, elevated low-density lipoprotein (LDL) cholesterol is associated with a higher risk of heart / arterial disease compared to high-density cholesterol (HDL), which is protective **[24]**. The links and mechanisms between saturated fatty acids, cholesterol, and coronary heart disease are complicated and often contradictory, as individual fatty acids are associated with positive, neutral, and negative effects on heart disease **[23]**.

Forouhi et al. **[25]** found an even chain of saturated fatty acids (C14:0, C16:0, and C18:0), which were beneficial in their effects, while saturated fatty acids with an odd chain (C15:0 and C17:0) in low concentrations) were indirectly associated with the occurrence of type 2 diabetes *mellitus*. Khaw et al. **[26]** also reported that saturated fatty acids were associated with a risk of ischemic disease.

Not all fatty acids uniformly affect human health, suggesting that additional subgroups and identifying specific functions of individual fatty acids may help identify risk factors for human health **[27]**.

Monounsaturated fatty acids in the selected beef thigh and roast beef muscles

Average proportion and statistical evaluation of the monounsaturated fatty acids proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef is shown in Table 4.

The monounsaturated fatty acid proportion from the total proportion of the intramuscular fat fatty acids in the thigh and roast beef muscles was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The result of the comparison shows that the monounsaturated fatty acid proportion from the total proportion of fatty acids of intramuscular fat in the muscles of the thigh and roast beef muscles is different in the groups statistically not significant F (0.55^{-}, p>0.05). The thighs and roast beef muscles did not affect the monounsaturated fatty acid proportion.

Table 4 Average proportion and statistical evaluation of the monounsaturated fatty acids proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x}$ ±SD, %	Scheffe´s test, α = 0.05	
			Beef thigh – *m. quadriceps femoris*	Roast beef – *m. longissimus dorsi*
Beef thigh – *m. semimembranosus*	6	48.17 ±2.89	p >0.05	p >0.05
Beef thigh – *m. quadriceps femoris*	6	49.24 ±0.62	p >0.05	
Roast beef – *m. longissimus dorsi*	6	48.44 ±1.11		
Analysis of variance		F (0.55, p >0.05)		

Note: m. – muscle, n – multiplicity, SD – standard deviation, p >0.05 – no statistically significant difference.

The monounsaturated fatty acid proportion from the total proportion of fatty acids of intramuscular fat in the *m. semimembranosus* of the thigh reached the average value of 48.17%, and in the *m. quadriceps femoris* of the thigh slightly higher, 49.24%. The average monounsaturated fatty acid proportion from the total fatty acid proportion of intramuscular fat in *m. longissimus dorsi* of the roast beef was found to be 48.44%.

The difference in the proportion of monounsaturated fatty acids from the total proportion of intramuscular fat fatty acids between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant (p >0.05). Also, the difference in the proportion of monounsaturated fatty acids from the total proportion of fatty acids of intramuscular fat between *m. semimembranosus* of thigh and *m. longissimus dorsi* of the roast beef was not statistically significant (p >0.05). But also, the difference in the proportion of monounsaturated fatty acids from the total fatty acid proportion of intramuscular fat between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef was not statistically significant (p >0.05).

Statistical evaluation of the results of the proportion of monounsaturated fatty acids from the total proportion of intramuscular fat fatty acids in the thigh and roast beef muscles based on the standard deviation revealed that the largest variation of the measured values was at *m. semimembranosus* of the thigh and the lowest at *m. quadriceps femoris* thigh (SD = 2.89 vs. SD = 0.62).

Vahmani et al. **[21]** report in their study a proportion of monounsaturated fatty acids of 35-45% in the intramuscular fat of mature bovine muscle, which are lower results compared to our achieved in the intramuscular fat of the thigh and roast beef.

It turned out, beef obtained from the farming system based on a diet with supplementary concentrate mixture contains monounsaturated fatty acids (with concentration and ratio) as organic/grazing-based alternatives. However, it is unclear why this difference occurs (potentially due to the supply of oleic acid from a conventional diet or de novo synthesis of oleic acid in muscle). There are no known reports of a relationship between higher monounsaturated fatty acids in conventional beef and human nutrition and health. Further research is needed in this area. Although organic meat/pastured meat has less monounsaturated fatty acids, it sometimes contains more vaccenic acid (t11 C18:1) **[28]**, leading to greater de novo synthesis of beneficial substances that can positively affect human health. However, further research is needed to assess this condition **[27]**.

Polyunsaturated fatty acids in the selected beef thigh and roast beef muscles

Average proportion and statistical evaluation of the polyunsaturated fatty acids proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi of the* roast beef is shown in Table 5.

Table 5 Average proportion and statistical evaluation of the polyunsaturated fatty acids proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x}$ ±*SD*, %	Scheffe´s test, $\alpha = 0.05$	
			Beef thigh – *m. quadriceps femoris*	**Roast beef – *m. longissimus dorsi***
Beef thigh – *m. semimembranosus*	6	13.37 ±0.23	$p > 0.05$	$p \leq 0.05$
Beef thigh – *m. quadriceps femoris*	6	13.64 ±0.16	$p \leq 0.05$	
Roast beef – *m. longissimus dorsi*	6	14.63 ±0.59		
Analysis of variance		F (18.39, $p \leq 0.001$)		

Note: m. – muscle, n – multiplicity, SD – standard deviation, $p > 0.05$ – no statistically significant difference, $p \leq 0.001$, $p \leq 0.05$ – a statistically significant difference.

The polyunsaturated fatty acid proportion from the total proportion of intramuscular fatty acids in the thigh and roast beef muscles was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The comparison result shows that the proportion of saturated fatty acids from the total proportion of fatty acids of intramuscular fat in the muscles of the thigh and roast beef muscle is different in the groups statistically significant F (18.39^{+++}, $p \leq 0.001$). The thighs and roast beef muscles affected the polyunsaturated fatty acid proportion.

The polyunsaturated fatty acid proportion from the total fatty acid proportion of intramuscular fat in the *m. semimembranosus* of the thigh reached the average proportion of 13.37%, and in the *m. quadriceps femoris* of the thigh slightly higher, 13.64%. The average proportion of polyunsaturated fatty acids out of the intramuscular fat, total fatty acid proportion in *m. longissimus dorsi* of the roast beef was found to be 14.63%.

The difference in the proportion of polyunsaturated fatty acids from the total proportion of intramuscular fatty acids between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant ($p > 0.05$). However, the difference in the proportion of polyunsaturated fatty acids from the total fatty acid proportion of intramuscular fat between *m. semimembranosus* of the thigh and *m. longissimus dorsi* of the roast beef was statistically significant ($p \leq 0.05$). Also, the difference in the proportion of polyunsaturated fatty acids from the total fatty acid proportion of intramuscular fat between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef was statistically significant ($p \leq 0.05$).

Statistical evaluation of the results of the polyunsaturated fatty acid proportion of the total intramuscular fatty acid proportion in the thigh and roast beef muscles based on the standard deviation revealed that the largest variation of the measured values was at *m. longissimus dorsi* of the roast beef and the lowest at *m. quadriceps femoris* of the thigh (SD = 0.59 vs. SD = 0.16).

Polyunsaturated fatty acid research has become very popular in human nutrition. Polyunsaturated fatty acids are categorized as having more than one double bond and most of them are divided into two main groups: omega-3 (n-3), which has a double bond between the third and fourth carbon from the terminal methyl group, and omega-6 (n-6) having a double bond between the sixth and seventh carbon from the terminal methyl group **[28]**.

The human body can metabolise and synthesise many fatty acids, but two major essential polyunsaturated fatty acids must come from the diet. These are n-3 polyunsaturated fatty acid α-linolenic acid (ALA) and n-6 polyunsaturated fatty acid linoleic acid (LA) **[29]**.

Intervention and observational studies show that replacing the saturated fatty acids in the diet with polyunsaturated fatty acids significantly reduces the risk of cardiovascular disease **[22]**, **[30]**.

The ratio of polyunsaturated to saturated fatty acids in the selected beef thigh and roast beef muscles

Average ratio and statistical evaluation of the polyunsaturated to saturated fatty acids ratio in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi of the* roast beef is shown in Table 6.

Table 6 Average ratio and statistical evaluation of the polyunsaturated to saturated fatty acids ratio in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x} \pm SD$	Scheffe´s test, $\alpha = 0.05$	
			Beef thigh – *m. quadriceps femoris*	Roast beef – *m. longissimus dorsi*
Beef thigh – *m. semimembranosus*	6	0.36 ±0.01	$p > 0.05$	$p \leq 0.05$
Beef thigh – *m. quadriceps femoris*	6	0.37 ±0.01	$p \leq 0.05$	
Roast beef – *m. longissimus dorsi*	6	0.40 ±0.02		
Analysis of variance		F (15.95, $p \leq 0.001$)		

Note: m. – muscle, n – multiplicity, SD – standard deviation, $p > 0.05$ – no statistically significant difference, $p \leq 0.001$, $p \leq 0.05$ – a statistically significant difference.

The ratio of polyunsaturated fatty acids to saturated fatty acids in the intramuscular fat of the thigh and roast beef muscles was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The comparison result shows that the ratio of polyunsaturated to saturated fatty acids in intramuscular fat in the thigh and roast beef muscles is different in the groups statistically significant F (15.95^{+++}, $p \leq 0.001$). The thighs and roast beef muscles affected the ratio of polyunsaturated fatty acids to saturated fatty acids.

The ratio of polyunsaturated fatty acids to saturated fatty acids of intramuscular fat in the *m. semimembranosus* of the thigh reached the average value of 0.36, and in the *m. quadriceps femoris* of the thigh slightly higher, 0.37. The average ratio of polyunsaturated fatty acids to saturated fatty acids in intramuscular fat in *m. longissimus dorsi* of the roast beef was found to be 0.40.

The difference in the values of the ratio of polyunsaturated fatty acids to saturated fatty acids in intramuscular fat between *m. semimembranosus* s and *m. quadriceps femoris* of the thigh was not statistically significant ($p > 0.05$). But the difference in the values of the ratio of polyunsaturated fatty acids to saturated fatty acids in intramuscular fat between *m. semimembranosus* of the thigh and *m. longissimus dorsi* of the roast beef was statistically significant ($p \leq 0.05$). Also, the difference in the values of the ratio of polyunsaturated fatty acids to saturated fatty acids in intramuscular fat between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the was statistically significant ($p \leq 0.05$).

Statistical evaluation of the results of the ratio of polyunsaturated fatty acids to saturated fatty acids in intramuscular fat in the thigh and roast beef muscles based on the standard deviation revealed that the largest fluctuation of values was at *m. longissimus dorsi* of the roast beef and almost half lower at *m. semimembranosus* and *m. quadriceps femoris* of the thigh (SD = 0.02 vs. = 0.01).

Vahmani et al. **[21]** interpret in their study the results of the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA, P : S) as typical low in beef, about 0.1, except in very poor animals for which the ratio of polyunsaturated to saturated fatty acids may be higher, about 0.5-0.7, which is higher than our results of 0.36 and 0.37 in the bovine thigh intramuscular fat or 0.40 in the roast beef intramuscular fat.

The recommended reference value for the ratio of polyunsaturated to saturated fatty acids (PUFA/SFA or P : S) is >0.7) **[31]**. The results of our research differ from the recommended reference value of the ratio of polyunsaturated to saturated fatty acids of the mentioned authors, i.e. 0.46 for the roast beef muscle and 0.36 and 0.37 for the bovine thigh muscle.

The fatty acid composition of meat, which consists of muscle and fat tissue, is important for two reasons. Firstly, it determines the nutritional value and affects various aspects of meat quality, including shelf life and taste. The nutritional value is partly determined by the ratio of polyunsaturated to saturated fatty acids **[32]**.

The ratio of polyunsaturated to monounsaturated fatty acids in the selected beef thigh and roast beef muscles

Average ratio and statistical evaluation of the polyunsaturated to monounsaturated fatty acids ratio in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi of the* roast beef is shown in Table 7.

The ratio of polyunsaturated fatty acids to monounsaturated fatty acids in the intramuscular fat of the thigh and roast beef muscles was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The comparison result shows that the ratio of polyunsaturated to monounsaturated fatty acids in intramuscular fat in the thigh and roast beef muscles differs in the groups statistically significant F (12.44^{+++}, $p \leq 0.001$). The thighs and roast beef muscles affected the ratio of polyunsaturated fatty acids to monounsaturated fatty acids.

Table 7 Average value of the ratio and statistical evaluation of the ratio of the polyunsaturated fatty acids to monounsaturated fatty acids in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x} \pm SD$	Scheffe´s test, $\alpha = 0.05$	
			Beef thigh – *m. quadriceps femoris*	**Roast beef – *m. longissimus dorsi***
Beef thigh – *m. semimembranosus*	6	0.27 ±0.01	$p > 0.05$	$p \leq 0.05$
Beef thigh – *m. quadriceps femoris*	6	0.28 ±0.01	$p \leq 0.05$	
Roast beef – *m. longissimus dorsi*	6	0.30 ±0.02		
Analysis of variance		F(12.44^{+++}, $p \leq 0.001$)		

Note: m. – muscle, n – multiplicity, SD – standard deviation, $p > 0.05$ – no statistically significant difference, $p \leq 0.001$, $p \leq 0.05$ – a statistically significant difference.

The ratio of polyunsaturated fatty acids to monounsaturated fatty acids of intramuscular fat in the *m. semimembranosus* of the thigh reached the average value of 0.27, and in the *m. quadriceps femoris* of the thigh slightly higher, 0.28. The average ratio of polyunsaturated fatty acids to monounsaturated fatty acids in intramuscular fat in *m. longissimus dorsi* of the roast beef was found to be 0.30.

The difference in the values of the ratio of polyunsaturated fatty acids to monounsaturated fatty acids in intramuscular fat between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant ($p > 0.05$). But, the difference in the values of the ratio of polyunsaturated fatty acids to monounsaturated fatty acids in intramuscular fat between *m. semimembranosus* of the thigh and *m. longissimus dorsi* of the roast beef was statistically significant ($p \leq 0.05$). Also, the difference in the values of the ratio of polyunsaturated fatty acids to monounsaturated fatty acids in intramuscular fat between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the was statistically significant ($p \leq 0.05$).

Statistical evaluation of the results of the ratio of polyunsaturated fatty acids to monounsaturated fatty acids in intramuscular fat of the thigh and roast beef muscles based on the standard deviation revealed that the largest fluctuation of values was at *m. longissimus dorsi* of the roast beef and the lowest at *m. quadriceps femoris* of the thigh (SD = 0.02 vs. SD = 0.01).

Differences in fat content affect the composition of fatty acids, regardless of the species or breed of the animal and the factors of nutrition and feeding. The content of saturated fatty acids and of monounsaturated fatty acids increases with increasing fat content faster than the content of polyunsaturated fatty acids, which leads to a decrease in the relative proportion of polyunsaturated fatty acids and consequently to changes in the ratio of polyunsaturated to saturated fatty acids (P : S) **[31]**.

For beef, there is a clear inverse relation between the ratio of polyunsaturated fatty acids to saturated fatty acids and total intramuscular fat. According to various literature sources, the ratio between beef's polyunsaturated and saturated fatty acids can fall to 0.05 for fat breeds. It can also rise to >0.5 for very lean breeds. This variation is much greater than the fact that beef's fatty acid profile is manipulated by using diet or the grazing.

In addition to the procedure of using cattle with the so-called lean meat, the only way to improve the ratio of polyunsaturated fatty acids to saturated fatty acids in ruminant meat, including cattle, is to prevent rumen bio-hydrogenation or to use feed material, feed supplements based on polyunsaturated fatty acids, respectively **[33]**.

The ratio of monounsaturated to saturated fatty acids ratio in the selected beef thigh and roast beef muscles

Average ratio and statistical evaluation of the monounsaturated to saturated fatty acids ratio in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi of the* roast beef is shown in Table 8.

The ratio of monounsaturated fatty acids to saturated fatty acids in the intramuscular fat of the thigh and roast beef muscles was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The comparison shows that the ratio of monounsaturated to saturated fatty acids in intramuscular fat in the thigh and roast beef muscles is different in the groups statistically insignificant F (0.36^{-}, p >0.05). The thighs and roast beef muscles did not affect the ratio of monounsaturated fatty acids to saturated fatty acids.

The ratio of monounsaturated fatty acids to saturated fatty acids in the intramuscular fat of the *m. semimembranosus* of the thigh reached the average value of 1.29, and in *m. quadriceps femoris* of the thigh slightly higher, 1.33. The mean ratio of monounsaturated fatty acids to saturated fatty acids in intramuscular fat in *m. longissimus dorsi* of the roast beef was found to be 1.31.

The difference in the values of the ratio of monounsaturated fatty acids to saturated fatty acids in intramuscular fat between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant (p >0.05). Nor does the difference in the values of the ratio of monounsaturated fatty acids to saturated fatty acids in intramuscular fat between *m. semimembranosus* of the thigh and *m. longissimus dorsi* of the roast beef was not statistically significant (p >0.05). Also, the difference in the values of the ratio of monounsaturated fatty acids to saturated fatty acids in intramuscular fat between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef was not statistically significant (p >0.05).

Table 8 Average ratio and statistical evaluation of the monounsaturated fatty acids to saturated fatty acids ratio in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x}$ ±SD	Scheffe´s test, α = 0.05	
			Beef thigh – *m. quadriceps femoris*	**Roast beef – *m. longissimus dorsi***
Beef thigh – *m. semimembranosus*	6	1.29 ±0.09	p >0.05	p >0.05
Beef thigh – *m. quadriceps femoris*	6	1.33 ±0.04	p >0.05	
Roast beef – *m. longissimus dorsi*	6	1.31 ±0.06		
Analysis of variance	F (0.36, p >0.05)			

Note: m. – muscle, n – multiplicity, SD – standard deviation, p >0.05 – no statistically significant difference.

Statistical evaluation of the results of the ratio of monounsaturated fatty acids to saturated fatty acids in the intramuscular fat of the thigh and roast beef muscles based on the standard deviation revealed that the largest fluctuation of values was at *m. semimembranosus* of the thigh and the lowest *at m. quadriceps femoris* of the thigh (SD = 0.09 vs. SD = 0.04).

Polyunsaturated fatty acids series n-3 in the selected beef thigh and roast beef muscles

Average proportion and statistical evaluation of the polyunsaturated fatty acids series n-3 proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi of the* roast beef is shown in Table 9.

The n-3 polyunsaturated fatty acid proportion from the total proportion of intramuscular fat fatty acids in the thigh and roast muscles was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The result of the comparison shows that the n-3 polyunsaturated fatty acid proportion of the total proportion of intramuscular fat fatty acids in the thigh and roast beef muscles is different in the groups of statistically not significant F (2.61, p >0.05). The thighs and roast beef muscles did not affect the proportion of n-3 polyunsaturated fatty acids.

The proportion of n-3 polyunsaturated fatty acids from the total intramuscular fatty acids in the *m. semimembranosus* of the thigh reached the average value of 0.57%, and in the *m. quadriceps femoris* of the thigh slightly higher, 0.58%. The average proportion of n-3 polyunsaturated fatty acids out of the total intramuscular fat fatty acid proportion in *m. longissimus dorsi* of the roast beef was found to be 0.63%.

The difference in the proportion of n-3 polyunsaturated fatty acids from the total proportion of intramuscular fatty acids between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant (p >0.05). Nor is the difference in the proportion of the n-3 polyunsaturated fatty acids of the total proportion of intramuscular fat fatty acids between *m. semimembranosus* of the thigh and *m. longissimus dorsi* of roast beef was not statistically significant (p>0.05). Also, the difference in the proportion of n-3 polyunsaturated fatty acids from the total proportion of intramuscular fatty acids between *m. quadriceps femoris* of the thighs and *m. longissimus dorsi* of the roast beef was not statistically significant (p >0.05).

Table 9 Average proportion and statistical evaluation of the n-3 polyunsaturated fatty acid proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x}$ ±SD, %	Scheffe´s test, α = 0.05	
			Beef thigh – *m. quadriceps femoris*	**Roast beef – *m. longissimus dorsi***
Beef thigh – *m. semimembranosus*	6	0.57 ±0.05	p >0.05	p >0.05
Beef thigh – *m. quadriceps femoris*	6	0.58 ±0.05	p >0.05	
Roast beef – *m. longissimus dorsi*	6	0.63 ±0.04		
Analysis of variance	F (2.61, p >0.05)			

Note: m. – muscle, n – multiplicity, SD – standard deviation, p >0.05 – no statistically significant difference.

Statistical evaluation of the results of the proportion of n-3 polyunsaturated fatty acids from the total proportion of intramuscular fat fatty acids in the thigh and roast beef muscles based on the standard deviation revealed that the greater variation of the measured values was *m. semimembranosus* a *m. quadriceps femoris* of the thigh and lower at *m. longissimus dorsi* of the (SD = 0.05 vs. SD = 0.04).

N-3 polyunsaturated fatty acids in beef contribute significantly to the overall human n-3 polyunsaturated fatty acid intake. Currently, several brands are applied to selling beef under a strict feed feeding practice code, e.g. pasture (PCAS system in Australia) **[34]**.

Beef is a source of long-chain n-3 essential polyunsaturated fatty acids, often under-consumed in the human diet **[28]**.

Consumption of the very long-chain n-3 polyunsaturated fatty acids reduces the risk of cardiovascular disease and demonstrates reduced arrhythmia, blood pressure, inflammation, platelet sensitivity, and dementia **[35]**.

Consumption of ruminant meat could be a good method to increase the intake of n-3 polyunsaturated fatty acids **[36]**. In contrast, beef from the organic and pasture system contains more n-3 polyunsaturated fatty acids than cattle from a system based on diets with a supplementary concentrate mixture, which benefits the health of the food consumer **[28]**.

This type of meat has price advantages over similar types obtained from conventional farming. Today, consumers are looking for products from natural breeding conditions and are willing to pay more. In the context of n-3 polyunsaturated fatty acids, the health benefits of n-3 polyunsaturated fatty acids need to be more clearly defined. Further studies are needed to indicate the value offered for beef production based on the rearing system, the application of welfare principles, and the type of feed **[37]**.

Polyunsaturated fatty acids n-6 in the selected beef thigh and roast beef muscles

Average proportion and statistical evaluation of the n-6 polyunsaturated fatty acid proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef is shown in Table 10.

The n-6 polyunsaturated fatty acid proportion from the total proportion of intramuscular fat fatty acids in the thigh and roast muscles was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The result of the comparison shows that the proportion of n-6 polyunsaturated fatty acids of the total proportion of intramuscular fat fatty acids in the thigh and roast beef muscles is different in the groups statistically significant F (11.79 $^{+++}$, $p \leq 0.001$). The thighs and roast beef muscles affected the dry matter proportion and the n-6 polyunsaturated fatty acid proportion.

The proportion of n-6 polyunsaturated fatty acids from the total intramuscular fat fatty acids in the *musculus semimembranosus* of the thigh reached the average value of 12.58%, and in the *m. quadriceps femoris* of the thigh slightly higher, 12.68%. The average proportion of n-6 polyunsaturated fatty acids out of the total intramuscular fat fatty acid proportion in *m. longissimus dorsi* of the roast beef was found to be 13.99%.

Table 10 Average proportion and statistical evaluation of the n-6 polyunsaturated fatty acid proportion in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x}$ ±SD, %	Scheffe´s test, $\alpha = 0.05$	
			Beef thigh – *m. quadriceps femoris*	Roast beef – *m. longissimus dorsi*
Beef thigh – *m. semimembranosus*	6	12.58 ±0.41	$p > 0.05$	$p \leq 0.05$
Beef thigh – *m. quadriceps femoris*	6	12.68 ±0.71	$p \leq 0.05$	
Roast beef – *m. longissimus dorsi*	6	13.99 ±0.60		
Analysis of variance		F (11.79, $p \leq 0.001$)		

Note: m. – muscle, n – multiplicity, SD – standard deviation, $p > 0.05$ – no statistically significant difference, $p \leq 0.001$, $p \leq 0.05$ – a statistically significant difference.

The difference in the proportion of the n-6 polyunsaturated fatty acids from the total proportion of intramuscular fat fatty acids between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant ($p > 0.05$). However, the difference in the proportion of n-6 polyunsaturated fatty acids from the total proportion of intramuscular fat fatty acids between *m. semimembranosus* of the thigh and *m. longissimus dorsi* of the roast beef was statistically significant ($p \leq 0.05$). Also, the difference in the proportion of the n-6 polyunsaturated fatty acids from the total proportion of intramuscular fat fatty acids between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef was statistically significant ($p \leq 0.05$).

Statistical evaluation of the results of the n-6 polyunsaturated fatty acid proportion of the total intramuscular fat fatty acid proportion in the thigh and roast beef muscles based on the standard deviation revealed that the greater variation of the measured values was *m. quadriceps femoris* and lower at *m. semimembranosus* of the thigh (SD = 0.71 vs. SD = 0.41).

According to the literature, there are many results on the content of n-6 polyunsaturated fatty acids from the comparison of beef obtained from the cattle breeding system in organic farming and free-range grazing in conventional rearing based on feed ration with a supplementary concentrate mixture, respectively. Some studies report increased levels of linoleic acid in beef from organic rearing **[38]** and others in conventional beef **[28]**. Importantly, in almost all publications, there is a difference in linoleic acid content between farming systems. Still, there is marginal interest in the management of cattle in each farming system, suggesting that management (and thus potential nutrition and feeding of cattle) has a very small effect on the linoleic acid content, the total concentration of n-6 polyunsaturated fatty acids in beef, respectively **[27]**.

The ratio of the n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids in the selected beef thigh and roast beef muscles

Average ratio and statistical evaluation of the n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi of the* roast beef is shown in Table 11.

The ratio of n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids in the intramuscular fat of the thigh and roast beef muscles was statistically evaluated according to the analysis of variance, comparing the critical value with the test characteristic, based on which the null hypothesis H_0 was rejected. The result of the comparison shows that the ratio of the n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids of the intramuscular fat in the thigh and roast beef muscles is different in the groups statistically not significant F (0.12, p >0.05). The thighs and roast beef muscles did not affect the ratio of n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids.

The ratio of n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids of intramuscular fat in the *m. semimembranosus* of the thigh reached the average value of 22.1, and in the *m. quadriceps femoris* of the thigh slightly higher, 21.92. The mean ratio of n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids in intramuscular fat in *m. longissimus dorsi* of the roast beef was found to be 22.41.

Table 11 Average ratio and statistical evaluation of the n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Group	n	$\bar{x}$ ±*SD*	Scheffeho test, α = 0.05	
			Beef thigh – *m. quadriceps femoris*	**Roast beef – *m. longissimus dorsi***
Beef thigh – *m. semimembranosus*	6	22.10 ±2.35	p >0.05	p >0.05
Beef thigh – *m. quadriceps femoris*	6	21.92 ±1.24	p >0.05	
Roast beef – *m. longissimus dorsi*	6	22.41 ±1.40		
Analysis of variance		F (0.12, p >0.05)		

Note: m. – muscle, n – multiplicity, SD – standard deviation, p >0.05 – no statistically significant difference.

The difference in the values of the n-6 polyunsaturated fatty acids ratio to n-3 polyunsaturated fatty acids in the intramuscular fat between *m. semimembranosus* and *m. quadriceps femoris* of the thigh was not statistically significant (p>0.05). Nor does the difference in the values of the n-6 polyunsaturated fatty acids ratio to n-3 polyunsaturated fatty acids in the intramuscular fat between *m. semimembranosus* of thigh and *m. longissimus dorsi* of the roast beef was not statistically significant (p >0.05). Also, the difference in the values of the ratio of n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids in intramuscular fat between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef was not statistically significant (p >0.05).

Statistical evaluation of the ratio of n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids in the intramuscular fat of the thigh and roast beef muscles based on the standard deviation revealed that the largest fluctuation of values was at *m. semimembranosus* and the lowest at *m. quadriceps femoris* of the thigh (SD = 2.35 vs. SD = 1.24).

The recommended reference value for the ratio between n-6 polyunsaturated fatty acids and n-3 polyunsaturated fatty acids is <5 **[31]**. Our research found a much wider ratio between n-6 to n-3 polyunsaturated fatty acids. The ratio between polyunsaturated fatty acids in the roast beef muscle was 22.41, and in the bovine thigh muscle, 21.92 and 22.1.

Correlations between examined variables in the musculus semimembranosus, m. quadriceps femoris of beef thigh and m. longissimus dorsi of the roast beef

The average value and statistical evaluation of the correlations between examined variables in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef is shown in Table 12.

A strong linear relation positive and statistically significant (p ≤0.01, p ≤0.001) was recorded between intramuscular fat and polyunsaturated fatty acids, between intramuscular fat and the ratio of polyunsaturated to saturated fatty acids, between intramuscular fat and the ratio of polyunsaturated to monounsaturated fatty acids,

between intramuscular fat and n-3 polyunsaturated fatty acids, between intramuscular fat and n-6 polyunsaturated fatty acids, also between saturated and monounsaturated fatty acids, between polyunsaturated fatty acids and the ratio of polyunsaturated to saturated fatty acids, between polyunsaturated fatty acids and the ratio of polyunsaturated to monounsaturated fatty acids, between polyunsaturated fatty acids and n-6 polyunsaturated fatty acids, also between the ratio of polyunsaturated to saturated fatty acids and the ratio of polyunsaturated to monounsaturated fatty acids, between the ratio of polyunsaturated to saturated fatty acids and n-6 polyunsaturated fatty acids, between the ratio of polyunsaturated to monounsaturated fatty acids and n-6 polyunsaturated fatty acids, but also between n-3 polyunsaturated fatty acids to n-6 polyunsaturated fatty acids.

Table 12 Average value and statistical evaluation of the correlations between examined variables in the *musculus semimembranosus* and *m. quadriceps femoris* of the beef thigh and in the *m. longissimus dorsi* of the roast beef.

Variable	Fat	SFA	MUFA	PUFA	PUFA/SFA	PUFA/MUFA	MUFA/SFA	n-3	n-6	n-6/n-3
Dry matter	0.38^{-}	0.03^{-}	0.23^{-}	-0.32^{-}	-0.35^{-}	-0.40^{-}	0.13^{-}	-0.30^{-}	-0.42^{-}	-0.02^{-}
Fat		0.04^{-}	-0.02^{-}	0.69^{++}	0.69^{+++}	0.60^{++}	0.09^{-}	0.58^{++}	0.75^{+++}	0.01^{-}
SFA			0.60^{++}	0.09^{-}	0.11^{-}	0.23^{-}	-0.41^{-}	-0.07^{-}	0.27^{-}	0.34^{-}
MUFA				-0.20^{-}	0.05^{-}	-0.25^{-}	0.23^{-}	-0.02^{-}	0.13^{-}	0.15^{-}
PUFA					0.93^{+++}	0.96^{+++}	-0.16^{-}	0.34^{-}	0.71^{+++}	0.25^{-}
PUFA/SFA						0.85^{+++}	-0.02^{-}	0.38-	0.77^{+++}	0.26^{-}
PUFA/MUFA							-0.28^{-}	0.28^{-}	0.68^{++}	0.27^{-}
MUFA/SFA								0.42^{-}	-0.09^{-}	-0.61^{++}
n-3									0.59^{++}	-0.63^{++}
n-6										0.25^{-}

Note: the numerical value is the result of r (correlation coefficient); – designation by upper index means no statistically significant difference in the correlation relation between the two variables (p >0.05); ++, +++ designation by upper index means statistically significant difference in the correlation relation between the two variables ($p \leq 0.01$, $p \leq 0.001$).

A strong linear relationship negative and statistically significant ($p \leq 0.01$) was recorded between the ratio of monounsaturated to saturated fatty acids, the ratio of n-6 to n-3 polyunsaturated fatty acids, and also between n-3 polyunsaturated fatty acids and the ratio of n-6 to n-3 polyunsaturated fatty acids.

Among all other variables, a trivial or mean positive or negative linear relation was recorded as statistically insignificant (p >0.05).

It is crucial to look for healthy foods that meet consumer quality requirements while respecting the doses for the ratio of polyunsaturated fatty acids to saturated fatty acids, n-6 to n-3 polyunsaturated fatty acids set by public health authorities to prevent cardiovascular and other diseases. Fat storage and fatty acid profiles have a major impact on meat quality assessment, and their relation to human health should be made carefully and with greater scientific support. However, further studies are needed to clarify the real impact of fat and fatty acid consumption on human health **[39]**.

CONCLUSION

The presented study is current in terms of research addressing the quality of beef in terms of the fatty acid profile of the health of the food consumer. *Musculus semimembranosus* and *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of the roast beef were selected to research meat obtained from the young bull. The results led to the conclusion based on which it can be stated that between *m. semimembranosus* and *m. quadriceps femoris* of the thighs are not statistically significant differences (p >0.05) in the investigated fatty acids. Statistically significant differences ($p \leq 0.05$) were found between *m. quadriceps femoris* of the thigh and *m. longissimus dorsi* of roast beef in polyunsaturated fatty acids, the ratio of polyunsaturated fatty acids to saturated fatty acids, and the ratio of polyunsaturated fatty acids to monounsaturated fatty acids. Strong, statistically significant ($p \leq 0.01$, $p \leq 0.001$) correlations were found mainly between intramuscular fat and polyunsaturated fatty acids, between intramuscular fat and the ratio of polyunsaturated fatty acids to saturated fatty acids, between intramuscular fat, and the ratio of polyunsaturated fatty acids to monounsaturated fatty acids. In conclusion, it can be stated that the muscles of the thigh and roast beef from young cattle are characterized by statistically significant differences in the proportion of fatty acids. The examined muscles meet the recommended values by the ratio of polyunsaturated fatty acids to saturated fatty acids, increasing and maintaining the health of the food consumer. Still, they pose a risk concerning cardiovascular diseases by the ratio of n-6 polyunsaturated fatty acids to n-3 polyunsaturated fatty acids.

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

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Biofuel production by *Candida tropicalis* from orange peels waste using response surface methodology

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ABSTRACT

Citrus fruits are widely consumed worldwide due to their nutritional and health benefits. However, the disposal of citrus waste poses significant environmental challenges. Orange peels (OP) are a substantial by-product of fruit processing and hold great potential as a source for bioethanol production, promoting investment in utilizing agricultural waste for biofuel purposes. OP offers a cost-effective substrate for producing value-added compounds, including bioethanol. Autoclaved-water treated OP biomass exhibited the highest release of reducing sugars (68.2%) this results supported by SEM images of that Autoclaving has definite effect on the structure of the OP particles. Among the five tested microbes, *Candida tropicalis* was selected as a promising bioethanol candidate due to its ethanol tolerance and ability to utilize xylose. Preliminary screening using Plackett-Burman Design (PBD) was conducted to identify six influential factors affecting the fermentation process at three levels, determining the optimum response region for bioethanol production by *C. tropicalis*. The significant variables were further investigated using Response Surface Methodology-Central Composite Rotatable Design (RSM-CCRD) at five levels, a novel approach in this study. The addition of cysteine and resazurin as reducing agents increased bioethanol production by 2.9 and 2.1 times, respectively, from the treated OP. Under the optimized conditions obtained from RSM-CCRD, bioethanol production reached 16.7 mg/mL per mg/ml reducing sugars. Implementing all the optimized conditions, including an initial pH of 5.75, 3% yeast extract, 2.25 g/L cysteine, 4% inoculum size, 0.6 g/L $ZnSO_4$, 0.29 g/L $MgSO_4$, 0.3 g/L $MnSO_4$, and substrate treatment with active charcoal before fermentation, the bioethanol yield increased by 2.2 times after three days of fermentation using co-cultures of *C. tropicalis* and *Kluyveromyces marxianus*. The fermentation process was conducted at 30 °C and 150 rpm. Exploring OP as a low-cost renewable substrate and employing efficient microorganisms open new avenues for bioethanol production.

Keywords: bioethanol, response surface, OP, submerged fermentation, SEM

INTRODUCTION

One of Biofuel production from agricultural waste materials is a highly effective solution for reducing both crude oil consumption and environmental pollution **[1]**. Each year, over 100 billion metric tons of biomass waste, including forestry residues, agricultural by-products, fruit processing waste, and other food processing waste, are generated globally **[2]**. Improper disposal of these waste materials can lead to severe health and environmental issues. Therefore, it is crucial to develop eco-friendly and efficient strategies for utilizing and managing various types of biomass waste. The increasing demand for alternative and sustainable energy sources, driven by concerns about energy security and environmental safety, has placed liquid biofuels, which account for approximately 40% of global energy consumption, among the prioritized renewable energies **[3]**, **[4]**.

Citrus fruits, particularly oranges, are widely cultivated and consumed worldwide, generating significant amounts of fruit waste. This waste, rich in sugars such as sucrose, glucose, and fructose, can be fermented to produce bioethanol. Oranges alone contribute to approximately 55% of global citrus fruit production. The potential of orange peels (OP) as a raw material for ethanol production has been extensively studied at both pilot plant and laboratory scales **[1]**. OP contains fermentable sugars like glucose, fructose, sucrose, and insoluble polysaccharides such as cellulose and pectin **[5]**. The low lignin content of OP makes it an ideal substrate for ethanol production; however, pectin requires pretreatment to release the sugars. Citrus-processing industries generate enormous amounts of waste yearly, with citrus peel waste accounting for nearly 50% of the wet fruit mass. Citrus waste holds significant economic value due to its abundance of flavonoids, carotenoids, dietary fiber, sugars, polyphenols, essential oils, ascorbic acid, and trace elements **[6]**. Hence, OP represents a promising substrate for numerous industrial applications.

Furthermore, environmental concerns, long-term economic sustainability, and national security have increased interest in renewable and domestically sourced fuels as alternatives to fossil fuels **[7]**. The depletion of global petroleum-based fuel reserves and the rising prices of such fuels have driven research on alternative fuel sources. In this context, bioethanol derived from renewable feedstock through bioconversion is widely recognised as a viable alternative fuel. The potential environmental benefits of replacing petroleum-based fuels with biofuels derived from renewable sources are significant driving factors for promoting biofuel production **[8]**. Orange peels can serve as a renewable source for bioethanol production, offering increased productivity and reduced processing costs while adding value to the orange juice industry waste. Most studies on bioethanol production through yeast fermentation have utilized Saccharomyces cerevisiae, which finds extensive applications in food and biofuels **[9]**, **[4]**. However, **[10]** reported a release of 10.924% using *S. cerevisiae* and *C. tropicalis* under optimum conditions, such as H_2O_2-pretreated corn stover (12%), inoculation (25%), pH of 5, and a temperature of 32 °C after 144 hours.

In biological experiments, it is important and advantageous to employ techniques that minimize costs by reducing the number of required experimental formulations to study specific characteristics **[11]**. The application of Response Surface Methodology (RSM) has demonstrated successful optimization of parameters for enzyme production, ethanol, and other bioprocesses **[12]**. Among the various second-order designs, Central Composite Design (CCD) is the most widely used class in RSM **[13]**. Surface plots, derived from fitting individual models to dependent variables, are created and overlaid to identify regions where acceptable predictions for independent variables coincide **[14]**. Thus, the primary objective of this study is to produce bioethanol from OP waste, starting with biomass pre-treatment and bio-treatment, followed by fermentation of liberated sugars using selected microorganisms. *Candida tropicalis* was investigated as a new biofuel candidate, exhibiting promising characteristics for bioethanol production from OP. The optimization of bioethanol production from pre-treated OP biomass was carried out using RSM-CCRD based on significant factors identified through preliminary PBD screening. Moreover, for the first time, the entire fermentation process was optimized using co-cultures of *Candida tropicalis* and *Kluyveromyces marixanus K77* as bioethanol producers from the OP substrate.

Scientific hypothesis

Production of bioethanol from treated orange peel is not less than or equal 20% using RSM-CCRD.

MATERIAL AND METHODOLOGY

Samples

The OP waste samples used in this study were collected from local household and orange juice shops present inside Sadat City, Minufiya Governorate. The collected fresh OP samples were washed with tap water and then dried in an oven at 60 °C for 24 hours till the final constant weight was achieved.

Chemicals

Chemicals used in this study were obtained from the following sources: Hydrochloric acid (HCl, 35%), sulfuric acid (H_2SO_4, 98%), sodium hydroxide (NaOH, 99%), calcium hydroxide ($Ca(OH)_2$, 85%), sodium potassium tartrate (99%), dextrose anhydrous(>99%), Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3, 99%), and 3,5-dinitrosalicylic acid (DNS, 99%) reagent were purchased from Central Drug House,India. Ammonium hydroxide (NH_4OH, 25%), gallic acid (98%), sodium citrate (99%), citric acid (99%), ammonium sulfate ($(NH_4)_2SO_4$, 99.5%), potassium dichromate($K_2Cr_2O_7$, 99.5%) and active charcoal powder were chemicals obtained from Biochem-Egypt. Cobalt chloride ($CoCl_2$, ATC) was obtained from Loba Chemie-India. Yeast extract, malt extract, agar, peptone, and Whatman filter paper (0.22µm) were obtained from El-Gomhouria Company Egypt. Ferrous sulfate ($FeSO_4.7H_2O$, 99%), manganese sulfate ($MnSO_4$, 98%), zinc sulfate ($ZnSO_4$, 99%), glycerol (98%), and potassium phosphate (K_2HPO_4, 99%) were purchased from El Nasr-Pharmaceutical CO. Egypt. Resazurin (75%) and cysteine(PTC) were obtained from Fisher Scientific.

Biological material

The microorganisms used in this study were obtained from GEBRI, University of Sadat City. They included *Geotrichum candidum*, *Rhizopus oryzae* NRRL 3563, *Candida tropicalis*, *Candida oleaphila* , *Kluyveromyces marixanus* K77, *Pichia anomala* J121, *Saccharomyces boulardii* CNCM I-745, and *Saccharomyces cerevisiae*.

Instruments

The MOD MARS 6 microwave sample preparation system (MARS 6 Synthesis, CEM) was used for sample preparation. The amino acid composition was determined using high-performance liquid chromatography "Agilent-1200", with a separating column InfinityLab Poroshell 120 HILIC 1.9 microns.

Laboratory Methods

Orange Peels Chemical Composition: Orange peels (OP) waste samples were collected from local households in Sadat City, Minufiya Governorate, Egypt, from 2016 to 2017. The proximate the proximate analysis of the collected OP that were used in this study is presented in Table 1. The moisture content of the dried samples was determined following the standard procedure of the US National Renewable Energy Laboratory **[15]**. The ash content was determined using AOAC methods **[16]**. Pectin analysis was conducted according to the method described by Sudhakar and Maini **[72]**. Acid detergent fiber (ADF), neutral detergent fiber (NDF), and acid detergent lignin (ADL) were determined following the method outlined by Cypriano **[17]**. All analyses were performed in triplicate, and the mean and standard deviation (SD) values were calculated using MS Excel.

Media and Microorganisms: Yeast Malt Peptone (YMP) and Potato-Dextrose Agar (PDA) media were utilized for maintaining fungal and yeast isolates. All media were prepared using double distilled water, and the pH was adjusted to 5.6 ±0.2. The fermentation media employed for bioethanol production **[18]** had the following composition (g/L): $(NH_4)_2SO_4$ 1, KH_2PO_4 1, Yeast extract 10. The fermentation medium was modified to include selected reducing agents such as resazurin and cysteine (0.5-1.5). All media were prepared as described, using double distilled water, and the pH was adjusted using NaOH (1N) or HCl (1N). The media were autoclaved at 121 °C for 20 minutes at 15 psi.

Physical, Chemical, and Water/Autoclaving Pre-treatment: The dried samples were subsequently ground using a spice-grinding machine and kept for all experiments (Figure 1).Alkaline hydrolysis using 2% NaOH was used with a biomass loading of 10% (w/v) in 250 mL screw-capped bottles. The bottles were then autoclaved for 30 minutes at 121 °C. After autoclaving, the treated samples were allowed to settle and cool. The biomass was then filtered and washed with distilled water. Ammonia pre-treatment was carried out using 15% ammonia with a solid-to-liquid ratio of 1:6 (g/mL; w/v) in 250 mL screw-capped bottles **[19]**. The biomass was placed in a water bath overnight at 60 °C.

Distilled water was hydrolysed, with a biomass loading of 10% (w/v) in 250 mL screw-capped bottles. The bottles were autoclaved for 30 minutes at 121 °C and a pressure of 1.5 atmospheres. The filtrate was allowed to settle and cool, and the biomass was filtered. Finally, the treated biomass was filtered, and the filtrate was adjusted to pH 4.5. The total reducing sugar content was measured using the DNS method with a spectrophotometer at λ540 nm **[20]**.

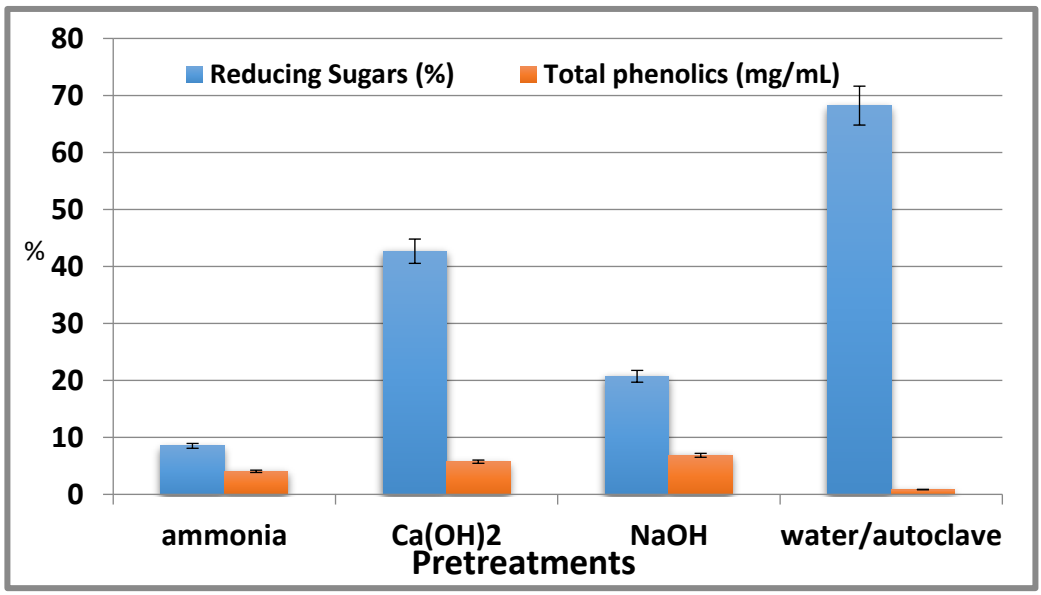

Figure 1 Pretreatments of orange-peels biomass (OP) showing released sugars yield (%), and total phenolic content (mg/mL) after treatment.

Bio-treatment of Orange Peel (OP) Biomass: Cultures of *Geotrichum candidum* and *Rhizopus oryzae* NRRL 3563 were grown on Potato-Dextrose Agar (PDA) plates for 5-6 days. One-week-old slants were mixed with a sterile solution of 0.05% Tween-80 in water to prepare the inoculum. The hydrolysis medium was then inoculated with four discs of *G. candidum* or *R. oryzae*, which had been freshly grown for 3-5 days at 30 °C **[21]**. All experiments were conducted in 250-mL conical flasks containing 5 g of OP biomass substrate and 100 mL of distilled water. The flasks were autoclaved at 121 °C for 20 minutes. The prepared microbial inoculum was added to the autoclaved OP biomass and incubated at 30 °C for 7 days on a rotary shaking incubator (New Brunswick, Canada) at 150 rpm. Samples were collected over a 6-day time course, and the biomass was separated by filtration using Whatman filter paper (No. 1). The filtrate was stored at -18 °C until it was assayed for total sugars using the DNS method.

Selection of Yeast for Fermentation: *Saccharomyces cerevisiae*, *Candida oleophila*, *Candida tropicalis*, *Kluyveromyces marixanus*, and *Pichia anomala* were assessed for their potential in producing bioethanol using the treated biomass over 4 days. To determine their ethanol tolerance, the selected strains were subjected to an ethanol tolerance test in glass-screw tubes containing YMP broth media with varying alcohol concentrations (1%, 2%, 3%, 4%, 8%, and 10%) **[22]**. The inoculated tubes were incubated under static conditions at 30 °C, and yeast viability was subsequently examined. After a 72-hour incubation at 30 °C, the microbial numbers were quantified as colony-forming units per milliliter (CFU/mL).

The chosen yeast strains were cultivated and maintained on YMP slants. After 48 hours of incubation at 30 °C, the grown cultures with an optical density of 0.55 at 600 nm (O.D.600 0.55) were utilized for inoculating the fermentation media at a volume of 2% (v/v). The fermentation medium was prepared in 100 mL screw-capped bottles using treated OP biomass. The medium composition consisted of a steam-autoclaved suspension of pretreated OP (50 mL), along with additional components such as KH_2PO_4 (0.1%), $(NH_4)_2SO_4$ (0.1%), and yeast extract (1%). The pH was adjusted to 5.5, and the bottles were autoclaved for 20 minutes at 121 °C **[23]**.

The autoclaved flasks were opened while still hot in a sterile environment within a biosafety cabinet to eliminate volatile compounds, such as D-limonene, which can hinder yeast growth. One of the screw-capped bottles served as a control without inoculation, while the others were inoculated with a seeding inoculum (inoculum load of 2%). Following inoculation, the screw-capped bottles were incubated at 30 °C in a shaking incubator with an agitation rate of 120 rpm for 5 days. On days 1, 2, and 3 of fermentation, the fermented broth was sampled and subsequently centrifuged for 15 minutes at 6000 rpm. The supernatant was collected, and the ethanol concentration was determined using the dichromate method.

Reducing Agent Selection: To optimize bioethanol production, two reducing agents, cysteine and resazurin, were evaluated following the method described by Anschau et al. **[24]**. In 100 mL screw-capped bottles, a solution of 50 mL treated OP biomass was combined with KH_2PO_4 (0.1%), $(NH_4)_2SO_4$ (0.1%), and yeast extract (1%). Various concentrations of cysteine and resazurin (0.5, 0.75, and 1 g/L) were added to the mixture, and the pH was adjusted to 5.5. The bottles were then autoclaved for 20 minutes at 121 °C. One bottle was left uninoculated as a control, and another without the reducing agent was included for comparison. All the inoculated bottles were incubated at 30 °C in a shaking incubator with an agitation rate of 120 rpm for 3 days. Daily samples were withdrawn for analysis.

Plackett-Burman Screening Design (PBD): Bioethanol production in the fermentation process is influenced by various factors, including nutritional and environmental conditions **[4]**. In the Plackett-Burman screening design (PBD), six variables were considered: initial pH, substrate concentration, reducing agent, inoculum size, agitation speed, and yeast extract concentration. Each variable was set at levels -1 and +1, representing low and high levels, respectively **[25]**. The PBD aimed to identify significant variables affecting bioethanol production, without considering the interaction effects between variables. The results of the PBD were analyzed using JMP version 8 software (SAS Institute Inc., Cary, NC, USA). All experiments were performed in triplicate, and the mean values of bioethanol production were recorded as the response (Plackett and Burman **[73]**). The experimental design consisted of 15 experiments, each with different settings for the six variables, as outlined in Table 2. The PBD utilized a first-order model, represented by Equation 1.

$$Y = \beta 0 + \Sigma \beta i x i \quad (1)$$

In the equation, Y represents the response (bioethanol production yield), β0 denotes the model intercept, βi represents the linear coefficient, and xi represents the level of the independent variable.

Central Composite Rotatable Design (CCRD): Building upon the results of the Plackett-Burman screening design (PBD), a Central Composite Rotatable Design (CCRD) was employed to further investigate the significant factors identified in the PBD, namely pH (X1), reducing agent (X2), and substrate concentration (X3). These factors were chosen as the main variables and were assigned five levels, coded as -2, -1, 0, +1, and +2. The

CCRD consisted of 17 trials, including three center points, and is presented in Table 3. A second-order polynomial function was used to predict the optimal conditions to establish the relationship between the independent variables and the bioethanol production as the response variable **[12]**. The function is represented by Equation 2:

$$Y = \beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2 + \beta_{13}X_1X_3 +\beta_{23}X_2X_3 +\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2 \quad (2)$$

In the equation, Y represents the predicted response (bioethanol production), β0 is the model constant, X_1, X_2, and X_3 are the independent variables, β_1, β_2, and β_3 are the linear coefficients, β_{12}, β_{13}, and β_{23} are the cross-product coefficients, and β_{11}, β_{22}, and β_{33} are the quadratic coefficients. Regression analysis of the experimental data was performed using JMP version 8 software (SAS Institute Inc., Cary, NC, USA) **[26]**. The coefficient of determination, R^2, was used to assess the quality of fit of the polynomial model equation. The experiments were conducted in triplicate, and the mean values were calculated. Other factors were kept constant throughout all the response surface methodology (RSM) experiments.

Enhancement of bioethanol production (Active charcoal, Metals addition, and Cocultures addition): To enhance bioethanol production, C. tropicalis was inoculated into sterilized 250 mL Erlenmeyer flasks containing 100 mL of YMP broth and incubated at 30 °C for 48 h at 120 rpm on a shaking incubator to obtain the seeding culture. This seeding inoculum was then transferred to sterilized 500 mL Erlenmeyer flasks containing 200 mL of YMP broth. The fermentation media were prepared using the optimized conditions determined from RSM-CCRD, which included 3% yeast extract and an inoculum size of 4% of O.D600 = 1. Other factors such as a reducing agent at a concentration of 2.25 g/L and an initial pH of 5.75 were also included. Additionally, the effect of metal supplementation was investigated by adding 0.6 g/L of zinc sulfate, 0.29 g/L of magnesium sulfate, and 0.3 g/L of manganese sulfate **[27]**. The fermentation mixture was then transferred to 1 L screw capped bottles and incubated at 30 °C and 120 rpm for 2 days.

To further enhance bioethanol production, 1 g of active charcoal was added to 1 L of pretreated OP biomass based on the method described by Ma et al. **[28]**. The mixture was allowed to settle for 2 hours, filtered, and then used in fermentation. The initial reducing sugar concentration was increased to 21.2 mg/mL while maintaining the same conditions as described earlier.

For coculture experiments, 24-hour-old cultures of *C. tropicalis* and *K. marxianus* were separately grown on YMP agar media. They were then used to inoculate 100 mL of YMP broth medium, followed by incubation at 30 °C for 24 hours at 120 rpm. Two bottles were inoculated with the 24-hour-old C. tropicalis seeding broth, and another two bottles were inoculated with the 24-hour-old *K. marxianus* seeding broth. These bottles were incubated for 3 days at 30 °C. After 3 days, two different yeast cultures were frozen for 12 hours and then inoculated with the other yeast culture (24-hour-old). Additional bottles were inoculated directly with the yeast strains without freezing and incubated for 3 days at 30 °C. Samples were withdrawn daily and centrifuged at 6000 g for 15 minutes. The supernatants were stored at -18 °C for further analysis. The experiment was repeated twice, and the average values of bioethanol production were determined along with their standard error.

Scanning electron microscope (SEM): To analyze the treated and untreated OP samples, they were first fixed using 2.5% glutaraldehyde and then dehydrated using a series of ethanol dilutions with agitation in an automatic tissue processor (Leica EM TP, Leica Microsystems, Austria). The samples were then dried using a CO_2 critical point drier (Model: Audosamdri-815, Tousimis, Rockville, Maryland, USA). Finally, the samples were coated with gold using a sputter coater (SPI-Module) based on the method described by Tan et al. **[29]**. The coated samples were observed using a scanning electron microscope (JSM-5500 LV; JEOL Ltd, Japan) in high vacuum mode at the Regional Center of Mycology and Biotechnology in Cairo, Egypt. HPLC analysis: High-performance liquid chromatography (HPLC) was employed to analyze sugars such as glucose, xylose, fructose, and others. Isocratic Milli-Q degassed deionized water was used as the mobile phase, flowing at a 0.8 mL/min rate. The analysis was conducted using a Refractive Index (RI) detector, which was maintained at a temperature of 65 °C. Samples were withdrawn at specific reaction times, and then they were diluted, centrifuged, and filtered through 0.45 µm membranes into HPLC vials, which were stored at 4 °C. The peaks corresponding to the sugars were detected and quantified based on the area and retention time, utilizing standards of glucose, fructose, sucrose, xylose, mannose, and galactose obtained from Sigma Aldrich.

Determination of reducing sugars: A DNS (3,5-dinitrosalicylic acid) reagent was prepared following the method described by Miller **[77]** to determine the content of reducing sugars. In this process, 1 g of DNS was dissolved in 20 mL of 2 M NaOH, and then 30 g of sodium potassium tartrate was slowly added. The mixture was diluted to a final volume of 100 mL using distilled water.

In a clean and dry test tube, 0.5 mL of the sample was combined with 0.5 mL of the DNS reagent. The resulting mixture was boiled for 5 minutes and then cooled to halt the reaction. The absorbance of the solution was measured spectrophotometrically at a wavelength of 540 nm, using a calibration curve prepared with a standard

glucose solution. The calibration curve was created using anhydrous glucose standard solutions with concentrations of 0.5, 1, 2, 3, 4, and 5 g/L. A blank solution prepared with distilled water was also used.The yield of reducing sugars (%) was calculated using the following formula, as outlined by Chen et al. **[30]**:

$$\textbf{Hydrolysis Yield } (\%) = \frac{(\textbf{Reducing sugars in mg/mL} * \mathbf{0.9} * \mathbf{100})}{\textbf{polysaccharide in substrate}}$$

Determination of total phenolics: In a clean and dry test tube, 3.16 mL of distilled water was combined with 40 µL of the sample. To this mixture, 200 µL of Folin-reagent was added and allowed to settle for 5 minutes. Next, 600 µL of a 20% Na_2CO_3 solution was added, and the mixture was left to settle for 2 hours in a clean, dark place. The absorption of the samples was measured using a spectrophotometer at a wavelength of 750 nm, as described by Blainski et al. **[31]**. All analyses were performed in triplicate, and the mean values and standard deviations (SD) were calculated using MS Excel.

Determination of bioethanol using the dichromate oxidation method: To prepare the potassium dichromate reagent, 62.5 mL of distilled water was slowly mixed with 162.5 mL of concentrated sulfuric acid. The mixture was then cooled under tap water. Subsequently, 17 g of potassium dichromate was added and diluted to a final volume of 250 mL. In a clean and dry test tube, 30 µL of the sample was diluted with 500 µL of distilled water. Then, 2 mL of the prepared potassium dichromate reagent was added, followed by 1 mL of 2 M sodium hydroxide. The mixture was incubated at 50 °C for 30 minutes. The samples were then measured using a spectrophotometer at a wavelength of 600 nm. The reagent was prepared by mixing 62.5 mL of distilled water with 162.5 mL of concentrated sulfuric acid. After cooling under tap water, 17 g of potassium dichromate was added, and the solution was diluted to a final volume of 250 mL. The samples were measured using a spectrophotometer at a wavelength of 600 nm, as described by Mushimiyimana et al. **[32]**.

Description of the Experiment: In accordance with the methodology outlined in the study, a total of 32 orange peel (OP) samples were meticulously prepared. These samples were further categorized into two groups: 15 derived from experimental sources and 17 from trials. Each of these samples underwent a chemical analysis, with three replicates conducted for every individual experiment, resulting in a total of 96 samples. The study rigorously adhered to the true experimental design framework, incorporating key elements such as randomization and control which involve three experimental treatments tested in BPD, RSM, and RSM-CCRD with the primary objective was to identify the treatment that yielded the highest efficiency in bioethanol production from OP.

Statistical Analysis

All experiments were performed in three replicates and the mean, standard deviation and analysis of variance (ANOVA) were used for statistical analysis of the experimental results. The significant differences between data obtained was tested at p <0. 05 using SPSS version 22 (IBM,USA).

RESULTS AND DISCUSSION

Chemical analysis of orange peels (OP) and its pre-treatment

The utilization of vegetable and fruit wastes for biofuel production has gained significant attention due to their abundance and high content of sugars, cellulose, and hemicellulose **[2]**. These wastes, including orange peels (OP), offer a cost-effective and sustainable alternative to fossil fuels. Previous studies have explored the potential of various agro-industrial wastes such as potato peel waste, rice straw, grape pomace, and apple pomace for bioethanol production using different microbial strains **[33]**.

In this study, the chemical composition of OP, based on dry weight, was analyzed (Table 1). The results revealed that OP contains substantial amounts of pectin, fiber, and saccharides, which can be effectively utilized in the fermentation process for bioethanol production. The presence of carbohydrates, cellulose, and pectin in OP was notable (Table 1). The relatively low lignin content of OP makes it easily hydrolysable. Fiber, pectin, cellulose, and total sugars collectively constitute approximately 71% of the total chemical composition. These findings align with the chemical analysis of OP conducted by **[5]**.

Table 1 Chemical composition of orange-peels biomass used in ethanol production[1].

Component	(%)
Moisture	12.1 ±0.21
Fiber	26.3 ±0.42
Total Sugars	12.6 ±0.90
Pectin	19.2 ±0.32
Lignin	0.4 ±0.02
Cellulose	13.07 ±0.35
Protein	7.8 ±0.32
Fat	12.3 ±0.15
Ash	2.1 ±0.07
Hemicellulose	1.4 ±0.04
Neutral detergent fibre	10.3 ±0.22
Acid detergent fibre	8.9 ±0.22
Total digestible nutrients	85.3 ±0.68
Net energy (Mcal/kg)	2.03

Note: [1]Values are means of three replicates (±SD) and analysis are measured on dry weight bases.

The chemical composition analysis highlights the potential of OP as a valuable feedstock for bioethanol production. The high content of sugars and cellulose indicates the availability of fermentable substrates for microbial conversion into bioethanol. Furthermore, the presence of pectin offers additional fermentable sugars, contributing to the overall bioethanol yield. The composition of OP supports its suitability as a renewable and sustainable resource for biofuel production, emphasizing its significance in waste valorization and energy sustainability.

The utilization of lignocellulosic biomass for producing value-added products holds great promise due to its abundant availability as unused biomass and its cost-effectiveness **[1]**. Importantly, this approach does not compete with food production or require additional land use, making it an environmentally sustainable option. Among the critical steps in the conversion of lignocellulosic biomass to bioethanol, pre-treatment plays a crucial role in enhancing the overall process efficiency by facilitating hydrolysis and increasing the yield of fermentable sugars, ultimately impacting ethanol production and production costs.

Various pre-treatment methods, including physical, chemical, and biological approaches, are currently employed in lignocellulosic biomass processing **[4]**. In the case of orange peels (OP), pre-treatment effects are believed to arise from the reduction of cellulose crystallinity and the increased porosity of the biomass, which promote easier hydrolysis and the release of sugars **[34]**. During pre-treatment, the complex and highly cross-linked structure of lignin, which consists of phenolic monomers, undergoes breakdown, producing different phenolic compounds **[35]**.

The pre-treatment step in the production of bioethanol from lignocellulosic biomass is crucial for maximizing the efficiency of subsequent hydrolysis and fermentation processes. By reducing cellulose crystallinity, increasing biomass porosity, and facilitating the breakdown of lignin, pre-treatment enables improved access to fermentable sugars and enhances overall ethanol yields. The selection of appropriate pre-treatment methods tailored to specific biomass feedstocks is essential for optimizing the efficiency and cost-effectiveness of the bioethanol production process.

This study investigated various pre-treatment methods, including water/autoclaved, NaOH, $Ca(OH)_2$, and ammonia (Figure 1). Each pre-treatment method exhibited distinct effects on the treated substrate. Mechanical size reduction, for instance, improved the efficiency of downstream processes by making the substrate biomass more susceptible to hydrolysis, thereby increasing the yield of monomeric sugars **[36]**.

The results indicated that the highest release of reducing sugars (68.2%) was achieved by subjecting 10 g of OP substrate to water/autoclaved pre-treatment for 30 minutes, resulting in a phenolic content of 0.84 mg/mL. Conversely, the lowest release of reducing sugars (8.5%) was observed with 15% ammonia pre-treatment in a water bath at 60 °C overnight (Figure 1). Steam-based hydrolysis, conducted under high temperature and pressure, led to the partial hydrolysis of soluble fractions, thereby concentrating the insoluble fractions for subsequent separation and bioconversion **[8]**.

Alkali and acid pre-treatments caused the degradation of ester and glycosidic bonds, resulting in the partial de-crystallization of cellulose and the liberation of individual monosaccharides. These treatments also induced alterations in the lignin structure and partial solvation of hemicellulose, enhancing cellulose digestibility and lignin solubilization **[37]**. On the other hand, water-autoclaved pre-treatment involved water penetration into

biomass cells, cellulose hydration, and the dissolution of hemicellulose and lignin, thereby facilitating the disruption of the lignocellulosic structure **[38]**.

Due to the complex nature of lignocellulosic biomass, various physical and chemical pre-treatment methods were investigated, followed by enzymatic hydrolysis to break down the biomass into simple sugars. These pre-treatments increased the material's porosity and reduced the crystallinity of cellulose **[38]**. de la Torre et al. **[39]** reported that orange waste contains several monosaccharides, and under specific conditions (pH 5.2, 50 °C, 300 rpm agitation speed, and enzyme concentrations of 6.7% w/w), glucose yields exceeding 80% were achieved. The pre-treatment method that yielded the highest amount of sugars while minimizing the presence of inhibitory compounds was selected for the subsequent fermentation process.

SEM analysis: The SEM analysis conducted at a scale of 500 µm (Figure 2) confirmed that autoclaving steam pre-treatment induced a significant change in the morphology of OP biomasses. A clear distinction can be observed between the morphology of the OP samples before (A, A') and after (B, B') the pre-treatment process. The pre-treatment increased the amorphous portion and a corresponding decrease in the crystalline part of the biomasses. The SEM micrographs reveal noticeable cracks on the surface of the fibers and splitting of the surface near the center.

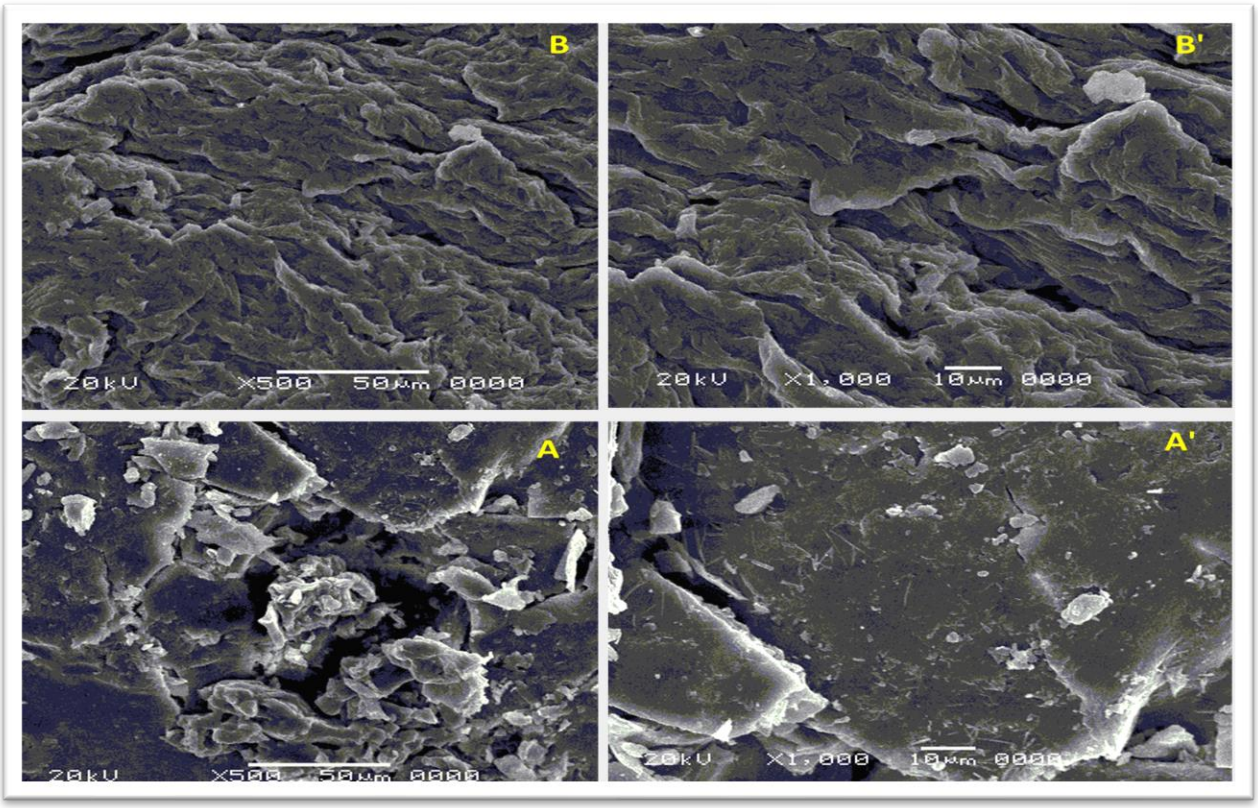

Figure 2 SEM micrograph of orange-peels biomass before (A, A') and after (B, B') water hydrolysis (autoclaving) treatment. Note: boundaries of the biomass after the pre-treatment (B, B'), while the untreated samples appear densely packed in comparison (A, A').

These observations are consistent with the findings of Li et al. **[40]**, who examined the effect of microwave treatment on OP morphology using SEM micrographs at a scale of 1000 µm before and after pre-treatment. They reported that OP exhibited a smooth surface before treatment, which transformed into a cracked and porous structure after microwave treatment. Similarly, Xu et al. **[41]** documented that steam explosion hydrolysis changed corn stover's morphology, transitioning from a smooth surface with a high crystal structure before treatment to a cracked and porous structure after treatment.

Fungal bio-treatment hydrolysis: The advancements in modern biotechnology have contributed significantly to reducing the cost of enzyme production, thereby enhancing the economic viability of the hydrolysis process **[74]**. As enzymes constitute a substantial portion of the production cost, minimizing their usage during hydrolysis can play a crucial role in cost reduction **[5]**. Filamentous fungi are a prominent source of cellulases and hemicellulases **[42]**. Fungi such as *Aspergillus niger*, *Geotrichium candidium*, and *Rhizopus oryzae*

have demonstrated the ability to produce cellulase and pectinase enzymes for the hydrolysis of lignocellulosic substrates **[43]**.

In this study, *R. oryzae* and *G. candidium*, previously recognized as producers of cellulase and pectinase, were inoculated into a medium containing untreated OP biomass. The cultures were incubated at 30 °C with an agitation rate of 150 rpm (Figure 3). For the incubation, both fungi exhibited distinct hydrolysis patterns, and the release of reducing sugars from the biotreated OP increased over time, reaching its peak values of 87.6% and 40.7% on the 2nd and 3rd days, respectively. The release of reducing sugars can be attributed to the enzymatic alteration or degradation of cellulose and pectin by the fungal enzymes, which act on cellulose's reducing and non-reducing ends. Additionally, the action of the enzymes opens up the cell wall structure, facilitating the subsequent hydrolysis of biopolymers **[6]**. Both fungal species are recognized for their ability to produce extracellular enzymes that degrade cell walls, making them valuable for industrial applications **[42]**. The genus *Rhizopus* includes several species employed for industrial enzyme production, such as glucoamylases, cellulases, and tannases, as well as organic acids **[44]**. Currently, this microorganism is primarily used to produce lactic and fumaric acid, as well as enzymes like lipases, amylases, and cellulases **[45]**. *Geotrichum sp*., on the other hand, has been reported as a proficient producer of active polygalacturonase among yeasts **[46]**.

However, the overall results depicted in Figure 3 indicate that *G. candidium* and *R. oryzae* require approximately 2 and 5 days to achieve a reducing sugar content of 51.9% and 43.1%. Consequently, water-autoclaving hydrolysis was employed before further optimization of bioethanol production.

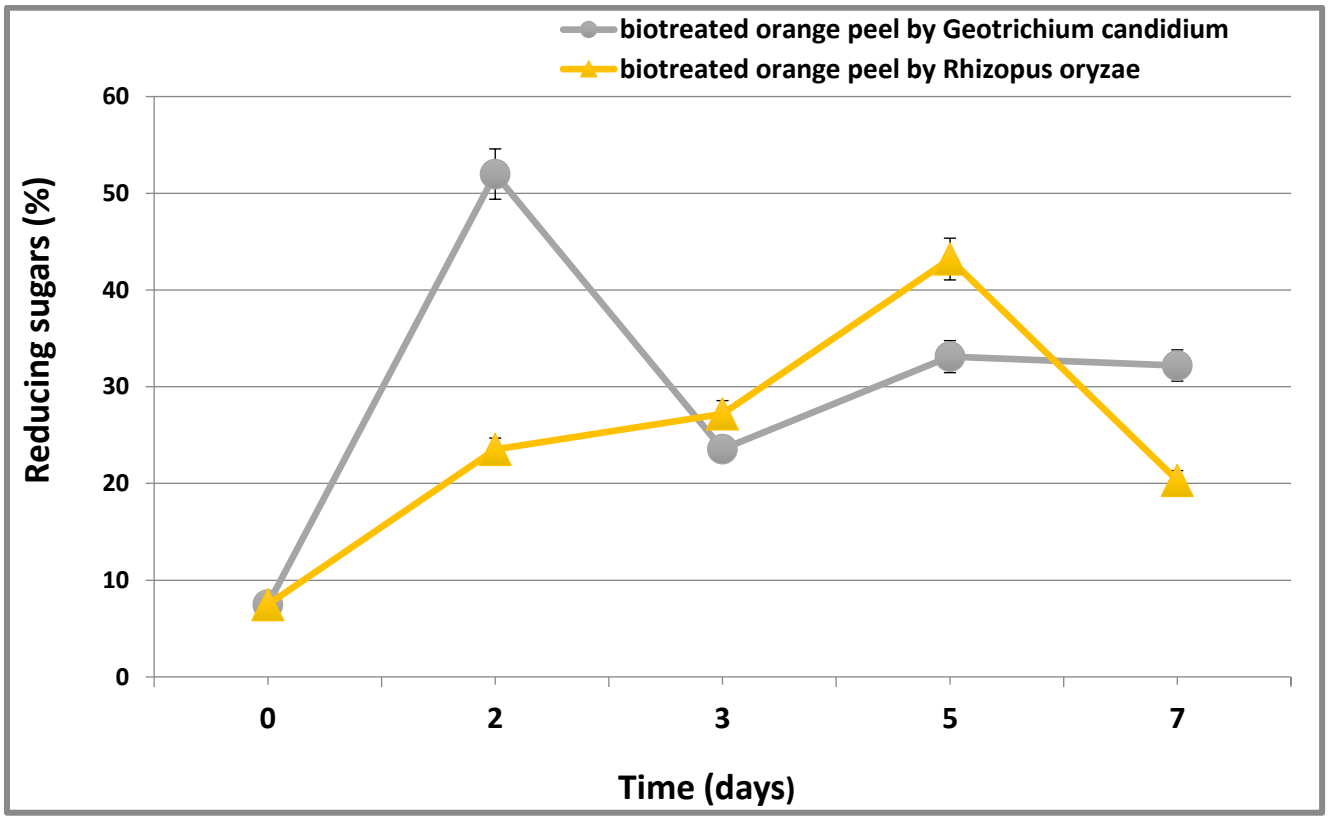

Figure 3 Hydrolysis of OP biomass using *Rhizopus oryzae and Geotrichium candidium* through time course of 7 days, incubated at 30 ºC and 150 rpm.

Selection of yeast used for bioethanol production: The selection of yeast for bioethanol production was crucial due to the specific requirements of the OP biomass, which contains xylose as one of its main sugars. *S. cerevisiae*, a commonly used microorganism for bioethanol production, was found to be unable to ferment xylose. Therefore, a preliminary experiment was conducted to evaluate different yeast species for their bioethanol production using treated OP biomass over a 4-day time course.

The results (Figure 4) showed that *C. tropicalis* exhibited the highest bioethanol production, with a 0.315 mg/mL concentration. *K. marxianus* and *C. oleophila* followed closely with bioethanol concentrations of 0.227 and 0.20 mg/mL on day 3, respectively. Industrial-scale fermentation of treated OP biomass requires microorganisms capable of functioning at high ethanol concentrations. Therefore, the alcohol tolerance of the newly identified candidates, *C. oleophila* and *C. tropicalis*, was compared with that of the well-known bioethanol producer *S. cerevisiae*, from 1 to 10%.

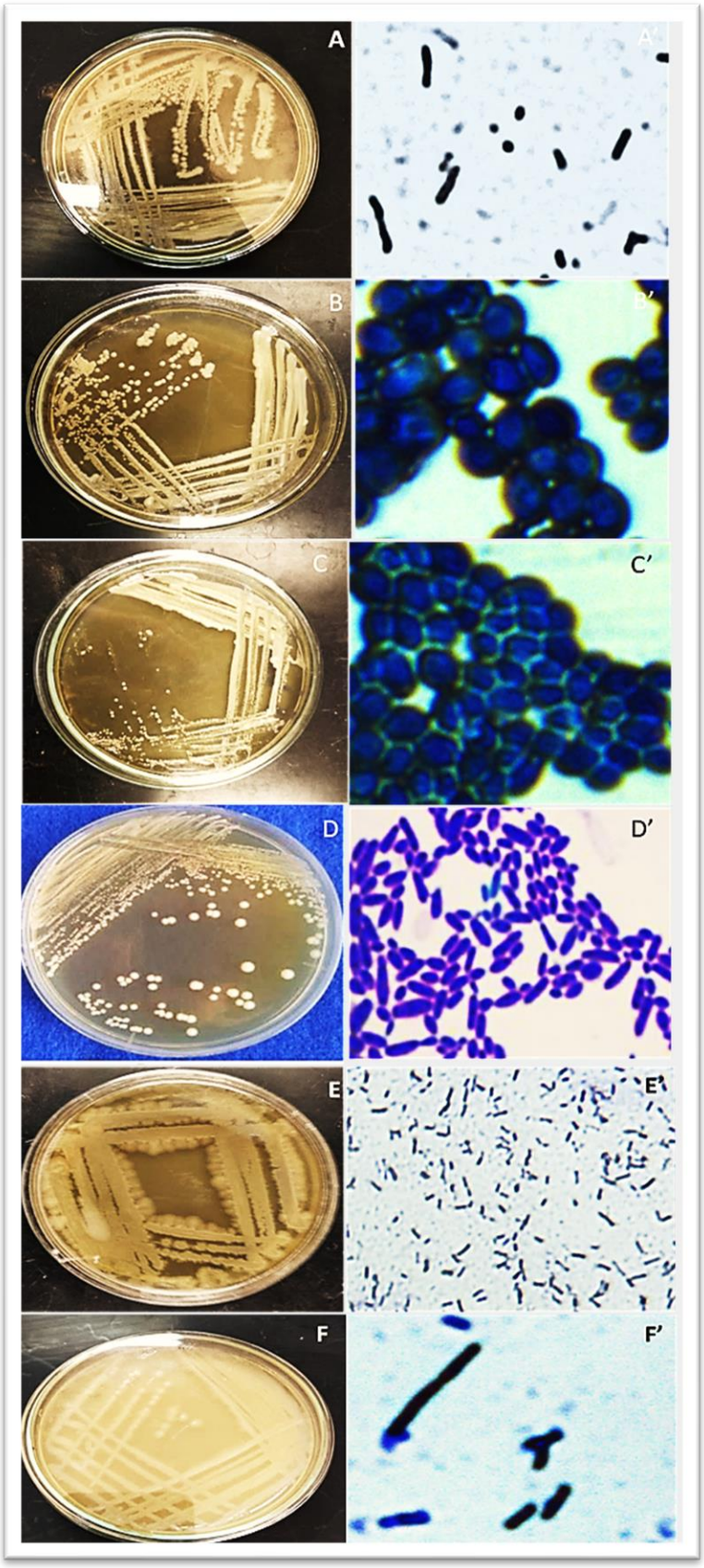

Figure 4 Yeasts used during the study, on YMP medium (A) and under light microscope stained with simple stain at 1000x magnification (A'); *C. oleophila* (A, A'); *S. cerevisiae* (B, B'); *S. boulardii* (C, C'); *Pichia anomala.* (D, D'); *C. tropicalis* (E, E') and *Kluyveromyces* marixanus (F, F').

In addition to alcohol tolerance, utilising xylose, which is released from hemicellulose during pretreatment, was a crucial factor. Based on its good ethanol tolerance and xylose utilization capabilities, *C. tropicalis* was selected for further investigations. Most published studies on bioethanol production predominantly focus on *S. cerevisiae* **[4]**. Therefore, *C. tropicalis* was chosen as a novel bioethanol producer, considering its bioethanol production of 0.315 mg/mL and its ability to utilize xylose. Subsequently, the strain was further adapted to alcohol concentrations ranging from 2 to 12%.

C. tropicalis is a species of yeast belonging to the *Candida genus*, while *S. cerevisiae* belongs to the *Saccharomyces* genus. Both are single-celled eukaryotes with various industrial applications. *S. cerevisiae* finds wide use in food, ingredients, biofuels, pharmaceuticals, and functional genomics, while *C. tropicalis* has diverse applications in industries such as food production **[47]**. *C. tropicalis* has been observed to produce high levels of xylitol, reaching 36 g/L in semi-synthetic conditions within 59 hours of fermentation **[48]**. Although *C. tropicalis* has not been extensively studied for biofuel production from OP biomass, it has been used in other applications, including the food industry.

Kluyveromyces, another genus of yeast in the *Saccharomycetaceae* family, has wide-ranging applications such as bioethanol production, low-lactose milk production, and heterologous protein production **[49]**. Studies involving simultaneous saccharification and fermentation using a co-culture of *S. cerevisiae* and *C. tropicalis* resulted in a bioethanol yield of 10.924% under optimal conditions, using H_2O_2-pretreated corn stover (12% concentration), 25% inoculum, pH 5, and 32 °C for 144 hours **[10]**. Additionally, *Candida tropicalis* MK-160 strain was found to produce higher titers of xylanase and 5.45% ethanol when grown on sugarcane bagasse and wheat bran **[50]**.

Since xylose constitutes a significant portion (30-40%) of the hemicellulose in OP biomass, efficient bioconversion of xylose is essential for economically feasible biomass conversion. Native xylose-utilizing yeasts such as *Candida shehatae*, *Scheffersomyces stipitis*, and *Spathaspora passalidarum* have been studied for their ability to convert xylose to xylulose through the oxidoreductase pathway **[51]**.

Candida tropicalis, an important *Candida* yeast species, can be found in plants and the digestive systems of mammals. It is considered an osmotolerant yeast due to its robust cell walls and broad environmental tolerance in terms of pH and ionic strength **[52]**. It possesses ascorbate oxidase enzyme activity and is amenable to biosensor construction due to its easy manipulation, rapid growth rate, and ability to utilize different carbon sources **[53]**.

HPLC analysis (Figure 5) revealed that different treatment methods resulted in distinct sugar profiles. Among them, the autoclaved water treatment exhibited higher levels of reducing sugars and relatively fewer inhibitory compounds, making it the preferred choice for fermentation purposes.

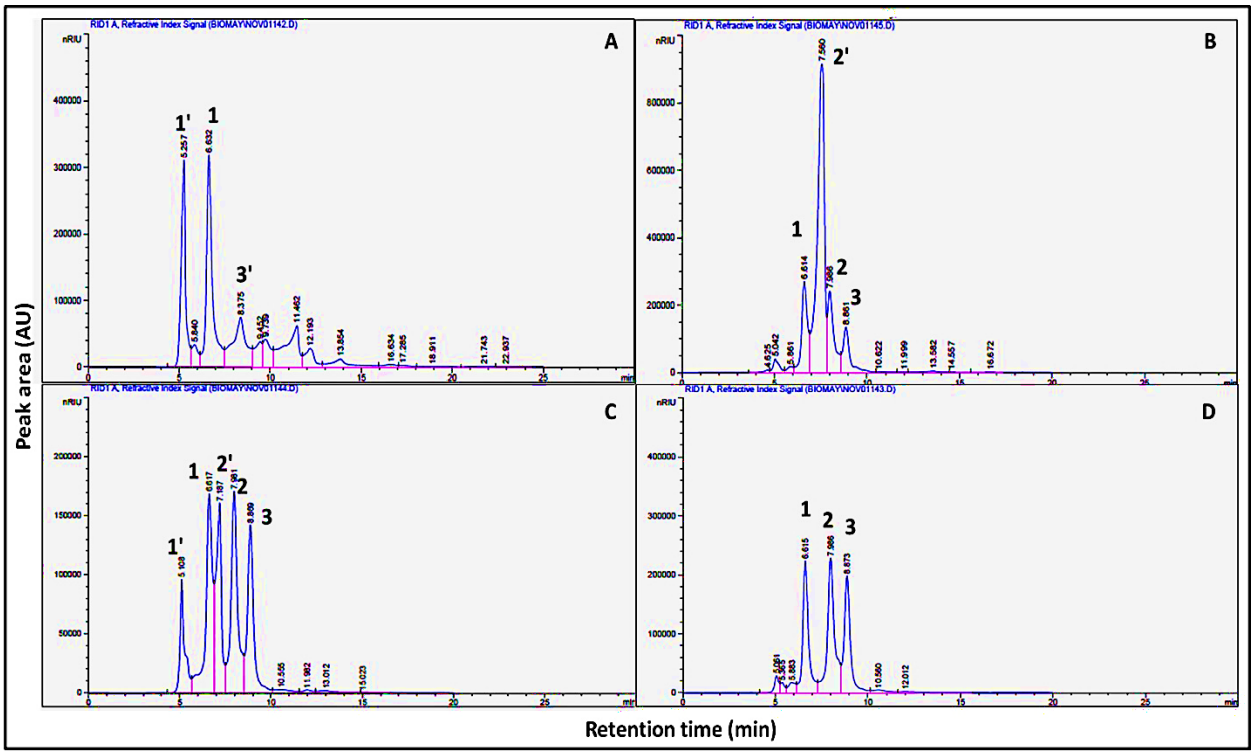

Figure 5 HPLC analysis of treated orange-peels biomass showing different sugar profiling of liberated sugars; biomass treated with calcium hydroxide (A), biomass treated with ammonia (B), autoclaved treated biomass with

water (C) and untreated biomass control (D); identified peaks are 1: sucrose, 1': xylose; 2: glucose; 2': galactose; 3: fructose; 3': mannose.

Effect of reducing agent on the fermentation process

The inclusion of reducing agents in the fermentation process plays a crucial role by reducing the redox potential of the cells. This, in turn, scavenges oxygen and alters the electron flow, ultimately influencing the production of bioethanol **[54]**. In this study, two reducing agents, cysteine and resazurin, were added to treated OP at concentrations of 0.5, 0.75, and 1 g/L. The addition of cysteine increased alcohol production from 0.31 mg/mL to 0.55 mg/mL, while resazurin resulted in a production of 0.41 mg/mL. These findings align with previous research conducted by Alriksson et al. **[55]**, who reported that the addition of 4 mM cysteine, glycine, and glutamic acid increased the fermentation rate from 119.6 mg/L to 236.1 mg/L of glutathione and reduced the biomass rate from 25.3 g/L to 25 g/L in cane molasses. Similarly, Hossain et al. **[56]** observed that the addition of 5 mM dithionite and 17.5 mM sulfite as reducing agents to sugarcane bagasse hydrolysate improved the SSF process from 0.9 to 3.9 g/L/h. Based on these results, cysteine at concentrations of 0.5 g/L and 1.5 g/L was selected for further optimization of the bioethanol production process from OP biomass.

Screening of bioethanol production using PBD

The production of bioethanol is influenced by various factors, including temperature, sugar concentration, fermentation time, pH, inoculum size, and agitation rate. This study employed a Plackett-Burman design (PBD) to identify the key factors affecting bioethanol production by *C. tropicalis* from treated OP biomass. Six variables were investigated, including initial pH, cysteine (reducing agent) concentration, inoculum size, yeast extract percentage, agitation speed, and substrate concentration. The fixed medium constitutions included temperature, KH_2PO_4, and ammonium sulfate.

The results in Table 2 and Figure 6 demonstrated a wide range of bioethanol production, from 1.93 to 6.03 mg/mL. This variation can be attributed to the interactions among the tested variables. The highest bioethanol production of 6.03 mg/mL was achieved at run number 4, with the following conditions: initial pH of 6, cysteine concentration of 1.5 g/L, agitation speed of 170 rpm, inoculum size of 3.5% (O.D600 0.9), yeast extract percentage of 3%, and substrate volume of 50 mL, after a fermentation time of 2 days.

Analysis of variance (ANOVA) using the Fisher test was conducted to assess the effects of the independent variables on bioethanol production, and statistically significant results were determined based on a p-value <0.05. The obtained model F-value of 8.3797 indicated the significance of the model for bioethanol production, with a p-value of 0.0108. A smaller p-value indicates a higher significance of the corresponding coefficient. The analysis suggested that pH, reducing agent concentration, and substrate concentration were significant factors with a positive effect, as illustrated in the pareto chart (Figure 6).

Using JMP version 8, the first-order model equation (Equation 3) for the PBD was determined as follows:

$$Y = -0.0287 + 0.00577X1 + 0.0075X2 + 0.00012X3 - 0.00137X4 - 0.00138X5 + 0.00028X6 \quad (3)$$

Y represents the bioethanol amount in the equation, while X1, X2, X3, X4, X5, and X6 correspond to pH, cysteine concentration, agitation speed, inoculum size, yeast extract percentage, and substrate concentration, respectively.

PBD has been widely employed for screening various parameters in bioprocesses, including enzyme production and ethanol production **[48]**, **[12]**. However, different authors have reported different results, which can be attributed to variations in yeast strains and substrates used. For example, El-Sayed et al. **[75]** utilized PBD to optimize ethanol production from *Ulva lactuca* using *S. cerevisiae*, and they found that inoculum size and sugar concentration significantly affected bioethanol production, resulting in a yield of 12 ±0.5 g/g of sugar/L. **[57]** employed PBD to investigate the effect of medium components on bioethanol production by *Wickerhamia* sp. from potato waste.

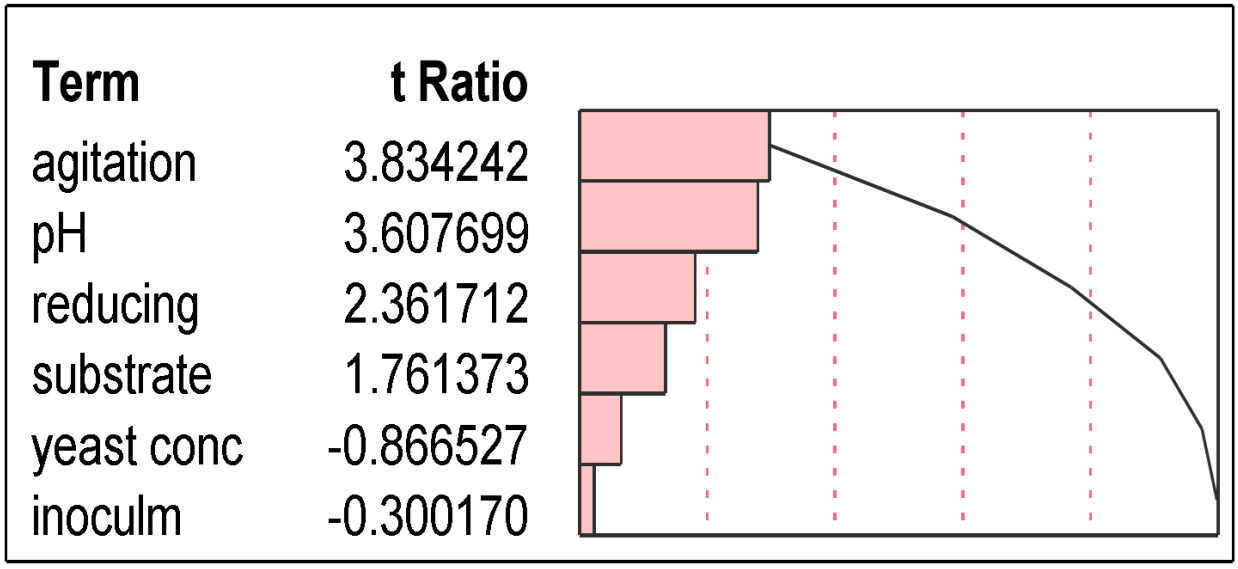

Figure 6 Pareto-chart rationalizing the effect of each variable on bioethanol production by *Candida tropicalis* using treated orange-peel biomass as substrate by PBD. The calculated F-ratio was higher than the theoretical one for the regression model (ANOVA), indicating its significance.

Table 2 Plackett-Burman experimental design matrix, and the actual values of bioethanol production by *C. tropicalis* using pretreated orange peel as substrate, after 2 days of fermentation at 30 °C.

Exp.no.	Pattern	Initial H	Cysteine (g/L)	Agitation speed (rpm)	Inoculum (O.D)	Yeast extract (%)	Substrate (mL)	Actual Bioethanol (mg/mL)	Predicted Bioethanol (mg/mL)
1	−−−+−−	4	0.5	70	0.9	1	30	1.937266	1.454627
2	+−−−+−	6	0.5	70	0.2	3	30	2.654305	2.580505
3	−+−−+−	4	1.5	70	0.2	3	30	2.000164	2.119252
4	−−+−−+	4	0.5	170	0.2	1	50	4.176442	3.637194
5	+++−−−	6	1.5	170	0.2	1	30	5.434407	5.194974
6	++−−−+	6	1.5	70	0.2	1	50	3.861951	4.427616
7	+−−+−+	6	0.5	70	0.9	1	50	2.968797	3.44221
8	−+++−−	4	1.5	170	0.9	1	30	2.968797	3.748315
9	−−+−++	4	0.5	170	0.2	3	50	2.591407	3.316414
10	+−+++−	6	0.5	170	0.9	3	30	3.434243	3.888788
11	−+−+++	4	1.5	70	0.9	3	50	2.704624	2.660176
12	++++++	6	1.5	170	0.9	3	50	6.03823	5.415118
13	0	5	1	120	0.55	2	40	3.861951	3.490432
14	0	5	1	120	0.55	2	40	3.861951	3.490432
15	0	5	1	120	0.55	2	40	3.861951	3.490432

Note: **: Significant p <0.01.

They achieved a bioethanol yield of 33.1 mg/mL under 30 °C, 150 rpm for 96 hours. Factors such as malt extract, tryptone, KH_2PO_4, Na_2HPO_4, NaCl, and $CaCl_2.2H_2O$ showed positive effects on the fermentation process, while $MgSO_4.7H_2O$ and $(NH_4)_2SO_4$ showed negative effects. Similarly, Yu et al. **[58]** used PBD to optimize bioethanol production from potato waste using *S. cerevisiae*, and they obtained a bioethanol yield of 36.85 mg/mL at 30 °C, 150 rpm after 48 hours. In their study, yeast extract, malt extract, and $MgSO_4.7H_2O$ positively affected bioethanol production, while KH_2PO_4 and $CaCl_2.2H_2O$ had negative effects.

In another study, **[1]** utilized Taguchi orthogonal array statistical design to optimize parameters for biomass pretreatment of sweet lime peel biomass, including solid loading, time of exposure, and sulfuric acid concentration. The optimized parameters of 17% (w/v) solid loading, 0.25% (v/v) acid concentration, and 60 minutes of steam exposure were used for enzymatic hydrolysis of the pretreated sweet lime peel. Subsequent fermentation using baker's yeast resulted in the release of 7.09 mg of reducing sugar/mL of hydrolysate, with a

bioethanol yield of 18% at 30 °C after 72 hours. However, Oberoi et al. **[5]** conducted primary hydrolysis of OP using acid concentrations ranging from 0 to 1.0% (w/v) at 121 °C and 15 psi for 15 minutes. The hydrolysate obtained from hydrolysis was then fermented using parameters optimized through response surface methodology (RSM), resulting in an ethanol yield of 0.25 g/g on a biomass basis.

Overall, PBD and other statistical designs have proven valuable in exploring and optimizing the parameters involved in bioethanol production from different substrates, leading to improved yields and process efficiency.

Optimization of Bioethanol production using RSM

RSM has proven to be effective in optimizing parameters for the production of enzymes and ethanol in biological systems **[5]**. To successfully produce bioethanol from OP biomass, optimising important fermentation parameters such as pH, substrate concentration, reducing agent, and agitation rate is crucial. Agitation rate plays a role in nutrient permeability and ethanol removal in the fermentation broth, with 150-200 rpm being the common range for yeast cell fermentation. However, excessive agitation can hinder yeast cell metabolic activities **[59]**, so an agitation rate of 150 rpm was selected for subsequent experiments. Similarly, sugar concentration affects fermentation, but excessive levels can lead to steady rates due to the limited uptake capacity of microbial cells **[4]**.

After identifying the most significant variables influencing bioethanol production using PBD, a CCRD was conducted, with day 2 chosen as the optimal day. The major variables identified from PBD were pH (X1), reducing agent (X2), and substrate concentration (X3), while the agitation speed remained at 150 rpm. These factors were tested at five levels (-2, -1, 0, +1, and +2) using 17 trials and three center points (Table 3). The significance of the model was assessed using the F-test and ANOVA, and the response surface quadratic model demonstrated statistical significance with a *p*-value of 0.011. The signs and statistical significance of coefficients ($p < 0.05$) were used to interpret the data, considering the positive or negative effects on the response and the presence of antagonistic or synergistic interactions between factors. The model achieved a determination coefficient (R^2) of 0.92, explaining 92% of the total variations and showed excellent agreement between experimental results and predicted values. A second-order polynomial model (Equation 4) was fitted to the experimental bioethanol production results to predict the optimal point within the experimental constraints.

Run 8 represented the optimum conditions for bioethanol production, including 3% yeast extract, 3.5% inoculum size with OD600 of 0.9, initial pH of 5.75, 2.25 g/L cysteine, initial reducing sugar concentration of 2.56 mg/mL, 85 mL reaction volume, 30 °C temperature, 2-day fermentation time, and agitation rate of 150 rpm. Under these optimized conditions, bioethanol production increased from 6.03 to 16.71 mg/mL. The model proved statistically valid in explaining all bioethanol production within the investigated experimental ranges (Table 3, Figure 7). The Pareto chart provided insights into the magnitude and ranking of factor estimates. Regression coefficients for the tested variables indicated positive and negative effects for pH and reducing agent concentration, respectively. Three-dimensional response surface plots based on the model equation were used to understand the interaction among variables and determine the optimal levels of each factor for bioethanol production from OP biomass (Figure 7). A more horizontal 3D surface and perfect interaction indicate the highest significant effect **[11]**.

Researchers have widely utilized RSM for optimizing bioethanol production from various substrates, leading to different yields depending on the substrate and yeast used **[60]**. For instance, **[23]** employed RSM to optimize bioethanol production from pineapple peels using *S. cerevisiae*. The optimum conditions were determined as pH 6, 5 g/L ammonium sulfate, and 6 g/L yeast loading, resulting in a maximum bioethanol yield of 5.82%.

In another study, **[61]** applied RSM to optimize bioethanol production from cellulase-treated sugarcane bagasse using *Candida wickerhamii*. Under the optimal conditions of pH 5.7, 33 °C temperature, and 50 mg/mL substrate concentration for 104 hours, a bioethanol yield of 4.28 mg/mL was achieved.

Sharma et al. **[62]** investigated the fermentation parameters influencing bioethanol production from kinnow waste and banana peels using simultaneous saccharification and fermentation with cocultures of *Pachysolen tannophilus* MTCC 1077 and *S. cerevisiae*. They determined that an inoculation rate of 6% (v/v) *S. cerevisiae*, incubation time of 48 hours, agitation time of 24 hours at 30 °C, and 4% (v/v) *P. tannophilus* resulted in optimal conditions, yielding 26.84 mg/mL of bioethanol.

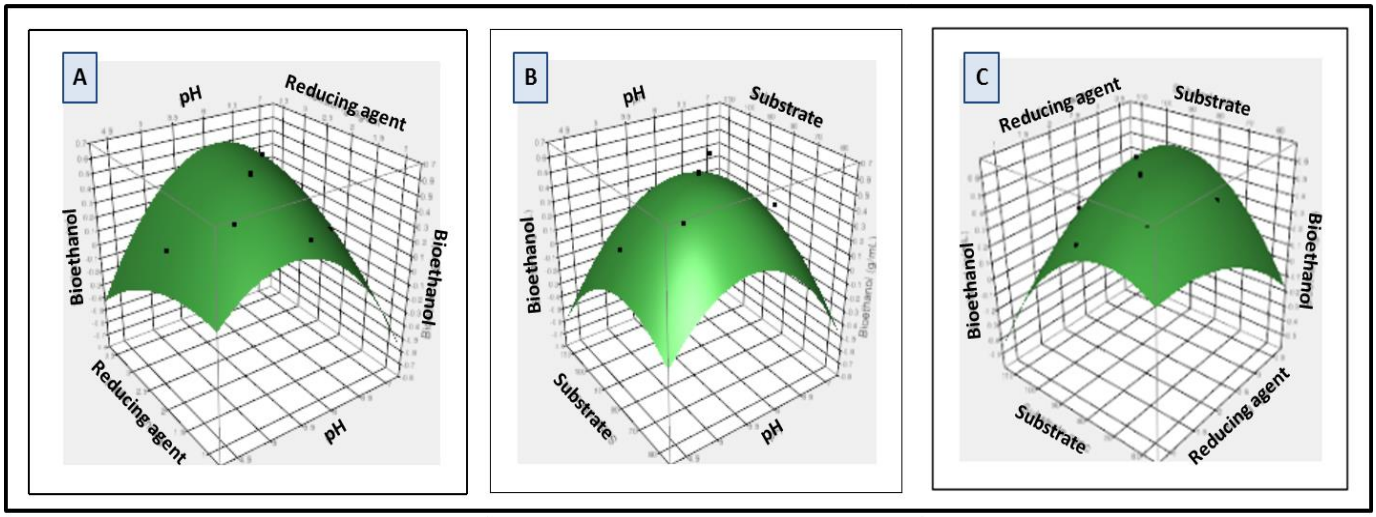

Figure 7 Response surface plot showing the effect of initial pH and reducing agent (A), pH and substrate conc. (B), reducing agent and substrate conc. (C) on the production of bioethanol by *Candida tropicalis* under three variables using pre-treated orange-peel biomass as substrate, other variables are held at zero level.

Table 3 Optimization of bioethanol production using RSM by *C. tropicalis* under three variables by CCRD-design matrix, with actual and predicted values from pretreated orange peel as substrate, after 2 days of fermentation at 30 °C.

Trial	Pattern	Initial pH	Reducing agent (mg/mL)	Reaction Substrate (mL)	Actual Bioethanol (mg/mL)	Predicted Bioethanol (mg/mL)
1	−−−	5	1.5	70	14.3807	14.50829
2	+−−	6.5	1.5	70	8.999076	8.206792
3	−+−	5	3	70	8.210543	8.604322
4	−−+	5	1.5	100	5.296981	7.063348
5	++−	6.5	3	70	12.96179	10.55903
6	+−+	6.5	1.5	100	5.858309	4.828137
7	−++	5	3	100	8.339737	8.495629
8	+++	6.5	3	100	15.28061	14.51662
9	0	5.75	2.25	85	16.71066	15.73255
10	0	5.75	2.25	85	14.1535	15.73255
11	0	5.75	2.25	85	16.48791	15.73255
12	00a	5.75	2.25	59.77	9.94487	11.22784
13	00A	5.75	2.25	110.23	8.678316	8.295341
14	0a0	5.75	0.99	85	12.04183	11.69251
15	0A0	5.75	3.519	85	13.62558	14.8749
16	a00	4.49	2.25	85	8.678316	6.91852
17	A00	7.019	2.25	85	4.022854	6.682645

Note: *: Significant p <0.01.

Furthermore, Raja Sathendra et al. **[60]** utilized RSM to optimize bioethanol production from palm wood using *Kluyveromyces marxianus*. The optimal conditions were found to be pH 5, temperature 45 °C, agitation rate of 156 rpm, 3.2% inoculum size, and 8% (v/v) substrate concentration, leading to a maximum bioethanol yield of 22.90 mg/mL. Similarly, Jambo et al. **[63]** employed RSM to optimize bioethanol production from *Eucheuma cottonii* using *S. cerevisiae*. The optimal conditions were determined as a 12% (v/v) inoculum size, pH 5.2, temperature of 32 °C, and a fermentation time of 72 hours, resulting in a bioethanol yield of 9.77 mg/mL.

Likewise, Sininart Chongkhong **[64]** utilized RSM to optimize bioethanol production from banana peels using *S. cerevisiae*. The optimal conditions were identified as pH 4.8, 28 °C temperature, and a yeast loading of 4% (w/w) for a fermentation duration of 192 hours, achieving a bioethanol yield of 9.2%.

Enhancement of the bioethanol production using successive cocultures and metal addition

The bioethanol production was significantly improved by implementing a series of enhancements, including successive cocultures and metal addition. Initially, bioethanol production increased from 6.03 to 16.71 mg/mL under optimized conditions determined by response surface methodology (RSM). These conditions included 3% yeast extract, a 3.5% v/v, O.D600 (0.9), inoculum size, initial pH of 5.75, 2.25 g/L cysteine, and 2.56 mg/mL initial reducing sugar. This fermentation was carried out by *C. tropicalis* at 30 °C and 150 rpm for 2 days.

Further optimization was conducted by introducing selected metals into the fermentation process. Studies have shown that certain metal ions, such as Zn+2, Mg+2, and Mn+2, have the potential to enhance bioethanol production and the fermentation process **[76]**. For example, Alamri et al. **[65]** demonstrated that the addition of zinc, magnesium, and manganese at their respective optimum concentrations of 0.6 g/L, 0.2-0.3 g/L, and 0.03 g/L induced ethanol production from date molasses. Specifically, *Hanseniaspora guilliermondii* KKUY-0036 and *H. uvarum* KKUY-0078 achieved ethanol yields of 6.48% and 6.11%, respectively. This enhancement can be attributed to the activation of alcohol dehydrogenase enzymes by zinc ions, which are essential for ethanol formation. Magnesium ions directly influence various aspects of yeast physiology, including sugar consumption, ethanol production, yeast growth, and responses to environmental stress. They act as size transducers, growth enhancers, and stress protectants during the fermentation process **[65]**.

To further improve bioethanol production, the substrate filtrate was treated with activated charcoal before fermentation. Activated charcoal is commonly used as an adsorbent to remove inhibitors, such as furans and phenolic compounds, from lignocellulosic hydrolysates due to its high adsorption capacity and strong hydrophobicity **[66]**. This treatment helps to create a more favorable environment for bioethanol production.

Additionally, successive coculturing of *C. tropicalis* and *K. marxianus* was employed as a strategy for enhancement. Co-culture processes involve the simultaneous cultivation of two different yeast strains in the same reaction mixture, leading to improved ethanol production compared to pure cultures. The use of co-cultures, such as combining pentose-utilizing yeasts like *Pichia stipitis* and *Pichia fermentans* with *S. cerevisiae*, allows for the utilization of both hexose and pentose sugars, thereby enhancing ethanol production **[4]**, **[67]**.

Overall, by combining these optimization strategies, the bioethanol yield was increased to 87.66% after 3 days of fermentation, as shown in Table 4. This represents a significant improvement compared to the initial conditions and demonstrates the effectiveness of successive cocultures and metal addition in enhancing bioethanol production.

Activated charcoal is commonly used as an adsorbent in lignocellulosic hydrolysates to remove inhibitors with higher hydrophobicity than sugars and aliphatic carboxylic acids, such as furans, furfurals, and phenolic compounds. This is due to its strong hydrophobicity and high adsorption capacity **[66]**. The effectiveness of active charcoal treatment in enhancing butanol production was investigated by Zhang et al. **[68]**. They found that the addition of 5.0% (w/v) active charcoal removed 77.9% of furan derivatives and 98.6% of aromatic monomers, resulting in an increased butanol yield of 0.22 g per g sugar.

Co-culture processes involve the simultaneous cultivation of two different yeasts in the same reaction mixture, and they have shown to enhance ethanol production compared to pure cultures **[69]**. In the case of bioethanol production, co-cultures often combine pentose-utilizing yeasts like *Pichia stipitis* and *Pichia fermentans* with *S. cerevisiae* to utilize both hexose and pentose sugars **[67]**, **[4]**.

In the present study, the overall results (Table 4) demonstrated that under the optimized conditions of an initial pH of 5.75, 3% yeast extract, 4% inoculum size (O.D600 1), 2.25 g/L cysteine, 0.6 g/L $ZnSO_4$, 0.29 g/L $MgSO_4$, and 0.3 g/L $MnSO_4$, along with treating the OP filtrate with active charcoal before fermentation, the bioethanol yield was increased to 87.6%. This represents a 4-fold increase compared to the PBD method and a 1.84-fold increase compared to RSM. Notably, this enhancement was achieved through the successive co-culture of *C. oleophila* and *K. marixanus,* which was applied for the first time in this study.

Similar approaches utilizing co-cultures have been explored in previous studies. For example, **[33]** produced bioethanol from apple pomace hydrolysate by co-culturing *Trichoderma harzianum*, *Aspergillus sojae*, and *S. cerevisiae*. They found that the optimal conditions for maximum bioethanol production included a 4% (v/v) inoculation rate of *S. cerevisiae* and 6% (w/v) inoculation rates of *T. harzianum* and *A. sojae*, along with vented aeration and 200 rpm agitation speed. Patle and Lal **[70]** reviewed various bioethanol-producing strains isolated from raw honey, molasses, grapes, and apple, and demonstrated that mixed cultures of *Zymomonas mobilis* and *Candida tropicalis* using these substrates were promising for bioethanol production. Wu et al. **[71]** reported a maximal ethanol concentration of 48.98 mg/mL and productivity of 2.23 g/L/h under optimal conditions of 5% *Kluyveromyces marxianus* K21 inoculum at 40 °C after 22 hours.

Furthermore, Sindhu et al. **[2]** highlighted the limitation of pentose fermentation by yeasts such as *Z. mobilis* and *S. cerevisiae*, which efficiently ferment glucose but cannot consume xylose. However, yeasts like

Scheffersomyces stipitis, *Pichia stipitis*, and *Candida shehatae* can consume xylose and produce bioethanol. This limitation can be overcome by using genetically modified yeast or a co-culture of two yeast strains. For example, co-culturing *S. cerevisiae* and *S. stipitis* using a rice husk hydrolysate containing pentose and hexose sugars resulted in an ethanol yield of 0.42 $g.g^{-1}$ **[2]**. Co-culturing *S. cerevisiae* with *A. niger* was also considered a cost-competitive method for simultaneous saccharification and fermentation, resulting in the production of 35.2 mg/L of bioethanol using potato waste as a substrate **[57]**, **[2]**.

Table 4 Optimization of bioethanol production from treated OP using *C. tropicalis* under all detected optimizedconditions[1].

Experiment	Treatment Conditions of OP	Reducing sugars (g/L)	Ethanol (mg/mL)	Ethanol Yield[2] (%)
Plackett-Burman Design (PBD)	pH of 6, yeast extract (3 g/L), cysteine (0.5 g/L), substrate volume of 30 mL, inoculum size (3.5%), and OD_{600} (0.9), kept at 30 °C for 2 days fermentation	**1.30**	**6.03**	**21.55**
Response surface methodology (RSM)	pH of 5.57, yeast extract (3 g/L, cysteine 2.25 g/L, substrate volume 85 mL, inoculum size 3.5%,) OD_{600} of 0.9, kept at 30 °C for 2 days fermentation.	**5.80**	**16.7**	**34.73**
Successive Co-Culture addition *Kluyveromyces marxianus* was incubated 3 days, frozen, then *C. tropicalis* was added and incubated for 3 days at 30 °C	Treated with 1 g/L active charcoal before fermentation for 2h. Then Addition of metals**;** $ZnSO_4$ (0.6 g/L), $MgSO_4$ (0.29 g/L) and $MnSO_4$ (0.3 g/L), yeast of 24 h age and similar optimized condition as in RSM for inoculum size, and microbial obtical density, pH, and incubation temperature.	**0.93** **1.12**	**2.44** **2.26** **4.70**	**38.11** **49.55** **87.66**

Note: [1]Values are means of triplicates, [2]ethanol yield calculated based on reducing sugar (reducing sugar/ethanol)*100.

CONCLUSION

In conclusion, this study explored bioethanol production from agro-industrial waste, specifically OP wastes, using selected microorganisms. The findings suggest that OP wastes, which have a high cellulose content, hold promise as a low-cost substrate for bioethanol production. Water/autoclaving pre-treatment was found to be the most effective, resulting in the lowest phenolic content and the highest sugar yields of 68.2%. *C. tropicalis* was investigated as a potential candidate for bioethanol production, and two reducing agents, cysteine and resazurin, were introduced for the first time. The addition of cysteine and resazurin increased bioethanol production by 2.9 and 2.2 times, respectively. By using a combination of Plackett-Burman design (PBD) and response surface methodology-central composite rotatable design (RSM-CCRD), the significant factors influencing bioethanol production were identified and optimized. The optimized conditions included an initial pH of 5.75, 3% yeast extract, 2.25 g/L cysteine, 4% inoculum size, 0.6 g/L $ZnSO_4$, 0.29 g/L $MgSO_4$, and 0.3 g/L $MnSO_4$. Under these conditions, a maximum bioethanol yield of 87.6% was achieved using successive co-culturing of *C. tropicalis* and *K. marxianum*, with an incubation period of 3 days at 30 °C. To the best of our knowledge, this is the first study to utilize both *C. tropicalis* and *K. marxianum* for bioethanol production from OP. These co-cultures demonstrated their effectiveness in bioethanol production. Overall, this study serves as an important initial step in utilizing OP wastes for bioethanol production, and it highlights the potential of co-cultures as an efficient approach in bioethanol production. Further research in this field can build upon these findings and contribute to the development of sustainable and cost-effective bioethanol production processes.

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Effect of extract of ginger root and liquorice on the microbiological safety of mutton liver pâté

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ABSTRACT

This work aimed to evaluate the effect of ginger root (*Zingiber officinale*) and liquorice (*Glycyrrhiza glabra*) extract in liver pates on their microbiological safety, water activity and pH values. Four samples of pates were produced: control (without extracts), variant 1 (addition of 1% liquorice root and 2% ginger root), variant 2 (2% liquorice root, 3% ginger root), variant 3 (3% liquorice root, 4% ginger root). The number of mesophilic aerobic and facultative anaerobic microorganisms, lactobacilli, moulds, yeasts, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* on the day of production and after 1, 3, 6 and 12 months of storage were determined. According to the experimental data, the studied microbiological safety indicators were within the permissible standards during the entire period of storage. The lowest microflora growth was observed in variants 1 and 4. With increasing storage time of the samples, a decrease in the value of water activity and an increase in the pH value was observed. Sensory analysis showed a positive trend in pates' taste, texture, and aroma when introducing sheep fat and plant extracts into the recipe. According to the overall sensory analysis score, variant 2 received the highest score (8.5), while the control sample received the lowest score (7.9). The aroma, consistency and juiciness of the pâtés of variant 2 were significantly better (p <0.05). The studies confirmed the prospects of improving the microbiological stability of liver pâté using different combinations of plant extracts.

Keywords: pate, liquorice root, ginger root, microbiological safety, water activity, pH

INTRODUCTION

Liver pâtés are widely popular among the population of many countries and are considered a delicacy **[1]**. They are made using pork liver **[2]**, duck and chicken liver **[3]**, and ostrich liver **[4]**. However, information on the use of lamb liver and its impact on the quality characteristics of pâtés is very limited **[5]**. Liver pâtés, as ready-to-eat products, have a high risk of food safety concerns. Due to their high nutritional value and significant water content, pâtés can cause foodborne illness due to the excessive growth of pathogenic microorganisms **[6]**. Consequently, they require careful microbiological safety control. In addition to the above, liver pâtés have a low antioxidant and relatively high-fat content (up to 60%), increasing susceptibility to lipid oxidation and rancidity. They also have a high content of non-heme iron (up to 30 mg/g product), which is a promoter of oxidation in meat and meat products and can affect the rate of product spoilage **[7]**. The factors above contribute to instability during storage, susceptibility to oxidation, formation of lipid-derived volatiles, and corresponding changes in colour, aroma, taste, and nutritional properties **[7]**, **[8]**.

Several studies indicate that the use of plant extracts with antimicrobial activity can increase the microbiological safety and shelf life of products such as sausages **[9]**, ready-to-eat foods **[10]** and liver pâtés **[11]**, **[12]**. Positive results of the use of *Arbutus unedo* fruit extracts as an inhibitor of lipid oxidation and pathogenic

microflora in pates **[4]**, the use of *Morus alba* plant leaf extract in liver pates to prolong the shelf life in refrigerators in terms of microbiological safety, resistance to oxidation, etc. are reported **[11]**.

The antimicrobial and preservative potential of 1% and 2% turmeric extract in ready-to-eat foods **[10]**, the antimicrobial effect of liquorice extract against the common foodborne pathogen *Listeria monocytogenes* **[13]**, and aqueous extracts of *Glycyrrhiza glabra*, *Cuminum cyminum*, *Zingiber officinale*, *Origanum majorana* and *Petroselinum crispum* against several Gram-positive and Gram-negative bacterial isolates **[14]** were reported.

The most widely used plants with a strong antibacterial and immune-stimulating effect are liquorice root (*Glycyrrhiza glabra L.*) and ginger (*Zingiber officinale*) **[15]**, **[16]**, **[17]**, **[18]**, **[19]**. Licorice root is known for its anti-inflammatory, antimicrobial, antiviral, anti-allergic, antioxidant and anticancer properties. In traditional medicine, it is used to treat peptic ulcers, asthma, pharyngitis, and malaria, to relieve abdominal pain, and insomnia and against infections of various etiologies **[15]**, **[16]**, **[17]**.

The main active ingredient of liquorice – glycyrrhizin in the highest quantity (up to 23%) is contained in the plant's roots. In liquorice root are found flavonoids, glycinamide A and B16, glucose (up to 15.2%), sucrose (up to 11%), starch, resinous substances, gum, high content of organic acids – salicylic, synaptic, ferulic, caffeic, etc. Coumarins, alkaloids, tannins, steroids, estradiol, vitamins C and B, potassium, calcium, etc., are also determined **[17]**.

Ginger root is also known in Asian traditional medicine, especially in Chinese medicine, in West African folk medicine for its positive effect on inflammatory diseases: cough, colds, rheumatoid arthritis in stomach diseases: dyspepsia, colic, gastroparesis, etc. Analysis of the data shows that ginger root and liquorice are considered by scientists mainly in the composition of food products to prolong the shelf life **[19]**, **[20]**, **[21]**, **[22]**, **[23]**, **[24]**.

The combination of liquorice extract and ginger, according to the data presented in the works of Saeedifar and Mosayebi, has a synergistic effect in the treatment of several diseases, including cancer, increasing apoptosis, the action of lymphocytes infiltrating the tumour, suppression of growth of tumour masses **[25]**.

In our previous work **[26],** we studied the effect of liquorice and ginger root on liver pate's nutritive, chemical and technological properties. The purpose of this work is to study the effect of the extract of ginger root (*Zingiber officinale*) and liquorice (*Glycyrrhiza glabra*) in liver pates on their microbiological safety, water activity and pH values.

Scientific hypothesis

This research hypothesises that the incorporation of liquorice (*Glycyrrhiza glabra*) and ginger root (*Zingiber officinale*) extracts into liver pâtés will enhance their microbiological safety and stability by reducing pathogenic microorganism growth, inhibiting lipid oxidation and maintaining desirable water activity and pH levels, ultimately improving their overall quality characteristics.

MATERIAL AND METHODOLOGY

Samples

To produce mutton liver pâtés, the following components are used:
Mutton liver and butter (72% of fatness) were purchased from Magnum supermarket (Almaty, Kazakhstan).
Dried liquorice root was purchased in Zerde Phyto LLP (Kazakhstan).
Fresh ginger root was purchased from the local supermarket "Magnum" (Almaty, Kazakhstan).

Chemicals

Agar nutrient media: meat-and-peptone agar (State Research Center for Applied Microbiology and Biotechnology, Moscow, Russia).

Biological material

Escherichia coli (NCTC 12241/ATCC 25922), Salmonella typhimurium (NCTC 12023/ATCC 14028), and Staphylococcus aureus (NCTC 12973/ATCC 29213) test strains.

Instruments

Aqualab 4TE water activity meter (Decagon Devices Inc.).
pH meter HI 99163 (Hanna Instruments Inc.).
Ultrasonic homogenizer (Ultrasonic Homogenisers HD 4100, Germany).
Meat grinder MIM-300 (Russia).
Meat cutter ZB-40 (Hualian Machinery, China).
Manual sealing machine MZ04 (Russia).
Autoclave "Malysh Nerzh" with an electronic block EBU (22 l) (Russia).

Laboratory Methods

Microbiological indicators: All studies were conducted in a microbiological box under aseptic conditions. On the day of production and after 1, 3, 6 and 12 months of storage, the quantity of mesophilic aerobic and facultative anaerobic microorganisms (total viable count), lactobacilli, moulds, yeasts, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* was determined by Koch's cup method. All samples were stored in a refrigerated chamber at 4 °C before sampling.

The pre-diluted sample solution was diluted in sterile tubes with 9 ml of physiological solution and plated in Petri dishes with agar nutrient media: meat-peptone agar for determination of total viable count, Chapek-Dox medium for mould fungi, Saburo medium for yeast, and MRS medium for lactobacilli. Incubation was performed at 37 °C and 25 °C (for moulds).

In addition, *Escherichia coli* (NCTC 12241/ATCC 25922), *Salmonella typhimurium* (NCTC 12023/ATCC 14028), and *Staphylococcus aureus* (NCTC 12973/ATCC 29213) test strains were plated as controls. The test strains were purchased from The National Collection of Type Cultures (NCTC, American Type Culture Collection (ATCC)). The research was conducted at the Kazakhstan Association of Human Microbiome Researchers, Nazarbayev University (Astana) **[27]**.

Water activity was measured on an Aqualab 4TE water activity meter (Decagon Devices Inc.). Samples were ground beforehand, weighed, and evenly distributed on the device cup **[28]**.

Determination of pH: The potentiometric method determined the pote's actiome acidity (pH). Twice, the ground sample was mixed with distilled water in the proportion 1:10, followed by stirring on a magnetic stirrer for 30 minutes. pH after extraction was determined on a device HI 99163 (Hanna Instruments Inc.) **[29]**.

Sensory analysis: A sensory analysis was conducted by 30 trained Kazakh Research Institute of Processing and Food Industry employees, with panellists aged 21 to 58 years (15 males and 15 females). The pâté samples were cut into cubic-shaped pieces and distributed to the panellists in plastic disposable dishes, a glass of water, unsalted crackers, and a knife. The samples were evaluated using a 10-point scale, where 0 points corresponded to "highly undesirable" and 10 points corresponded to "highly desirable". To conduct the sensory evaluation, each sample was served in triplicate **[30]**.

Description of the Experiment

Preparation of the extract: Plant ingredients comply with the requirements of the technical regulations of the Customs Union TR CU 021/2011 No. 880 and TR CU 022/2011 No. 881 "On food safety", applicable in the Republic of Kazakhstan. All plant experiments were conducted with relevant institutional, national, and international guidelines and legislation. Dried liquorice root was purchased in Zerde Phyto LLP (Kazakhstan) and milled to powdery. Sifted twice through a sieve with a pore diameter of 1 mm, weighed and added distilled water in a ratio of 1:4. Fresh ginger root was purchased in the local supermarket chain "Magnum" (Republic of Kazakhstan, Almaty), washed, dried, ground in a blender and processed with an ultrasonic homogenizer (Ultrasonic Homogenisers HD 4100, Germany) with distilled water (hydromodule 1:4) (Figure 1).

The obtained extract was centrifuged (1000 rpm, 10 min). The supernatant was poured into a volumetric flask (Figure 2). The precipitate was again poured with water in a 1:2 ratio, repeatedly treated with an ultrasonic homogenizer, and centrifuged. The supernatant was added to the previous extract. The extract was then stored at 2 °C until analysis.

Production of pâté: Mutton liver and other ingredients were purchased from Magnum supermarket (Almaty, Kazakhstan). Production of pâté was conducted in the meat processing shop at Kazakh Research Institute of Processing and Food Industry. Four samples of pâté were produced: control (without extracts), variant 1 (adding 1% licorice root and 2% ginger root), variant 2 (2% liquorice root, 3% ginger root) and variant 3 (3% liquorice root, 4% ginger root) (Table 1).

Table 1 Recipe of control and experimental samples of pâté.

Ingredient	Control	Experimental samples		
		Variant 1	Variant 2	Variant 3
Mutton liver	65	65	65	65
Butter (72.5% fat)	10	10	10	10
Unfiltered broth from boiling lamb's liver	25	25	25	25
Spices and materials, g per 100 kg of raw materials				
Licorice root extract	-	1	2	3
Ginger root extract	-	2	3	4
Black pepper powder	0.1	0.1	0.1	0.1
Iodized table salt	0.1	0.1	0.1	0.1

Bile ducts and film were removed from the mutton's liver. Then it was soaked in running water for 2 hours to remove blood clots, sliced and blanched in hot water for 25 minutes. Afterwards, the liver was washed in cold water and chopped in a meat grinder MIM-300 (Russia). Onions were peeled, washed, sliced, and parboiled in vegetable oil for 10-15 minutes. All the ingredients were ground in a cutter ZB-40 (Hualian Machinery, China) for 5-7 minutes.

Homogenized paste mass was filled into cylindrical tin cans (diameter 72.8 mm, wall height 95 mm) (Figure 3), sealed with tin lids on manual sealing machine MZ04 (Russia), and sterilized on autoclave "Malysh Nerzh" with an electronic block EBU (22 l) (Russia) at a pressure of 0.25 MPa and sterilization temperature 117 °C.

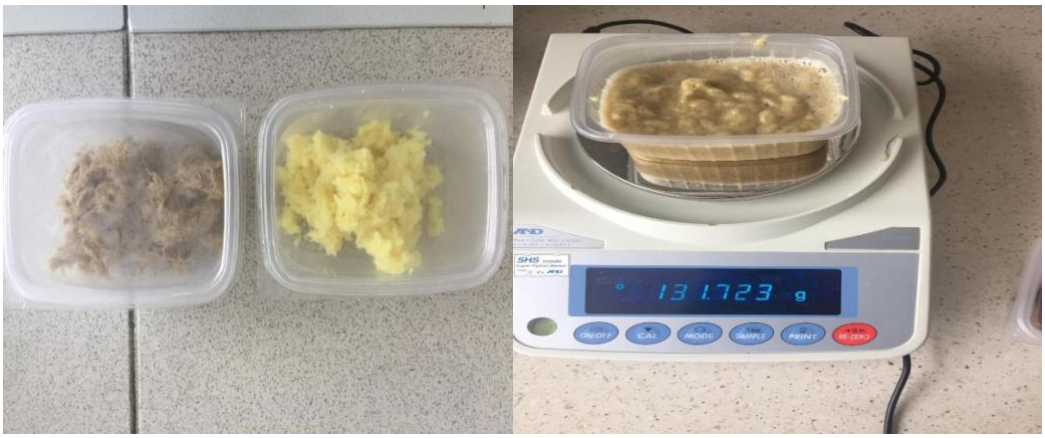

Figure 1 Processed ginger and licorice root.

Figure 2 Extracts and pate samples: a) ready extracts after centrifugation, b) control sample pate, c) experimental sample pate (2% licorice root – 3% ginger root).

Figure 3 Homogenized pâté mass.

Number of samples analyzed: To analyze the microbiological safety of mutton liver pâté, 60 samples of pâté were studied.

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.

Design of the experiment: At the beginning of the experiment, we analyzed the microbiological parameters of extracts depending on incubation time, the microbiological safety of extracts and liver pates. Water activity and pH of pâté samples during storage, and sensory analysis of liver pates with the addition of licorice root and ginger were studied.

Statistical Analysis

The experiments were performed in triplicate. Standard deviation values were indicated for all measurements. Differences in the measurements of the experimental and control groups were calculated using analysis of variation (one-way ANOVA) using the Tukey test. A p-value of <0.05 was considered significant.

RESULTS AND DISCUSSION

The effect of the extract of liquorice root and ginger and their different proportions in the composition of pâtés are shown in Table 2 and Table 3.

Table 2 Microbiological parameters of licorice extract sample after 7 days of storage (incubation time – 48 hours).

Indicator	Test strains	Growth rates
Total viable count, CFU/g	-	nd
Lactobacillus	cg	nd
Mould fungi	-	nd
Staphylococcus aureus	cg	nd
Escherichia coli	cg	nd
Salmonella	cg	nd
Yeast	-	nd

Note: cg – confluent growth.; nd – not detected.

Table 3 Microbiological parameters of licorice extract (incubation time – 48 hours).

Storage time	Indicator	Test strains	Pate samples			
			Control	Variant 1	Variant 2	Variant 3
Day of production	Total viable count, CFU/g	-	nd	nd	nd	nd
	Lactobacillus	cg	nd	nd	nd	nd
	Mould fungi	-	nd	nd	nd	nd
	Staphylococcus aureus	cg	nd	nd	nd	nd
	Escherichia coli	cg	nd	nd	nd	nd
	Salmonella	cg	nd	nd	nd	nd
	Yeast	cg	nd	nd	nd	nd
After 1 month	Total viable count, CFU/g	-	nd	nd	nd	nd
	Lactobacillus	cg	nd	nd	nd	nd
	Mould fungi	-	nd	nd	nd	nd
	Staphylococcus aureus	cg	nd	nd	nd	nd
	Escherichia coli	cg	nd	nd	nd	nd
	Salmonella	cg	nd	nd	nd	nd
	Yeast	cg	nd	nd	nd	nd
After 2 month	Total viable count, CFU/g	-	nd	nd	nd	nd
	Lactobacillus	cg	nd	nd	nd	nd
	Mould fungi	-	nd	nd	nd	nd
	Staphylococcus aureus	cg	nd	nd	nd	nd
	Escherichia coli	cg	nd	nd	nd	nd
	Salmonella	cg	nd	nd	nd	nd
	Yeast	cg	nd	nd	nd	nd

Table 3 Cont.

Storage time	Indicator	Test strains	Pate samples			
			Control	Variant 1	Variant 2	Variant 3
After 3 month	Total viable count, CFU/g	-	nd	nd	nd	nd
	Lactobacillus	cg	nd	nd	nd	nd
	Mould fungi	-	nd	nd	nd	nd
	Staphylococcus aureus	cg	nd	nd	nd	nd
	Escherichia coli	cg	nd	nd	nd	nd
	Salmonella	cg	nd	nd	nd	nd
	Yeast	cg	nd	nd	nd	nd
After 6 month	Total viable count, CFU/g	-	nd	nd	nd	nd
	Lactobacillus	cg	nd	nd	nd	nd
	Mould fungi	-	nd	nd	nd	nd
	Staphylococcus aureus	cg	nd	nd	nd	nd
	Escherichia coli	cg	nd	nd	nd	nd
	Salmonella	cg	nd	nd	nd	nd
	Yeast	cg	nd	nd	nd	nd
After 12 month	Total viable count, CFU/g	-	$4x10^2$	$2x10^1$	nd	nd
	Lactobacillus	cg	nd	nd	nd	nd
	Mould fungi	-	nd	nd	nd	nd
	Staphylococcus aureus	cg	nd	nd	nd	nd
	Escherichia coli	cg	nd	nd	nd	nd
	Salmonella	cg	nd	nd	nd	nd
	Yeast	cg	nd	nd	nd	nd

Note: cg – confluent growth.; nd – not detected.

Table 4 presents acceptable microbiological safety standards for plant extracts and liver pâtés.

Table 4 Regulated standards of microbiological safety of extracts and liver pates (TR CU 021/2011 and TR CU 034/2013) **[31]**, **[32]**.

Indicator	Standards, not more
Plant extracts	
Total viable count, CFU/g	$5x10^3$
Mold fungi, CFU/g	100
Escherichia coli	1.0 in 1 g
Yeast	50
Liver pates	
Total viable count, CFU/g	$1x10^3$
Nonspore-forming microorganisms, including lactic acid and/or mold fungi, and/or yeasts	Not allowed in 1 g
Staphylococcus aureus	Not allowed in 1 g
Escherichia coli	Not allowed in 1 g
Salmonella	Not allowed in 1 g

According to the results of experimental studies, during the storage period of pate samples, the least growth of microflora was observed in experimental samples with 1% liquorice root – 2% ginger root and 3% liquorice root – 4% ginger root. All samples during the storage period complied with the regulated norms of industry regulatory documents.

There were no *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* moulds and yeasts in all experimental samples, which indicated compliance of conditions and modes of production to sanitary requirements. On the 12th month of storage, the growth of total viable count up to $4x10^2$ and $2x10^1$ CFU/g was detected in the control and variant 1, respectively. Similar results on the reduction of total microbial count and Pseudomonas number were obtained by Bilska et al. when studying 0.2% and 0.6% of *Morus Alba* leaf extract in liver pâtés **[11]**.

Martin-Sánchez et al. showed a reduction in the growth of mesophilic anaerobic bacteria with the addition of 7.5% date paste and/or annatto extract in liver pâté **[33]**. These findings suggest that plant extracts containing certain amounts of phenolic and biologically active compounds may suppress the growth of unwanted microflora. Studies conducted by Islam et al. **[34]** and Nicolic et al. **[35]** confirmed the bactericidal effect of ginger extracts against foodborne pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.*, and others. Extract of ginger root acts as an antioxidant and antimicrobial agent against pathogenic bacteria **[36]**, **[37]**, **[38]**.

Experimental data obtained by Chabuck et al. **[14]** confirmed the antimicrobial activity of liquorice water extract against *E. coli* (25mm), *Staphylococcus saprophyticus* (23mm), and other microbial pathogens, as well as its effectiveness in combination with antibiotics. Liquorice contains several active components, such as glycyrrhizin, liquiritigenin, licochalcone A, and glabridin, which have been shown to have potent effects in inhibiting the activities of Gram-positive bacteria and Gram-negative bacteria **[39]**, **[40]**, **[41]**. Schilling et al. noted that adding rosemary and green tea extracts effectively suppressed lipid oxidation and slowed bacterial growth in pork sausages **[42]**. Riel et al. added parsley extract powder as a substitute for sodium nitrite in mortadella-type sausages and revealed a reduction of the growth of certain bacteria in the sausages **[43]**.

It is well known that water activity plays a crucial role in preserving food products **[44]**. It affects the growth and development of microorganisms and the rate of physicochemical processes during storage. Water activity is the best indicator for determining the potential growth of microorganisms **[45]**, **[46]**. A product may have a relatively high percentage of water content, but if this water is chemically "bound" with hygroscopic substances, it is not available for developing microorganisms. The kinetics of microbiological and biochemical processes, including those responsible for food spoilage, depend on the water activity level **[47]**, **[48]**.

The experimental samples on the day of production, at the 1st month and 12th month of storage, had significant differences from the control batch. However, at 1, 3 and 6 months of storage, all samples showed insignificant differences between the batches (p <0.05). The water activity values indicated a downward trend in water activity with increasing storage time (Table 5). The values of the control samples were higher than all samples of the experimental batches. Alirezalu et al. found that during a 45-day storage period, no significant differences were observed in the water activity values of sausages that included mixed plant extracts **[49]**, **[50]**.

Table 5 Water activity of pâté samples during storage.

Storage time	Control	Experimental		
		Variant 1	Variant 2	Variant 3
Production day	0.98^{a} ±0.00	0.97^{a} ±0.00	0.97^{a} ±0.00	0.97^{a} ±0.00
1 month	0.97^{a} ±0.02	0.97^{a} ±0.00	0.96^{a} ±0.00	0.96^{a} ±0.02
2 months	0.95 ^{a} ±0.01	0.96^{a} ±0.00	0.96 ^{a} ±0.01	0.96 ^{a} ±0.00
3 months	0.95 ^{a} ±0.01	0.96^{a} ±0.01	0.96 ^{a} ±0.01	0.95 ^{a} ±0.01
6 months	0.95 ^{a} ±0.01	0.96 ^{a} ±0.01	0.95 ^{a} ±0.01	0.95^{a} ±0.01
12 months	0.95^{a} ±0.01	0.95^{a} ±0.01	0.95^{a} ±0.01	0.94 ^{a} ±0.01

Note: Identical letters in the column mean that the test showed no significant difference between the batches (p >0.05).

The pH values of the control samples were slightly lower than those of the experimental samples (Table 6). The tendency to grow pH with the increase of plant extracts in the composition of the batch is visible. This trend continued at the beginning of storage (day of production) and during the entire storage period. Similar results were obtained by Ibrahim et al. in the study of Jojoba (*Simmondsia Chinensis*), *Jatropha curcas*, *Panax ginseng* and ginger (*Zinger officinale*) on the pH of lamb cutlets **[51]**. Adding liquorice root extract and ginger root extract to the experimental sample appeared to have an initial effect on both water activity and pH, with lower water activity and higher pH. However, after an initial adjustment period, the samples eventually reached similar water activity levels and relatively stable pH values. In conclusion, the addition of liquorice root extract and ginger root extract to the mutton liver pate did not significantly affect the water activity and pH values. The control and experimental samples showed stable water activity and pH levels during the 12-month storage period.

Table 6 pH of pate samples during storage.

Storage time	Control	Experimental		
		Variant 1	Variant 2	Variant 3
Production day	5.90 ±0.11[a]	5.98 ±0.13[b]	6.15 ±0.09[c]	6.18 ±0.07[d]
1 month	5.89 ±0.05[a]	5.95 ±0.03 [a]	6.06 ±0.01 [a]	6.15 ±0.07 [b]
2 months	5.94 ±0.17[a]	5.94 ±0.03 [b]	6.05 ±0.06 [b]	6.17 ±0.02 [c]
3 months	5.94 ±0.02[a]	5.99 ±0.05[b]	6.14 ±0.09[c]	6.22 ±0.07[d]
6 months	5.98 ±0.04[a]	6.05 ±0.08 [a]	6.26 ±0.04 [a]	6.31 ±0.05[d]
12 months	6.01 ±0.01[a]	6.07 ±0.11[b]	6.25 ±0.02[c]	6.34 ±0.09[d]

Note: [a-d] Values with different letters mean a significant difference between the batches (p <0.05). Identical letters mean that the test showed no significant difference between the batches (p >0.05).

Sensory analysis was conducted to evaluate the experimental batch of pâté compared to the control. The experimental batches of pâté were evaluated by trained panellists as being lighter in colour, having a spreading consistency, and being juicier (Table 7).

Table 7 Sensory analysis of liver pates with the addition of licorice root and ginger.

Parameter	Control	Experimental		
		Variant 1	Variant 2	Variant 3
Color (light – dark)	8.1 ±0.30[a]	8.1 ±0.20[a]	8.0 ±0.22[b]	7.5 ±0.01[b]
Smell (aroma) (intense – faint)	8.8 ±0.21[a]	8.9±0.20[a]	9.0 ±0.28[b]	8.8 ±0.32[a]
Taste (sweet – salty)	8.3±0.32[a]	8.1 ±0.21[a]	8.3 ±0.16[a]	8.2 ±0.29[a]
Consistency (firm – soft)	6.0±0.31[a]	6.3 ±0.20[b]	8.0 ±0.18[c]	8.2 ±0.30[c]
Juiciness (dry – juicy)	7.8 ±0.28[a]	8.2 ±0.33[b]	8.8 ±0.45[c]	9.0 ±0.37[c]
Particles in the mass (insignificant – noticeable)	9.0 ±0.37[a]	9.0 ±0.20[a]	9.0 ±0.21[a]	8.9 ±0.30[a]
Overall score	7.9 ±0.41[a]	8.0 ±0.30[a]	8.55 ±0.27[b]	8.4 ±0.33[c]

Note: [a-d] Values with different letters mean a significant difference between the batches (p <0.05). Identical letters mean that the test showed no significant difference between the batches (p >0.05).

According to the sensory evaluation results (Table 7), all experimental and control samples did not show critical differences in taste and the presence of particles in the liver paste. Differences were noticeable in the evaluation of colour: the experimental samples with 2% liquorice root-3% ginger root (8.0 points) and 3% liquorice root – 4% ginger root (7.5 points) had a pale colour. The texture of the control sample (6.3 points) was evaluated as harder and drier than the experimental samples. Tasters rated the sample with 3% liquorice root and 4% ginger root as the most tender and juicy (8.2 and 9.0 points, respectively). With the increase of plant ingredients in the experimental samples, there was a trend towards a more pronounced tender and spreading consistency. As a result of the sensory analysis of the liver paste samples showed positive changes in consistency, juiciness, aroma, and overall evaluation with the introduction of plant extract ingredients into the recipe. Several studies reported comparable findings, indicating that using rosemary and green tea extracts **[42]**, **[52]**, **[53]** elderberry extract **[54]**, **[55]** guarana seed extracts **[56]** did not deteriorate the overall acceptability of meat products among consumers.

CONCLUSION

Product safety is one of the main selection criteria when consumers choose ready-to-eat foods. Adding a new ingredient to the traditional component composition of the product can significantly vary the microbiological indicators, which in turn are closely related to the values of water activity and pH. Microbiological parameters of experimental and control samples were within the regulated norms during 12 months of storage at 4 °C. Among the experimental samples, the sample containing 2% liquorice root – 3% ginger root, and 3% liquorice root – 4% ginger root showed the most optimal indicators of inhibition of undesirable microflora. During the storage period, a decrease in water activity and increased pH value were observed. The sensory analysis findings indicate that adding lard and plant-based ingredients to the pâté recipe had a beneficial effect on its consistency, juiciness, aroma and overall rating. The results of experimental studies have shown the prospects of using extracts of ginger root and liquorice as new and natural ingredients with functional properties to improve the microbiological stability of liver pates. Future research should investigate optimizing ingredient ratios, texture effects, and nutritional profiling to enhance the sensory appeal and nutritive value of liver pâtés containing liquorice and ginger extracts.

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Seaweed-based films for sustainable food packaging: properties, incorporation of essential oils, applications, and future directions

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ABSTRACT

Seaweed-based films have emerged as a promising solution for sustainable food packaging due to their renewable sourcing, biodegradability, and functional properties. This review provides an in-depth analysis of seaweed-based films, focusing on their properties, incorporation of essential oils, applications in food packaging, and future directions. The advantages of seaweed-based films include their renewable and abundant source, biodegradability, and favorable barrier properties. The review explores the physical and mechanical properties, barrier properties, and safety considerations of seaweed-based films. Additionally, it discusses the incorporation of essential oils into seaweed-based films and their potential benefits. Current and potential applications of seaweed-based films in food packaging, ranging from fresh produce to dairy products, are examined, along with the advantages and challenges associated with their use. A comparison with other sustainable packaging options is provided. Furthermore, the review highlights future research directions in developing seaweed-based films, such as improving mechanical properties, extending shelf life, scaling up production, reducing costs, and innovation in formulation. Overall, seaweed-based films offer a promising and sustainable alternative for food packaging, with ongoing research and development driving their advancement and potential for a more environmentally friendly packaging industry.

Keywords: seaweed-based films, sustainable packaging, food packaging, biodegradability, renewable sourcing, barrier properties, essential oils

INTRODUCTION

The growing need for sustainable packaging has become increasingly apparent in recent years. With environmental concerns such as plastic pollution and climate change at the forefront, there is a pressing demand for innovative packaging solutions that minimize negative impacts on the planet. One promising approach is using edible films and coatings, offering functional and sustainable benefits.

Edible films and coatings are thin layers of materials that can be consumed with packaged food or easily removed before consumption. They provide a protective barrier against external factors such as moisture, oxygen, and microbial contamination while also extending the shelf life of perishable products. These films can be made from various natural sources, including proteins, polysaccharides, lipids, and composite materials **[1]**.

Among the different types of edible films, seaweed-based films have gained significant attention as an environmentally friendly option. Seaweed, a type of marine macroalgae, possesses unique properties that make it well-suited for sustainable packaging applications.

One of the key advantages of seaweed-based films is their renewable and abundant source. Seaweeds are fast-growing marine plants that can be cultivated without arable land, freshwater, or pesticides. They have a high growth rate, allowing for sustainable harvesting without depleting natural resources. This makes seaweed an attractive alternative to traditional packaging materials derived from fossil fuels or limited resources **[2]**. Furthermore, seaweed-based films offer inherent biodegradability and compostability. Unlike conventional plastic packaging, which can persist in the environment for hundreds of years, seaweed films can naturally decompose through microbial activity, contributing to a more circular economy. This feature aligns with sustainable packaging principles, where materials should have minimal environmental impact at the end of their life cycle **[3]**. In summary, seaweed-based films offer a range of advantages for sustainable packaging. They are derived from renewable resources, biodegradable, and exhibit favorable barrier properties. Using seaweed as a packaging material, we can reduce reliance on fossil fuel-derived plastics and contribute to a more environmentally friendly packaging industry. The following sections will explore the properties of seaweed-based films in more detail and discuss their potential applications in food packaging **[4]**. Additionally, the review will assess the current and potential applications of seaweed-based films in food packaging. From extending the shelf life of fresh produce to enhancing the preservation of meat and dairy products, seaweed-based films offer a versatile and sustainable packaging solution across various food categories. The advantages and challenges associated with using seaweed-based films in food packaging will be analyzed, along with a comparison to other sustainable packaging options **[2]**.

Finally, the review will identify future research directions in developing seaweed-based films and provide a comprehensive conclusion summarizing the key points discussed. By examining the potential of seaweed-based films for sustainable packaging, this review aims to contribute to the ongoing efforts to reduce the environmental impact of packaging materials in the food industry **[4]**.

Properties of Seaweed-Based Films

In recent decades, scientists have discovered an abundance of unique compounds in marine (often termed the mother of the genesis of life) species that show promise as constituents in newly developed medicines, foods, packaging materials, and textiles that may be used to promote wellness for people. The term "seaweed" describes a wide variety of macro or multicellular marine plants and algae found in the ocean, rivers, ponds, and other bodies of water **[5]**.

Plastic is one of the most popular materials because of its many applications, long lifespan, high resilience, and relatively inexpensive cost **[6]**. However, regular plastics harm marine ecosystems since they are produced using non-renewable resources **[7] [8]**, **[9]**, **[10]**. Bioplastics might solve these issues; however, biomass-based first and second-generation bioplastics significantly influence land-use change, directly and indirectly **[11]**, **[12]**. As for bioplastics, seaweed is known as a 3rd generation feedstock because it requires no additional land for growth **[13]**, **[14]**. The composition of seaweed (Chemical) includes minerals ranging between 7 to 37.5%, water ranging starting from 80% and uptake of 90%, protein constitutes between 3 and 14.5%, carbohydrates present up to 50%, and Lipids constitute 1 to 3% **[15]**, **[16]**. Types of seaweeds are demonstrated in Figure 1.

Seaweed has been used extensively in bio-packing, food, and biomedical applications because of its biocompatibility, bio-absorbability, biodegradability, and nontoxicity. Both enzymatic association and non-enzymatic degradation of these polymers are possible. Alginate, agar, and carrageenan are three hydrocolloid polysaccharides produced from seaweed that have several uses as biopolymeric films **[17]**. Carbon and nitrogen cycling are only two examples of the ecological benefits and ecosystem provisioning that may be gained through seaweed agriculture. Thereby perhaps aiding the fight against eutrophication, ocean acidification, and climate change **[18]**, **[19]**.

Due to their excellent effects on ecological concerns and other distinctive qualities, materials that come into touch with food have great potential to benefit from fresh creative technologies like edible films. Biopolymers derived from seaweed have shown promise as a high-quality and reasonably priced alternative to petroleum-based polymers. Essential oils (EOs) are a natural, non-toxic alternative to synthetic preservatives that may be used to enhance the functioning of biopolymer-based packaging. Food packaging prevents food from spoiling due to environmental causes, especially when bioactive chemicals like EOs are included in biodegradable packaging materials **[20]**.

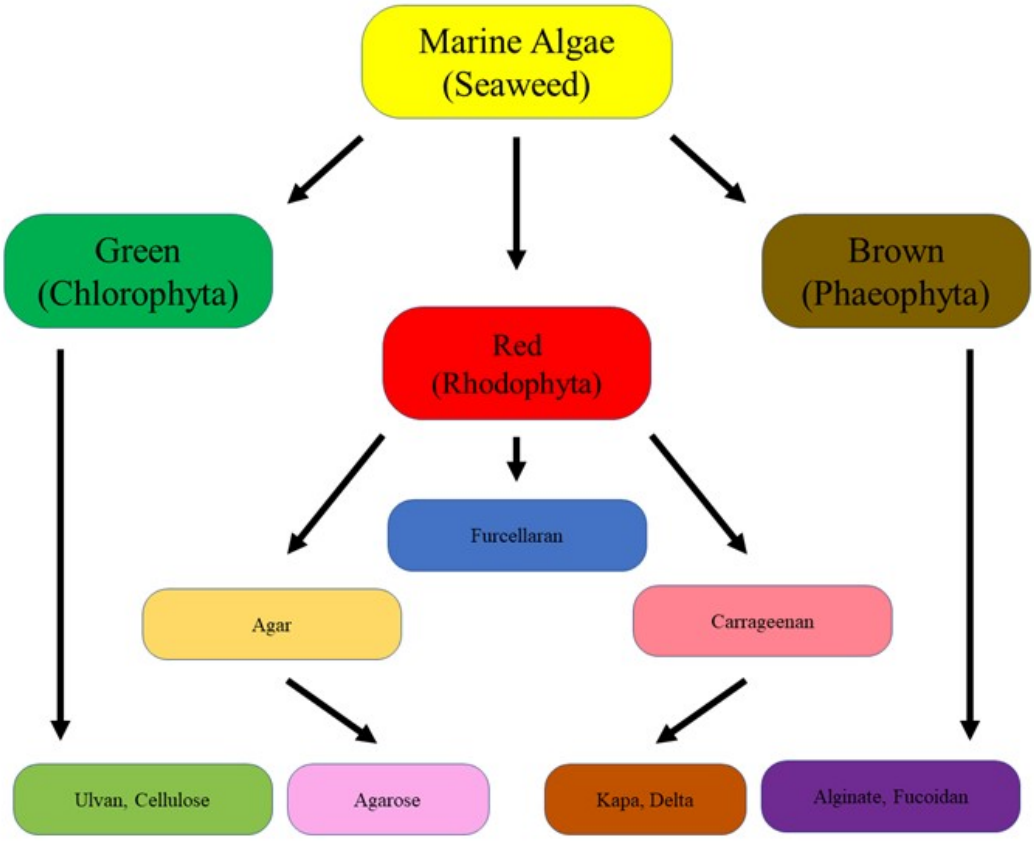

Figure 1 Overview of Seaweed.

Growing seaweeds is frequently seen as a way to address problems with food security, such as climate change, a lack of arable land, food scarcity, and wasteful fertilizer usage. The future bio-economy might benefit significantly from a thriving seaweed sector since it would allow for more efficient food production, new goods, and employment creation. With an average selling price of USD 0.48 per kg for brown seaweed, USD 0.40 per kg for red seaweed, and USD 0.80 per kg for green seaweed (all wet weight), seaweed farming accounted for approximately 31% of the entire approximately 120 million tons of aquaculture sector output (35.5 million tons) in 2019. More than half of all harvested seaweed is utilized to make hydrocolloids, whereas only around half is consumed by humans. About 30 MM tons of seaweed are eaten annually for various uses, including the food and pharmaceutical industries, giving rise to a worldwide seaweed market worth about USD 11 billion annually. The price of seaweeds is predicted to rise further in the near future **[21]**, **[22]**, **[23]**.

As a possible bioresource, seaweed has been the subject of recent research and discussion among bioplastics. Alginate has been studied by Rinaudo **[24]** as a potential solution for food packaging and more recently, in addition to alginate, Carina and Sharma **[2]** use polysaccharides as food packaging material isolated from different seaweed to further the current research on seaweed. The overall polymer condition of seaweed and its characteristics to form various plastic kinds are described by Zhang and Show **[25]**, Pacheco and Cotas **[26]**. Seaweed polymers are extracted and reviewed by Shravya and Vybhava Lakshmi **[27]**, Lim and Yusoff **[28]** for application in bioplastic manufacturing. Experimental research on the manufacture of seaweed-based polymers has been conducted in certain studies.

Albertos and Martin-Diana **[29]**, Aragão Rebouças Júnior and Turan **[30]**, Lim and Hii **[31]** tried out making edible films out of red and brown seaweed, brown seaweed alginate-based bioplastics. These studies review the various types of seaweed-based plastics. However, they seldom analyze the manufacturing and end-of-life (EoL) implications on the environment quantitatively, instead focusing on the physical qualities and prospective techniques to create seaweed-based plastics, with some even employing experimental data. Ayala and Thomsen **[32]** explore the potential for recycling some biorefinery byproducts for film production and the consequences on the environment of a novel seaweed-based plastic manufactured from *Saccharina latissima*. Carbon

balancing and life cycle assessment are used to evaluate this possibility. A summary of edible packaging based on of seaweed biopolymers is provided in Table 1.

Previous research by Zhang and Thomsen **[33]** has highlighted the significance of maximizing the value of the complete seaweed biomass. That is significant from both an ecological and a monetary viewpoint. In the food industry, where biomass has a high economic value, there is a considerable demand for seaweed biomass. Since seaweed biomass is used at a far lower cost in plastic manufacture, its value rises when it is put to good use, including byproducts from bio-crude extraction **[34]**.

Seaweed-based films are highly biodegradable, meaning they can be naturally broken down by environmental microorganisms **[35]**. They offer an eco-friendly alternative to conventional plastic films that often persist in the environment for extended periods. Seaweed is a renewable resource that can be sustainably harvested, making seaweed-based films a sustainable choice **[36]**. This characteristic aligns with the principles of environmental conservation and resource efficiency. One unique property of seaweed-based films is their water solubility **[36]**. This characteristic makes them suitable for single-use applications, such as dissolvable packaging units for detergents, personal care products, or food items.

Seaweed contains natural compounds, such as alginates and polyphenols, which exhibit antimicrobial properties **[35]**. When incorporated into seaweed-based films, these compounds can help inhibit the growth of bacteria and fungi, making them useful for food packaging where microbial spoilage is a concern. The versatility of seaweed-based films allows for modifications through blending with other biopolymers or incorporating additives **[36]**. This flexibility enables the films to be tailored to specific requirements, such as mechanical strength, gas permeability, or moisture resistance. So, seaweed-based films offer a sustainable and biodegradable alternative to conventional packaging materials. Their properties, including biodegradability, barrier properties, flexibility, water solubility, antimicrobial properties, and versatility, make them a promising solution for reducing plastic waste and environmental impact in various industries.

Table 1 Summary of edible Packaging based on of seaweed biopolymers.

Derivative	Biopolymer	Application	Ref
Gelidium sesquipedale	Agar	Active Pack	**[37]**
Kappaphycus sesquipedale	Carrageenan	Edible coating	**[38]**
Laminaria sesquipedale	Alginate	Edible coating	**[39]**
	Sodium alginate	Packaging	**[40]**
	Chitosan	Edible coating	**[41]**
Furcellaria lumbricalis	Furcellaran	Packaging film	**[42]**

Mechanical Properties

Food packaging films must meet strict standards for mechanical properties such as tensile strength, modulus of elasticity, and deformation upon break. The results of these tests reveal how well the film maintains its structural integrity under the numerous pressures that arise during the preparation, transportation, and storage of food in packaging. The elastic modulus measures the force per unit area required to stretch a film sample to a certain size. Measured in terms of force per unit area, tensile strength may be used to evaluate the resistance of a film to tearing. And the proportion of change in running time due to a break is given by the elongation at the break **[43]**.

PVA and sodium alginate were combined to create the new substance. A universal tensile machine was used to analyze the samples' tensile properties, with the crosshead moving at a rate of 50 mm/min. Each sample had an initial length of 40 mm, a breadth of 10 mm, and a 100-150 m thickness. The results showed that the tensile modulus was increased by 210 MPa (from 510 MPa to 700 MPa) when PVA and SA were used together. The addition of the polysaccharide resulted in a modest improvement in tensile strength from 41 MPa to 44 MPa and an increase in elongation at break from 53% to 57%. The rigid polysaccharide added to the polymer mix decreased its flexibility, but the study found that it was still useful for most applications despite this **[44]**.

Jumaidin and Sapuan **[45]** in terms of research towards making an environmentally friendly agar film with varying amounts of agar. Tensile strength was measured with the crosshead moving at 4.5 mm/min at 22 °C and 51% RH relative humidity. Tensile strength increased from 10.5 MPa with no agar to 14.5 MPa with 32% agar and 13.5 MPa with 42% agar. Also, the tensile modulus rose progressively from 1500 MPa at 0% agar to 2000 MPa at 42% agar. The elongation at break was similarly affected by the incorporation of agar into the SPS, going from 2% at 0 .1wt% agar to 0.77% at 41 wt% agar. SPS and agar may be readily miscible owing to their same chemical structure and phase compatibility, which may explain the observed increase in tensile strength. The more complex network structure of agar explains why it has superior mechanical qualities versus SPS.

When it comes to evaluating film performance, mechanical qualities are just as critical as water barrier capabilities, particularly for packaging and plasticulture. Mulch film has to be sturdy enough to withstand the weight of the equipment used to lay it on the ground. Strong mechanical qualities are preferred to account for certain stress and deformation while handling and putting the films on the soil. The findings demonstrate notable distinctions between seaweed based-films and traditional mulch films. Films made from seaweed that was filled with commercial $CaCO_3$ had the highest TS, at 84.92%, followed by films made from seaweed that was filled with microbial-induced $CaCO_3$ (82.14%), the control group (72.73%), and the conventional mulch film **[46]**.

Mechanical parameters including tensile strength, modulus of elasticity, and stretch at break influence food packaging polymer film quality. The linkages and solubility of chemicals and intermolecular interactions between polymer chains during blending determine composite films' mechanical properties. Integrated chemicals may change the film matrix's structure, making it less dense and allowing components to interact beyond hydrogen interactions with water molecules. There have been a number of research looking at how adding seaweed polysaccharides affects mechanical quality. More than half of the trials found that adding seaweed polysaccharides to the matching polymer increased the material's tensile strength. Excellent mechanical qualities were achieved by using alginate and starch to create films for food packing **[47]**. Alginate-based agar films are mechanically strong **[48]**.

Thermal Properties

Seaweed may improve thermal characteristics, but how much depends on the other ingredients in the mixture. The impacts on melting and glass transition temperature might vary greatly due to varying degrees of miscibility, crystallinity, and overall interaction. Adding FUC to collagen enhanced the material's thermal characteristics, while adding alginate to PHB decreased them. However, the alginate/PHB mix exhibited no changes in thermal properties **[2]**.

Understanding how polymer chains interact can be gleaned from their thermal properties, including parameters like melting temperature and glass transition temperature. These properties play a vital role in determining the suitability of polymers for applications like food packaging. They offer valuable insights that guide the entire process **[49]**. Thermal analysis by Goonoo and Bhaw-Luximon **[50]** has shown that carrageenan containing blends are somewhat miscible in the amorphous areas but not the crystalline portions. This causes the crystallization temperature to rise and the enthalpy of crystallization to fall, both of which were previously greater in the KC/PHBV mix.

According to X-ray diffraction and differential-scanning calorimetry, adding sodium alginate to PVA film lowered the melting temperature **[44]**. Alginate inclusion into PLA did not affect thermal characteristics **[51]**. The addition of alginate to PHB decreased the thermal stability **[52]**. When combined, adding agar and sugar palm starch increased the glass transition and melting temperatures **[45]**. Different change in properties of films by incorporating seaweed is given in Table 2.

Table 2 Change in properties of polymer film by incorporation seaweed.

Source	Increase/Decrease in different properties	References
Allyl isothiocyanate	coating and gas barrier properties	**[53]**
Chitosan	flexibility, permeability and hydrophobicity	**[54]**
Alginate	homogeneous, lower moisture, light absorbance, respiration rate, thermal properties, heat distribution	**[55]**, **[56]**, **[57]**, **[58]**
Bio-nano composite film	tensile strength, water resistance, thermal stability	**[59]**
Nanocrystalline cellulose	water solubility, water contact angle, elongation	**[60]**
Carrageenan	tensile strength, thermal degradation, uv barrier properties, moisture content, elongation, antimicrobial activity	**[10]**, **[61]**
Agar	tensile strength, the contact angle of water, swelling ratio	**[62]**
Polylysine	stronger complexes through electrostatic attraction	**[63]**

Biodegradability and Composability

Polymers' biodegradability is determined by three factors: (a) their chain length (i.e., molecular weight, (b) the complexity of their chemical formula, and (c) their crystalline structure **[64]**.

The extremely rapid breakdown is typical for simple, low-molecular-weight amorphous polymers. Compostability, on the other hand, is associated with improved biodegradation in specially controlled environments with the right combination of factors, such as high humidity and temperature and the presence of

microorganisms **[65]**. Composting tests may last 180 days at a certain temperature **[66]**. Because composting produces material rich in nutrients, in contrast to landfills, which produce carbon dioxide and methane, It benefits society as a whole if we switch to biodegradable plastics instead of single-use ones. Plant-based biodegradable polymers retain their composability even after being tainted with food scraps, in contrast to traditional plastics, which lose their ability to be recycled and are instead thrown away in landfills **[67]**. Bio-based degradation of biopolymer is given in Table 3.

If microorganisms are present to help in decomposition, the compostability or biodegradability of a biopolymer will be enhanced. Different outcomes may be achieved via controlled (industrial facilities) and unmanaged (natural habitats; soil, water, landfill, compost) degradation techniques owing to differences in factors like UV light exposure and oxygen availability **[65]**.

Table 3 Bio Degradation of bio-based biopolymers.

Type	Biopolymer	Percentage of Biodegradable	Rank	Testing Environment	Reference
Bio-Based	Alginate	90	severe	compost	**[68]**
	PLA	84/10/13	severe/low/low	compost/soil/compost	
	PHB	79/64	severe/moderate	compost/soil	
	Chitosan	100	severe	aqueous	
	Starch Based	14	low	soil	
	PHA	48/5	moderate/low	soil/compost	

Essential oils (EOs) and seaweed-based films

Edible films made from seaweed are popular for food packaging due to their high quality and biodegradability. Essential oils (EOs), which have potent antibacterial and antioxidant properties, are often added to seaweed-based biopolymers to create functional and active packaging materials with improved performance, such as increased shelf life and enhanced nutritional characteristics. The production process of seaweed-based films incorporating EOs is illustrated in Figure 2. EOs can render seaweed films mechanically stronger, more water-repellent, more UVR-resistant, and more thermally stable **[20]**.

The use of EOs in food packaging has gained popularity as a natural alternative to synthetic preservatives. Adding EOs to the polymer matrix can allow for the slow release of these beneficial compounds into the packaged food, improving its quality and shelf life. Essential oils are complex compounds that can be extracted through distillation of plant material or mechanical processes. These molecules are small, volatile, and hydrophobic, with a molecular weight of less than 300 Da.

Edible films made from seaweed infused with essential oils have been developed for food packaging **[69]**. Wet processes provide films with higher transparency, homogeneity, and reduced WVP and opacity. Wet processes are commonly used to create films with high transparency and homogeneity and reduced water vapor permeability and opacity. The solvent casting method is the dominant wet film creation process, where film-forming chemicals are dissolved in a solvent, cast onto a plate, and dried to remove the solvent. Water, ethanol, acetic acid, and lactic acid are commonly used solvents for film formation. Film-forming compounds contain polysaccharides with hydroxyl and polar groups, which can bond through covalent, ionic, electrostatic, and hydrophobic interactions. Hydrogen bonding is crucial to polysaccharide film formation. The interactions between the film and food product depend on the film's thickness, structure, molecular weight, temperature, and production parameters **[70]**, **[71]**, **[72]**.

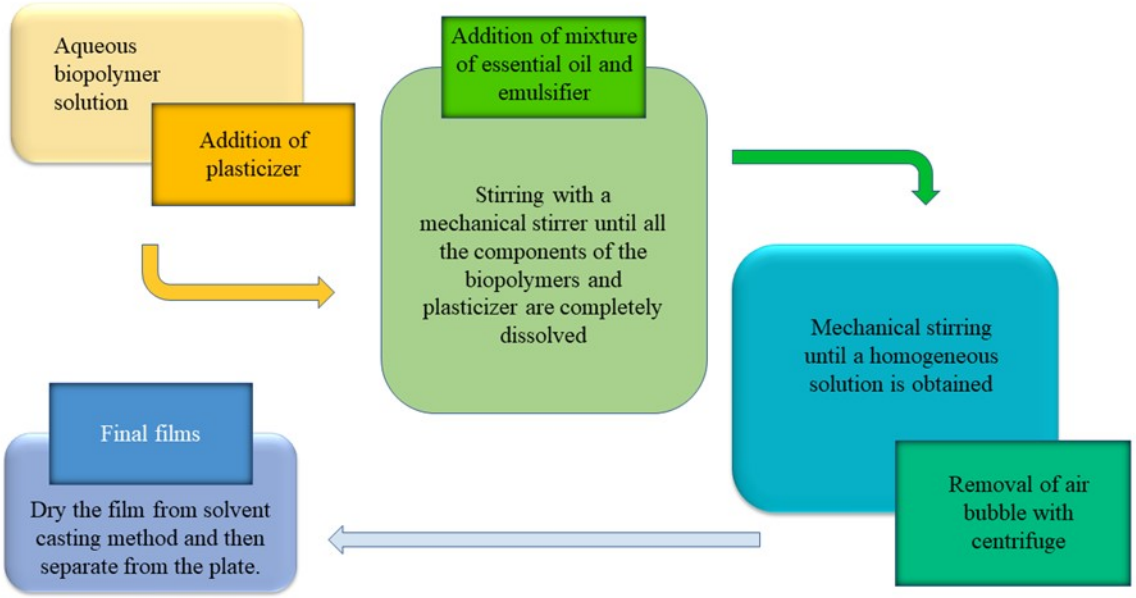

Figure 2 Overview of the production process of the seaweed-based film by incorporating essential oils.

Characterization of EOs loaded seaweed-based film

The seaweed biopolymer-based food packaging matrix containing EOs may be viewed by SEM, TEM, and AFM. Packaging materials' physical properties depend on the film's structure. The matrix must equally distribute antimicrobial essential oils (EOs) for the seaweed-based film toJ work. Seaweed polymer matrix-EO interaction improves film microstructure. EOs change seaweed-based film microstructure. Thyme and lemongrass oils made the alginate-based film surface rougher **[73]**.

Different surface morphology (SEM) was found for films made with alginate and cinnamon essential oil compared to films made with only alginate. The rough texture and hollow structures seen in the EO-containing film may be attributed to the upward migration of oil droplets upon exposure to air **[74]**.

Properties of edible films fabricated with EOs and seaweed derivatives

Essential oils can be used to impart color to seaweed polysaccharide-based films, although the films are typically colorless. In addition to adding color, essential oils can reduce Maillard reactions and dehydration by inhibiting microorganism growth and oxidative stress **[75]**.

Seaweed-based films' physical and functional properties are crucial to their practical use. The mechanical properties, oxygen and water vapor barrier properties, hydrophobicity, and solubility in water are among the most important physical characteristics of an active food packaging film. Essential oils significantly impact these properties, and their effect on seaweed-based films will be briefly discussed in this article. An overview of seaweed-based packaging films by incorporating EOs is summarized in Table 4. Table 4 provides an overview of seaweed-based packaging films incorporating essential oils, including their composition, properties, and potential applications.

Table 4 Overview of seaweed-based packaging films by incorporating EOs.

Overview of seaweed-based packaging films by incorporating EOs			
Source	EOs used	production method	Reference
Agar	0.12% tea tree, 10.0% clove, 2.0% neem	solvent casting	**[37]**, **[76]**, **[77]**
Alginate	0.02% cinnamaldehyde , 3.0% clove	coating, solvent casting	**[78]**, **[79]**
Sodium alginate	2% ginger, 0.6% oregano, 1% lemongrass	coating, solvent casting	**[80]**, **[81]**, **[82]**
Carrageenan	10.0% zataria multiflora , 3.0% rosemary, 1% cinnamon	coating, solvent casting	**[83]**, **[84]**, **[85]**

Seaweeds can accumulate hazardous substances due to industrialization and the leakage of petroleum chemicals into the water, which can include arsenic, lead, and mercury despite their numerous benefits. The European Commission Regulation (EC) No. 629/2008 has set a maximum cadmium level of 3 mg/kg dry weight in edible seaweeds. The regulation also limits the quantity of arsenic in a full meal to 10-40 mg per kilogram of dry weight, while other toxic metals have no specific rules **[86]**.

The United States Food and Drug Administration (FDA) has published a list of essential oils that can be used as a food flavoring and classified them as Generally Recognized As Safe (GRAS), meaning they are safe to ingest if used in approved levels (US FDA 2018). Regulation (EC) No. 1334/2008 of the European Commission provides guidelines to ensure the safety of flavorings, and a revised and updated list of permitted flavors was published in an annex to the regulation on October 1, 2012. Only ingredients on the Union-approved list, which is included in Regulation (EC) No. 1334/2008, may be added to food **[87]**, **[88]**.

Essential oils (EOs) can be safely used as ingredients in food at recommended levels, but higher levels can cause allergic reactions **[89]**. Essential oils (EOs) can be safely used as ingredients in food at recommended levels, but higher levels can cause allergic reactions **[90]**. Some health problems, such as eye, skin, and mucous membrane irritations and sensitivity to EOs containing aldehydes or phenols, have been linked to essential oils **[91]**. A previous study has suggested that clove essential oil may lower blood glucose, acidosis, and ketonuria, among other effects **[92]**. Therefore, the value and potential side effects of EOs should be assessed before using them in food. Applications of Seaweed-Based Films in Food Packaging Current and potential applications of seaweed-based films in food packaging Advantages and challenges of using seaweed-based films in food packaging Comparison with other sustainable packaging options **[93]**.

Applications of Seaweed-Based Films in Food Packaging

Fresh Produce Packaging: Shelf-life extension of fruits and vegetables: Seaweed-based films have shown great potential in extending the shelf life of fruits and vegetables. These films act as a barrier, preventing the exchange of gases and moisture between the packaged produce and the surrounding environment. By creating a modifJied atmosphere within the packaging, the respiration rate of the fruits and vegetables can be slowed down, effectively delaying the onset of spoilage **[2]**.

The barrier properties of seaweed-based films help to reduce water loss from the produce, minimizing shrinkage and maintaining turgidity. This can significantly extend the shelf life of perishable fruits and vegetables, allowing them to remain fresh and appealing for a longer duration. By preventing moisture loss, the films also help to retain the natural juiciness of the produce, enhancing their sensory quality **[4]**.

Preservation of texture and nutritional quality

One of the key challenges in fresh produce packaging is maintaining the desired texture and nutritional quality of the fruits and vegetables. Seaweed-based films provide an excellent solution to address these concerns. These films create a microenvironment that helps to preserve the texture and structural integrity of the produce, preventing softening, wilting, or browning **[93]**.

Furthermore, seaweed-based films have been found to preserve the nutritional quality of fruits and vegetables. They can act as a protective barrier against light, which reduces the degradation of light-sensitive nutrients such as vitamins and antioxidants. This ensures that the packaged produce retains its nutritional value and remains a healthy choice for consumers.

Seaweed-based films also offer a unique advantage in terms of their bioactive compounds. Seaweeds are rich in bioactive components, such as polyphenols, polysaccharides, and antioxidants, which have been associated with various health benefits. These compounds can potentially migrate from the seaweed-based films to the packaged produce, providing additional health-enhancing properties **[4]**.

In summary, seaweed-based films play a vital role in fresh produce packaging by extending the shelf life of fruits and vegetables and preserving their texture and nutritional quality. These films act as effective barriers, creating a modified atmosphere that slows down the respiration rate and minimizes moisture loss. By utilizing seaweed-based films, the freshness, sensory appeal, and nutritional value of packaged produce can be significantly enhanced, reducing food waste and improving consumer satisfaction **[4]**.

Meat and Seafood Packaging

Preservation and quality maintenance: Seaweed-based films have proven effective in preserving and maintaining quality for meat and seafood products. These films act as a protective barrier, preventing the entry of oxygen and moisture, which are major contributors to the degradation of meat and seafood.

By creating a modified atmosphere within the packaging, seaweed-based films help to inhibit the growth of spoilage-causing microorganisms and reduce oxidative reactions, thus extending the shelf life of the products.

The barrier properties of these films also minimize the loss of natural juices and flavors, helping to maintain the sensory attributes and overall quality of the packaged meat and seafood **[92]**.

Furthermore, seaweed-based films exhibit antimicrobial properties due to the presence of bioactive compounds in seaweeds. These compounds can inhibit the growth of pathogenic bacteria, reducing the risk of foodborne illnesses and enhancing the safety of the packaged meat and seafood **[94]**.

Extended shelf life for meat and seafood products

Seaweed-based films offer the advantage of significantly extending the shelf life of meat and seafood products. The barrier properties of these films help to prevent moisture loss and protect against external contamination, thereby maintaining the freshness and integrity of the packaged products.

In the case of meat, the use of seaweed-based films can prevent dehydration and the subsequent loss of weight, preserving the juiciness and tenderness of the meat. It also helps to minimize the growth of spoilage bacteria and delay the onset of microbial spoilage, leading to an extended shelf life **[95]**.

For seafood, which is highly perishable, seaweed-based films play a crucial role in maintaining its quality and extending its shelf life. The films act as a barrier to prevent the entry of oxygen, which can cause oxidation and deterioration of the seafood. This helps to preserve the flavor, texture, and color of the packaged seafood, making it more appealing to consumers and reducing the potential for waste **[2]**.

By utilizing seaweed-based films in meat and seafood packaging, the industry can benefit from increased product shelf life, reduced food waste, and improved overall quality and safety of the packaged products. These films provide a sustainable and effective solution to preserve the freshness and extend the availability of meat and seafood, ensuring that they reach consumers in optimal condition **[92]**.

Bakery and Confectionery Packaging

Preservation of texture and freshness in baked goods: Seaweed-based films offer valuable benefits in the packaging of bakery and confectionery products, specifically in preserving their texture and freshness. These films act as a protective barrier, preventing the exchange of moisture and gases between the packaged products and the surrounding environment.

Moisture is a critical factor in maintaining the desired texture of bakery items. Seaweed-based films help to regulate the moisture content by reducing moisture loss from the products and minimizing the absorption of moisture from the environment. This helps to prevent staleness, hardness, or dryness in baked goods, ensuring that they retain their softness, moistness, and overall appealing texture **[4]**.

Furthermore, seaweed-based films contribute to the retention of freshness in baked goods. These films create a microenvironment within the packaging that helps to slow down the staling process. They can prevent moisture migration between different components of the baked goods, such as the crust and the crumb, which helps maintain their distinct textures and flavors over time **[92]**.

Prevention of staleness and shelf stability

Seaweed-based films play a crucial role in preventing staleness and enhancing the shelf stability of bakery and confectionery products. Staleness is primarily caused by the absorption of moisture from the surrounding environment, resulting in changes in texture, taste, and overall quality.

Seaweed-based films act as a moisture barrier, effectively preventing the transfer of moisture into the packaged products. By minimizing moisture uptake, these films help to maintain the crispness, flakiness, and overall freshness of bakery items, such as cookies, pastries, and bread **[96]**.

Moreover, seaweed-based films contribute to the shelf stability of bakery and confectionery products by protecting them from external factors that can accelerate deterioration. These films act as a barrier against light, which can lead to the degradation of light-sensitive ingredients such as fats, colors, and flavors. By reducing exposure to light, seaweed-based films help preserve the packaged products' visual appeal, taste, and aroma **[97]**.

In summary, seaweed-based films offer significant advantages in the packaging of bakery and confectionery items. They preserve the texture and freshness of baked goods, preventing staleness and maintaining their appealing attributes. These films also contribute to shelf stability by acting as a moisture and light barrier, ensuring that the products remain fresh, flavorful, and visually appealing for a longer duration **[92]**.

Ready-to-Eat Meals Packaging

Convenience and freshness for ready-to-eat meals Seaweed-based films provide several advantages in the packaging of ready-to-eat meals, offering convenience and ensuring the freshness of the packaged food. These

films are highly versatile and can be tailored to accommodate different types of ready-to-eat meals, including pre-packaged salads, sandwiches, and complete meal kits.

Seaweed-based films offer a lightweight and flexible packaging solution, making them suitable for on-the-go consumption. The films are easy to handle and open, providing convenience for consumers who seek quick and hassle-free meal options. Additionally, these films can be designed with resealable features, allowing for multiple servings and maintaining the freshness of the remaining portions.

Furthermore, seaweed-based films help to preserve the freshness and quality of ready-to-eat meals. The films act as a barrier against moisture, preventing the loss of moisture from the food and the absorption of excess moisture from the environment. This helps to retain the desired texture, crispness, and juiciness of the packaged meals, ensuring an enjoyable eating experience for consumers.

Tamper-evident packaging for safety and integrity

Seaweed-based films offer tamper-evident packaging solutions for ready-to-eat meals, ensuring the safety and integrity of the packaged food. These films can be designed with features such as heat-sealed edges, tear strips, or tamper-evident labels that provide visible indicators of any tampering or unauthorized access to the package.

The tamper-evident properties of seaweed-based films are particularly important in ensuring food safety and building consumer trust. They offer protection against contamination and unauthorized opening of the packaged meals, reducing the risk of foodborne illnesses and maintaining the integrity of the food. The clear visibility of tampering indicators helps consumers make informed decisions about the safety and suitability of the product.

Seaweed-based films also contribute to sustainable packaging practices in the ready-to-eat meal sector. By utilizing renewable and biodegradable seaweed-based materials, these films offer an environmentally friendly alternative to traditional single-use plastics, reducing the overall environmental impact.

In conclusion, seaweed-based films provide convenient and fresh packaging solutions for ready-to-eat meals. They offer convenience to consumers on the go and help preserve the freshness and quality of the packaged food. Moreover, these films offer tamper-evident packaging features, ensuring the safety and integrity of the ready-to-eat meals. The utilization of seaweed-based films in this context aligns with sustainable packaging practices, contributing to a more environmentally friendly approach to food packaging.

Beverage Packaging

Single-serve sachets for liquid beverages: Seaweed-based films offer a practical solution for the packaging of liquid beverages, particularly in the form of single-serve sachets. These films provide a lightweight and flexible packaging option that is ideal for individual portions of beverages, such as energy drinks, juice concentrates, or instant coffee.

The use of seaweed-based films in single-serve sachets provides convenience for consumers. The films are easily tearable, allowing for effortless opening and pouring of the beverage. This makes them suitable for on-the-go consumption, providing a quick and convenient way to enjoy a refreshing drink.

Additionally, seaweed-based films contribute to the preservation of the beverage's quality. These films act as a barrier against oxygen and light, which are known to degrade the flavor, color, and nutritional content of beverages. By reducing exposure to these detrimental factors, seaweed-based films help to maintain the sensory attributes and overall freshness of the packaged liquid beverages.

Portability and sustainability in on-the-go packaging

Seaweed-based films offer portability and sustainability benefits in the packaging of beverages for on-the-go consumption. The films are lightweight and flexible, making them easy to carry and handle. They can be conveniently folded or rolled, occupying minimal space in bags or pockets, making them an excellent choice for portable beverages.

Moreover, seaweed-based films align with sustainable packaging practices. They are derived from renewable and biodegradable seaweed sources, making them environmentally friendly alternatives to traditional single-use plastics. The use of seaweed-based films helps to reduce the dependence on fossil fuel-based packaging materials, contributing to the overall reduction of plastic waste and environmental impact.

In summary, seaweed-based films offer practical and sustainable packaging solutions for beverages. Single-serve sachets made from these films provide convenience and ease of use for individual portions of liquid beverages. The portability and lightweight nature of seaweed-based films make them suitable for on-the-go consumption. Additionally, their sustainability benefits contribute to reducing plastic waste and promoting environmentally friendly packaging practices in the beverage industry.

Dairy Product Packaging

Oxygen and moisture barrier for dairy products: Seaweed-based films offer excellent oxygen and moisture barrier properties, making them an ideal packaging solution for dairy products. These films create a protective barrier that helps to prevent the entry of oxygen and moisture into the packaged dairy items, ensuring their quality and freshness.

Oxygen is known to contribute to the deterioration of dairy products by promoting oxidation and spoilage. Seaweed-based films act as a barrier against oxygen, reducing the exposure of dairy products to this element. This helps to maintain the flavor, texture, and nutritional integrity of dairy items, such as milk, cream, and dairy-based beverages.

Additionally, moisture can negatively impact the quality of dairy products by causing microbial growth, spoilage, and textural changes. Seaweed-based films provide an effective moisture barrier, minimizing moisture transfer between the product and the environment. By controlling moisture levels, these films help to extend the shelf life of dairy products and preserve their desired characteristics.

Sustainable packaging solution for cheese, yogurt, and butter: Seaweed-based films offer a sustainable packaging solution for a range of dairy products, including cheese, yogurt, and butter. These films are derived from renewable and biodegradable seaweed sources, making them an environmentally friendly alternative to conventional plastic packaging materials.

Cheese packaging can benefit from seaweed-based films due to their moisture regulation properties. These films help to maintain the proper moisture balance in the cheese, preventing drying out or excessive moisture absorption. This ensures that the cheese retains its texture, flavor, and overall quality during storage and transportation.

For yogurt packaging, seaweed-based films contribute to the preservation of freshness and consistency. These films provide an effective barrier against oxygen and moisture, preventing the growth of spoilage microorganisms and maintaining the smooth texture and taste of the yogurt. The sustainable nature of seaweed-based films aligns with the growing consumer demand for eco-friendly packaging options for dairy products.

Butter packaging can also benefit from seaweed-based films due to their ability to create a protective barrier against oxygen and moisture. These films help preserve butter's flavor, aroma, and texture, ensuring its quality and extending its shelf life. The use of sustainable seaweed-based films in butter packaging reflects a commitment to environmentally conscious practices in the dairy industry.

Future Directions and Conclusion

Improving the mechanical properties of seaweed-based films is crucial to ensure their suitability for food packaging and transportation. Further research and development efforts are needed to enhance their mechanical strength and durability. Scientists and engineers can explore innovative techniques, such as modifying the film's composition or processing conditions, to enhance its structural integrity and resistance to tearing or puncture. By improving these mechanical properties, seaweed-based films can effectively protect food products during storage, handling, and distribution.

Extending the shelf life of seaweed-based films is another important area of focus. Researchers can investigate methods to enhance the film's stability and usability over extended periods. This involves studying the factors that contribute to film degradation, such as moisture absorption or oxidation, and developing strategies to mitigate their effects. By understanding the degradation mechanisms and implementing appropriate protective measures, the shelf life of seaweed-based films can be prolonged, ensuring their functionality and quality throughout the entire food supply chain.

To meet the increasing demand for seaweed-based films, there is a need to scale up their production. Developing efficient and sustainable methods for large-scale cultivation and processing of seaweed is essential. This may involve optimizing cultivation techniques, exploring different seaweed species with desirable properties, and implementing advanced processing technologies. By increasing production capacity and streamlining the supply chain, the availability of seaweed-based films can be expanded, making them more accessible to the food packaging industry.

Cost reduction is a significant factor for the widespread adoption of seaweed-based films. Exploring cost-effective approaches in the production and processing of these films is essential to enhance their economic competitiveness compared to traditional packaging materials. This can involve optimizing production processes, utilizing locally available resources, and leveraging economies of scale. By finding ways to reduce production costs without compromising the film's quality and performance, seaweed-based films can become a financially viable and attractive option for food packaging applications.

Innovation in formulation is a key aspect of advancing seaweed-based films for food packaging. Researchers can experiment with composite materials and novel formulations to optimize the functional properties of these

films. The barrier properties, mechanical strength, and other desirable characteristics of seaweed-based films can be further enhanced by incorporating additives or modifiers, such as natural polymers or nanoparticles. Tailoring the formulation of these films for specific food packaging applications can lead to improved performance and wider versatility in the industry.

In conclusion, addressing the challenges related to the mechanical properties, shelf life extension, scaling up production, cost reduction, and formulation innovation is essential for the future development and widespread adoption of seaweed-based films in food packaging. By dedicating research efforts to these areas, we can unlock the full potential of seaweed-based films as sustainable and high-performing packaging materials, contributing to a more environmentally friendly and efficient food supply chain.Conclusion and Summary of Key Points Seaweed-based films present a promising solution for sustainable food packaging, addressing the need for environmentally friendly alternatives to conventional plastics. Their renewable sourcing, biodegradability, good barrier properties, and consumer acceptance make them attractive for various food packaging applications. Although challenges such as scalability, cost, mechanical strength, and shelf life need to be overcome, ongoing research and development efforts are driving the advancement of seaweed-based films. By further improving their properties and optimizing production processes, seaweed-based films have the potential to become a widely adopted and environmentally conscious packaging option, contributing to a more sustainable and circular economy.

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Determination of the fatty and amino acid composition of camel milk, milk powder and shubat

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ABSTRACT

Camel milk is considered an essential source of nutrition and an effective remedy with healing properties in treating several diseases. Shubat, a fermented drink made from camel milk, contains easily digestible proteins, determining its nutritional value. Meanwhile, few studies have analysed the fatty and amino acid composition of Bactrian camel milk, milk powder and shubat in Kazakhstan. In this paper, we used the gas chromatography-mass spectrometry method to determine milk the fatty and amino acid composition of Kazakhstan camel milk and camel milk powder and submit samples. As a result, significant differences in the fatty acid and amino acid compositions were observed among samples of raw milk, milk powder and shubat. differences were found in all amino acids. The most representative fatty acids in the three groups were C16:0, C18:0, C18:1n9c, C14:0 FAs. In camel milk samples, lysine (29.64%) was the highest in concentration among indispensable amino acids, followed by methionine (25.68%). Some polyunsaturated fatty acids (PUFAs) such as C18:3n3c, C20:4n6, C18:3n3c, C20:3n3c 8,11,14 were found only in shubat samples. Furthermore, we revealed a significant decrease in both dispensable (DAA) and indispensable (IDAA) contents in camel milk powder. Meanwhile, an increase in the quantitative content of amino acids has been observed in shubat, especially in threonine (166.86%), aspargine (156.34%), alanine (114.48%), etc. The results provide a theoretical basis for additional studies of camel milk composition of Bactrian camel in Kazakhstan.

Keywords: camel milk, milk powder, shubat, fatty acids, amino acids.

INTRODUCTION

Kazakhstan is a country where horse and camel breeding are traditionally practised. Compared to other animals, camels have a peculiar peculiarity and adaptability to our harsh climatic conditions **[1]**. As most of our country has desert and semi-desert zones. Camels can tolerate high heat, low temperature, and temporary waterlessness well. As the raw material base is expanding in the country now, it allows the exporting of camel breeding products **[2]**. The main regions where camels are bred are Aktobe, Atyrau, Mangistau, Kyzylorda, South Kazakhstan and Almaty regions. Historically, the camel has played an important role, supplying Kazakhs with milk, meat, wool, and leather. In Kazakhstan's western and southern regions, camel milk is the primary foodstuff. Kazakhstan is the only country where one-humped and two-humped camels are kept, and hybrids are produced **[3]**. In Figure 1, it is shown that from 1990 to 2022, the camel population increased by 59%. Camel milk is of the albumin type, so it is well-digested in the human body **[4]**. It is recognised that camel milk is an effective remedy with healing properties in treating gastritis, diabetes, asthma, tuberculosis, skin diseases, urinary problems and hepatitis. Milk consumption does not cause allergic reactions or gastrointestinal irritation **[5]**. Camel milk contains 5 to 6 % fat, proteins and other components determining its nutritional value. Camel milk usually contains short-chain fatty acids (C4-C12) in very low amounts compared to other types **[6]**. In terms of vitamin C content, it is significantly higher in vitamin C than milk from cows **[7]**. Camel milk is consumed by the inhabitants of arid

regions of the world, mainly as a fermented milk product called shubat or kymyran (from two-humped camels) or chal (from one-humped camels). Shubat is a sour milk drink made from camel's milk that is a traditional drink of Kazakhs **[8]**. Scientists have also proved the positive effect of shubat in diabetes; treatment with these products leads to the normalisation of the intracellular function of the pancreas, increasing the number of patients with normal glycaemic curves **[9]**, **[10]**. Due to changes in the economic and social status of the elderly and old people, the caloric content of their diet is significantly decreasing, which makes energy and protein-calorie deficiency among the most important problems. The energy deficit is aggravated by deficient protein intake, vitamins and mineral elements and an imbalance of polyunsaturated fatty acids **[11]**. Nowadays, to produce high-quality products using modern processing technologies, high-quality requirements are imposed on raw milk, the leading controlled indicators of which are physical, chemical and technological properties **[12]**. Therefore, this study aimed to research the fatty acid and amino acid composition of camel milk, camel milk powder and shubat samples collected from Almaty region in Kazakhstan.

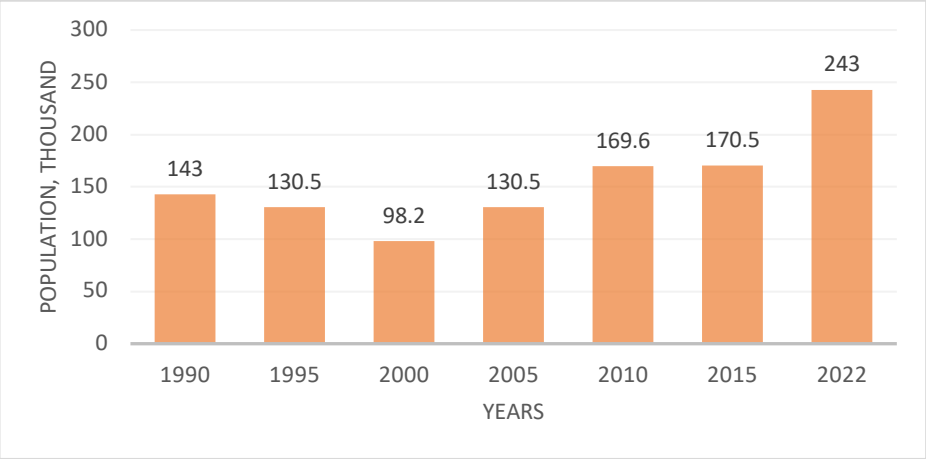


Figure 1 Number of camel population in Kazakhstan for 1990-2022.

Scientific Hypothesis

Camel milk provides nutrition through proteins, amino acids and fatty acids. There were significant differences in amino acid and fatty acid content between raw milk, dried milk and shubat samples from Kazakhstan.

MATERIAL AND METHODOLOGY

Samples

Fresh camel milk (n = 2), shubat (n = 2), milk powder (n = 2) samples were obtained from Bactrian camel breeding farm "Daulet-Beket" LLP, Akshi village, Almaty region, Kazakhstan November in 2022. Camels were pasture-fed. Samples were delivered in a thermos container to the laboratory.

Chemicals

All chemicals were purchased by “Laverna XXI century” (Moscow, Russia) and were of analytical grade quality. We used ethanol (purity ≥95%), isopropanol (purity ≥99.5%), sodium hydroxide (purity ≥99.5%), and methyl ether (purity ≥99%).

Instruments

A gas chromatograph “Shimadzu GC-2010 Plus” used for fatty acid analysis.

Laboratory Methods

Experiments were performed at the laboratory of the Kazakh-Japanese Innovation Centre of Kazakh National Agrarian Research University. Determination of fatty acid composition was carried out following GOST 32915-2014 “Milk and dairy products. Determination of fatty acid composition of the fat phase by gas chromatography method”. Briefly, the homogenisation was carried out with a blender for 3-5 min with maximum stirring. The separated hexane layer was transferred to a round bottom flask, then connected to a roundabout evaporator and the solvent was completely distilled off at a temperature of 70 ±2 °C. Methyl ether was added to the obtained fat fraction. 1 cm^3 of the fatty acid methyl ester solution was taken with a microsyringe and injected into a gas chromatograph “Shimadzu GC-2010 Plus”.

The following parameters were set for the chromatograph measurement: temperature of the flame ionisation detector – 260 °C; temperature parameters: 100 °C – 5 min, up to 210 °C – 8 min at a rate of 40 °C/min, up to 240 °C – 16.5 min at a rate of 10 °C/min; sample division flow 1/40. Analysis time – 60 min.

The qualitative and quantitative composition of amino acids was established by high-performance liquid chromatography (HPLC) **[13]**. To analyse the samples for total amino acid composition by HPLC, a precise sample of dry extract (~100 mg) was dissolved in 5 ml of 40% ethyl alcohol and incubated in an ultrasonic bath for 10 min. Aliquots (0.1-0.2 ml) were placed in a test tube. Then, they were dried in a water bath at 60 °C. The dried aliquots added 0.10 mL of 0.15 mol/l sodium hydroxide solution and then stirred. Next, 0.35 ml of phenylisothiocyanate solution in isopropyl alcohol and 0.05 ml of bidistilled water were added. The solution was mixed thoroughly again and left for 20 min at room temperature, then dried to dryness at 65 °C. The dry residue was dissolved in 1 ml of bidistilled water. The resulting solutions were subjected to chromatographic analysis.

Tryptophan was determined without derivatisation with phenylisothiocyanate by chromatographic analysis of a previously prepared solution of a suspension of the dry extract in ethyl alcohol 40%. To determine the sum of cysteine and cystine, the sample was pre-oxidised with supramuravic acid, and then cysteic acid was determined as a phenylthiocarbamate derivative. To obtain supramuric acid, one part of hydrogen peroxide and 9 parts of formic acid were mixed thoroughly in a 10 cm3 test tube. A suspension of the dry extract was placed in an evaporation cup, and 5 cm3 of oxidising agent was added and dried completely in a water bath at 60 °C. The dry residue was dissolved in 5 ml of 40% ethyl alcohol. An aliquot of the obtained solution was transferred into a test tube. Derivatisation with phenylisothiocyanate was carried out, filtered off, and the sample was injected into a chromatographic column.

Description of the Experiment

Sample preparation: Preparation of samples of camel milk, camel milk powder and shubat was carried out following GOST 32915-2014 “Milk and dairy products. Determination of the fatty acid composition of the fat phase by gas chromatography method”.

Number of samples analyzed: 6.

Number of repeated analyses: 2.

Number of experiment replication: 3.

Design of the experiment: In the experiment's first phase, we obtained the Bactrian camel raw milk, powder milk, and shubat samples from the "Daulet-Beket" LLP farm in the Almaty region. Then, we determined the fatty acid composition of raw milk, milk powder, and shubat. In the next phase, we detected the amino acid composition of these samples.

Statistical Analysis

The experiments were performed in three replications to get a true mean and support the hypothesis. Data were analyzed using the ANOVA in the SPSS software (Version 25.0, IBM Corporation, New York, USA). To reduce instance of a false positive, the threshold p-value for significance was adjusted after correction for multiple comparisons using the Bonferroni correction.

RESULTS AND DISCUSSION

Camel milk consumed by inhabitants of arid regions provides nutrition through proteins, amino acids and fatty acids **[14]**, **[15]**. Fatty acids are essential for the normal functioning of all body's systems: blood circulation and respiration to immunity and brain function. In addition, fatty acids are a membrane component of absolutely every cell in the organism **[16]**, **[17]**. Mass fractions of fatty acid (FA) composition of camel milk, camel milk powder and shubat are presented in Table 1. Significant differences (p <0.001; p <0.0001) were detected among all fatty acids after Bonforenni’s correction. Figure 2 shows the results of the milk fat chromatogram of camel milk, camel milk powder and shubat. The most representative fatty acids in the three groups were C16:0, C18:0, C18:1n9c, and C14:0 FAs (Table 1). Previous studies also observed the prevalence of C16:0, C18:0, and C14:0 in Mongolian Bacterian camel milk **[18]**. Of interest, we observed a higher amount of C18:1n9c in raw (28.9%), dry camel milk (31.14%) and shubat (27.51%) samples compared to those in Mongolian **[18]** and Turkish camel milk **[19]**. These differences may be due to the camel’s diet, breed and environmental factors **[20]**. The composition of fatty acids in dairy is mainly dependent on two processes: lipid metabolism in the rumen and the mammary gland. Recent studies have shown that dietary changes can affect the rumen and mammary gland microbiota. For instance, it has been reported that increasing the proportion of fresh forages, fibre and oilseeds in concentrates increases the level of fatty acids in raw milk **[21]**. Also, local breeds specific to certain areas demonstrate even higher levels of fatty acids than those traditionally considered the most productive species. In addition, genetics also influences the composition of the final raw material. Still, it is difficult to determine what the specific fatty acid content of milk will be without expensive and time-consuming analytical methods **[22]**.

Furthermore, significant differences were detected among all saturated fatty acids (SFAs), except for C14:0. Previously, **[23]** detected no statistically significant difference in the yield of SFAs in fermented and unfermented

milk. SFAs are fatty acids whose molecules are hydrogen-enriched. It is well known that excess saturated fatty acids increase blood cholesterol levels and contribute to obesity and the development of heart disease **[24]**.

Table 1 Mass fractions of fatty acid composition of camel milk, milk powder, and shubat % of total content.

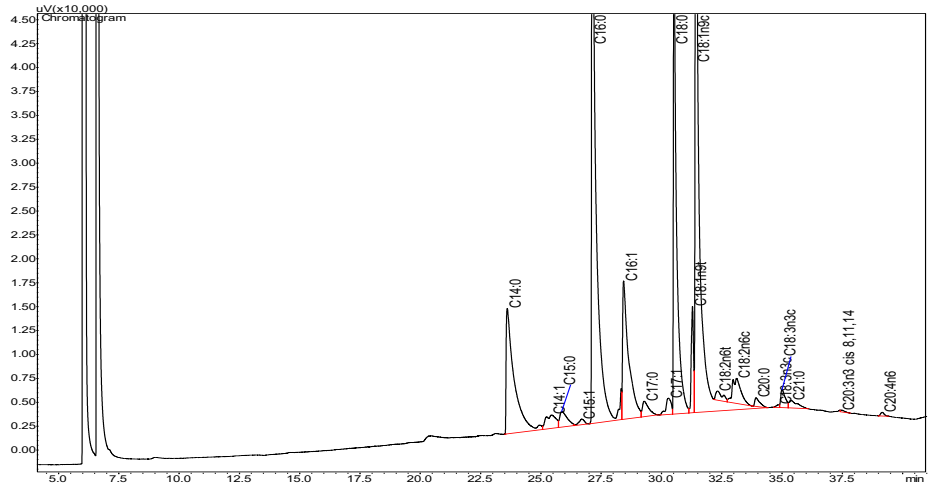
Fatty acid code	Classification	Camel milk, % ug/ml	Milk powder, % ug/ml	Shubat, % ug/ml
		Saturated fatty acids		
C14:0	Myristic	7.12 ±0.1	6.58 ±0	8.39 ±1.1
C15:0	Pentadecanoic	^{a}1.22 ±0.1	^{b}0.27 ±0	^{c}1.15 ±0.01
C16:0	Palmitic	^{a}28.48 ±0.1	^{b}7.44 ±0.01	^{c}26.18 ±0
C17:0	Margaric	^{a}1.71 ±0.1	^{b}0.39 ±0.01	^{c}0.94 ±0.01
C18:0	Stearic	^{a}17.65 ±0.09	^{b}17.35 ±0.01	^{c}14.6 ±0.01
C20:0	Arachidic	ND	ND	0.4 ±0.01
C21:0	Heneicosanoic	ND	^{a}1.50 ±0.01	^{c}0.63 ±0.01
		Monounsaturated fatty acids		
C14:1	Myristoleic, ω5	^{a}0.46 ±0.02	^{b}0.63 ±0.01	^{c}2.3 +0
C15:1	Pentadecenoic	^{a}0.55 ±0.02	^{b}25.03 ±0.01	^{c}0.31 ±0.03
C16:1	Palmitoleic, ω7	^{a}8.12 ±0.1	^{b}0.35 ±0	^{c}10 ±0
C17:1	Heptadecanoic acid	^{a}0.64 ±0.02	^{b}17.33 ±0.01	^{b}0.82 ±0.01
C18:1n9t	Elaidic	^{a}2.64 ±0.1	^{b}3.48 ±0.01	^{bc}2.29 ±0
C18:1n9c	Oleic	^{a}28.9 ±0.06	^{b}31.14 ±0.08	^{ac}27.51 ±0.6
		Polyunsaturated fatty acids		
C18:2n6t	Linolelaidic acid	^{a}1.31± 0.1	^{b}0.33 ±0.01	^{c}0.62 ±0.02
C18:2n6c	Linoleic, ω6	^{a}1.21 ±0.09	^{b}5.57 ±0.01	^{c}2.08 ±0.01
C18:3n3c	α-linolenic acid, ω3	ND	ND	0.08 ±0
C18:3n6c	γ-linolenic acid	0.29±0	ND	ND
C20:4n6	arachidonic	ND	ND	0.15 ±0.01
		Polyunsaturated fatty acids		
C18:3n3c	Linolenic	ND	ND	0.08±0.01
C20:3n3c 8,11,14	Eicosatetraenoic	ND	ND	0.08±0.01

Note: Data with different superscript letters display significant differences.

Unsaturated fatty acids (USFA) are monosaturated FAs (MUFAs) that have only one or polyunsaturated FAs (PUSFs) that have two or more double bonds between adjacent carbon atoms in their structure **[25]**. Shubat dominates in terms of increased content (C14:1 (2.3%), C16:1 (10%), C17:1 (0.82)) of MUFAs. Previous study has observed higher MUFA levels and low PUFA levels in camel milk **[18]**. MUFAs increase glucose absorption and thus prevent the development of diabetes and metabolic syndrome, prevent the development of breast cancer in women, and participate in strengthening the immune system. In addition, they reduce cholesterol levels in the blood and prevent the deposition of cholesterol plaques on the walls of blood vessels, thus reducing the risk of atherosclerosis **[26]**. One of the main monounsaturated fatty acids in camel milk is oleic acid – C18:1 (ω-9), which acts favorably on lipid metabolism, particularly cholesterol metabolism **[27]**.

The biological value of the lipid component of a product is characterized by its qualitative composition of fatty acids. The most significant biological significance of unsaturated fatty acids are PUFAs, the so-called essential FAs. The presence of polyunsaturated (essential) fatty acids in camel milk determines its usefulness and therapeutic effect. According to modern nutraceutical regulations, fats with a high content of PUFAs are considered biologically valuable **[28]**. The main benefit of PUFAs lies in their ability to strengthen the structure of cell membranes. They improve cellular activity, which naturally affects all organs and body systems **[28]**. PUFAs are primarily linoleic and linolenic acids. Importantly, a high amount (5.86%) of C18:2n6c is found in milk powder. It is well known that linoleic acid improves metabolism, regulates cholesterol levels and promotes muscle building **[29]**. Some polyunsaturated fatty acids (PUFAs) such as C18:3n3c, C20:4n6, C18:3n3c, C20:3n3c 8,11,14 were found only in shubat samples. These PUFAs promote estrogen production and increase immunity, affecting muscle tissue growth and repair **[30]**. Arachidonic acid has a high biological value in the nutrition of children. The absence or lack of it in the diet delays the physical development of the child **[31]**. During the fermentation process, complex biochemical processes take place in milk, as a result of which the chemical composition of the final product - shubat differs significantly from the chemical composition of the original raw milk. The content of beneficial fatty acids was higher in fermented milk than in unfermented milk **[23]**.

A)



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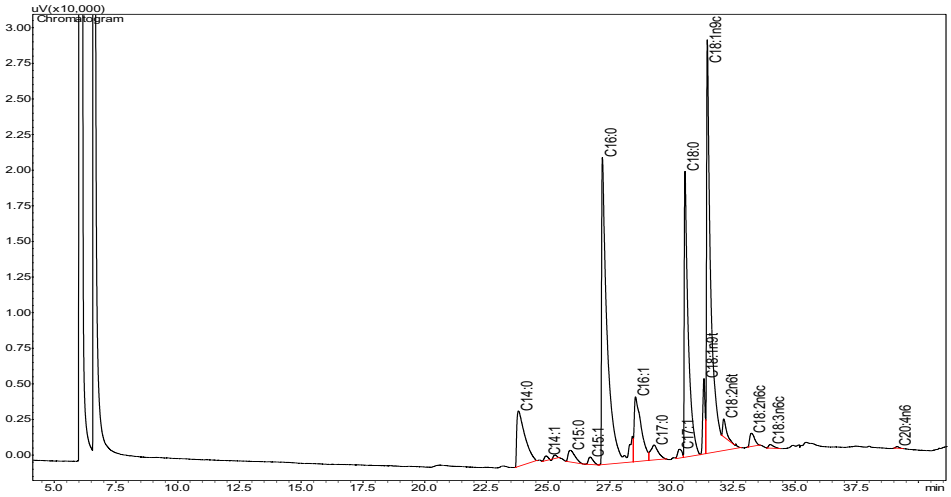

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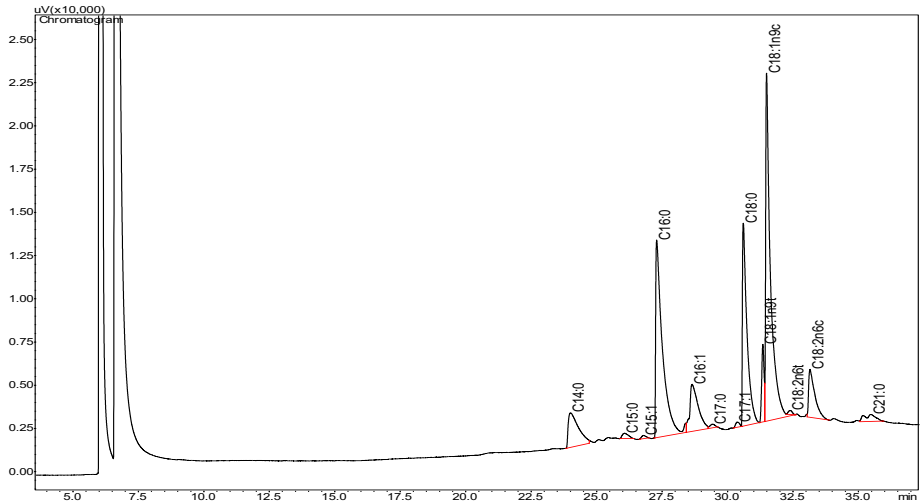


Figure 1 Mass fractions of fatty acid composition of camel milk, milk powder, and shubat % of total content. x-axis is a retention time (min), y-axis represents abundance.

Amino acids directly affect the nervous system, regulating mental performance, mood and sleep **[32]**, **[33]**, **[34]**. These components are essential for the formation of muscles, tendons and ligaments, as well as hair and skin. Without a sufficient amount of amino acids, active muscle growth is impossible **[35]**. Table 2 shows the concentrations of amino acids in the camel milk, milk powder and shubat samples. Figure 3 displays the results of a chromatogram study of the amino acid composition of camel milk, camel milk powder and shubat.

Table 2 Amino acid composition of camel milk, dry milk and shubat.

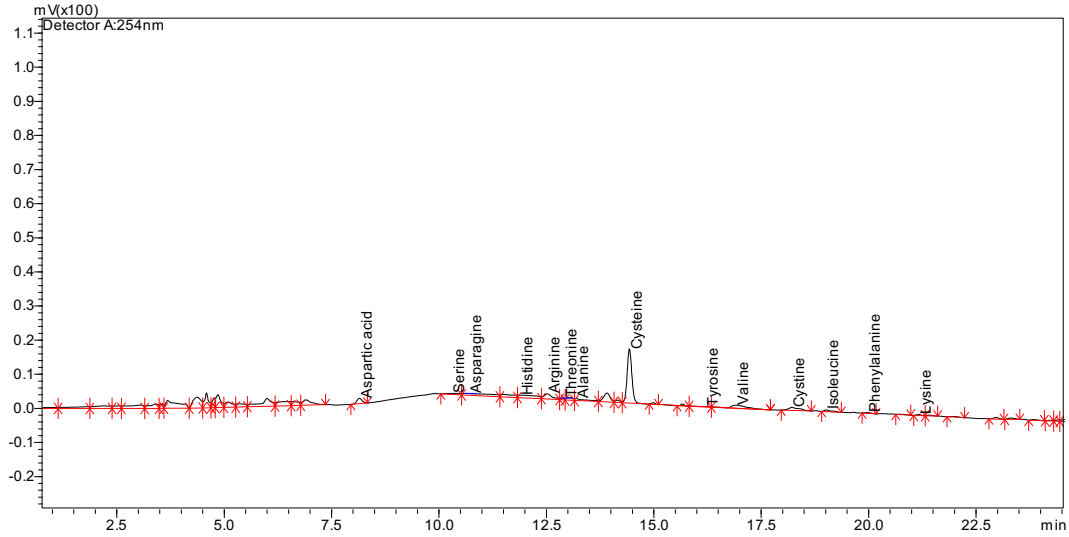
Amino acids	Camel milk	Camel milk powder	Shubat
Indispensable			
Valine	^{a}10.48 ±0.1	^{b}13.32 ±0.07	^{c}43.86 ±0.02
Isoleucine	^{a}13.98 ±0.09	^{b}2.14 ±0.1	^{c}25.34 ±0.1
Leucine	^{a}14.79 ±0.1	^{b}0.35 ±0.2	^{a}15.35 ±0.03
Lysine	^{a}29.64 ±0.1	^{b}0.91 ±0.04	^{c}44.86 ±0.05
Methionine	^{a}25.68 ±0.06	^{b}0.47 ±0.2	^{a}25.45 ±0.1
Threonine	^{a}20.88 ±0.06	^{b}1.3 ±0.06	^{c}166.86 ±0.07
Phenylalanine	^{a}20.65 ±0.06	^{b}1.15 ±0.03	^{c}59.92 ±0.03
Dispensable			
Alanine	^{a}88.96 ±0.02	^{b}2.88 ±0.02	^{c}114.48 ±0.01
Arginine	^{a}42.75 ±0.06	^{b}18.51 ±0.04	^{c}49.89 ±0.08
Asparagine	^{a}15.82 ±0.1	^{a}15.14 ±0.05	^{b}156.34 ±0.1
Aspartic acid	^{a}57.62 ±0.04	^{b}9.75 ±0.03	^{c}56.52 ±0.2
Glutamic acid	^{a}16.59 ±0.09	^{b}0.4 ±0.2	^{c}25.77 ±0.1
Histidine	^{a}13.74 ±0.04	^{b}10.98 ±0.01	^{c}11.62 ±0.2
Proline	^{a}0.94 ±0.03	^{a}0.34 ±0.2	^{b}1.36 ±0
Serine	^{a}13.68 ±0.2	^{b}2.3 ±0.04	^{a}14.4 ±0.1
Tyrosine	^{a}31.83 ±0	^{c}1.33 ±0.04	^{b}29.30 ±0.05
Cysteine	^{a}0.4 ±0.06	^{b}12.85 ±0.03	^{c}34.29 ±0.01
Cystine	^{a}16.64 ±0.07	^{b}20.54 ±0.04	^{c}41.80 ±0.01

Note: Data with different superscript letters display significant differences.

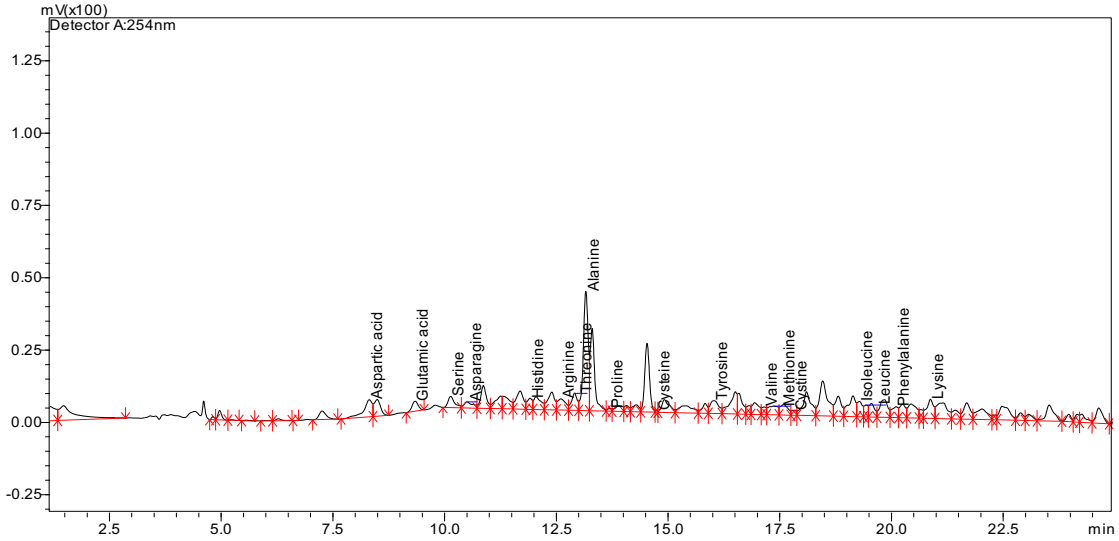
Significant differences were found in all amino acids. In camel milk samples, among indispensable amino acids, lysine (29.64%) was the highest in concentration, followed by methionine (25.68%). The results of our study indicate a higher amino acid content than those obtained on Saudi Arabian camels **[36]**. Among dispensable amino acids, cysteine was the least abundant (0.4%), consistent with the previous results **[18]**. Raw camel milk and shubat contain more essential amino acids than powdered milk. As is known, animal proteins play a crucial role in rational nutrition. Camel milk proteins are biologically valuable in digestibility and balanced amino acid composition.

We also revealed decreased dispensable (DAA) and indispensable (IDAA) contents in camel milk powder. It might be explained by denaturation changes of thermolabile protein substances of milk are possible during the drying process **[37]**. Milk processing can lead to denaturation, aggregation, and chemical alterations of amino acids. Prolonged heating causes a change in the charge and degree of hydration of protein molecules, as well as the release of active sites capable of interaction on their surface. The decrease in some amino acids may be due either to their degradation by heat or to their combination with other components **[38]**. Meanwhile, an increase in the quantitative content of amino acids has been observed in shubat, especially in threonine (166.86%), aspargine (156.34%), alanine (114.48%), etc. Threonine is essential for the synthesis of amino acids such as serine and glycine, which in turn are involved in the synthesis of collagen and elastin – proteins of connective and muscle tissue **[39]**. Thus, in fermentation and maturation, the amount of milk sugar is significantly reduced, and lactic acid, ethyl alcohol and carbon dioxide accumulate in shut **[40]**, **[41]**. In addition, proteinase enzymes of lactic acid bacteria and yeast hydrolyse casein and whey proteins of milk, turning part of them into polypeptides, peptides and free amino acids. This is how camel milk proteins are converted into easily digestible nitrogen-containing compounds. **[42]**. The essential amino acid content of shubat makes it a valuable source of protein while being low in fat and cholesterol. Moreover, the study by Bai et al. reported that the ratio of amino acids in shut was higher than that of camel milk **[43]**. Several studies describe the therapeutic and dietary value of preserved and reconstituted fermented milk products in the diet of the population in terms of their suppression of putrefactive processes in the intestine **[44]**. Of interest, traditional sour milk products such as koumis and shut were suggested as a remedy for intestinal diseases **[45]**.

A)



B)



C)

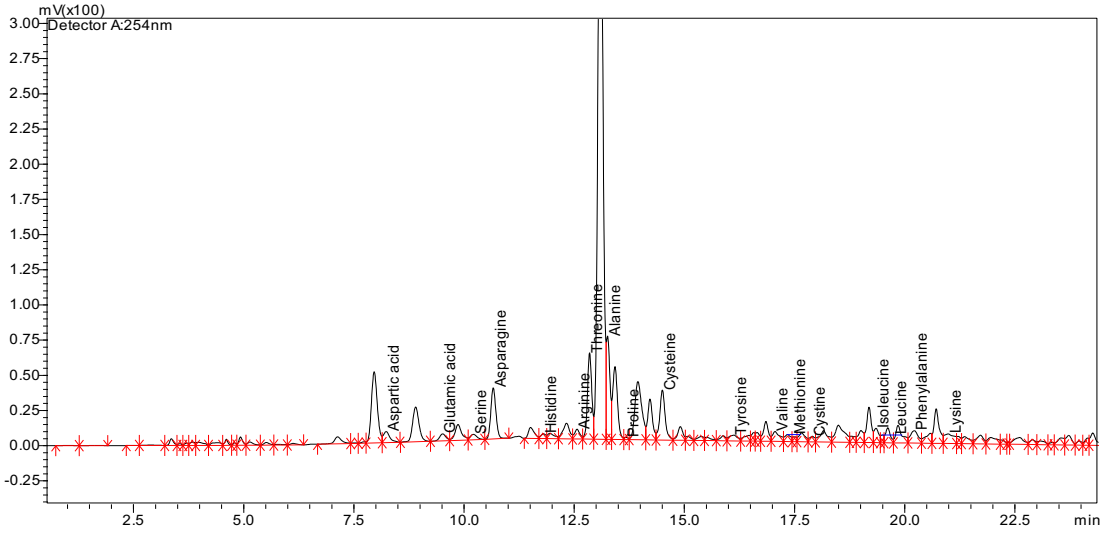


Figure 3 Chromatograms of amino acid composition of a) camel milk; b) camel milk powder; c) shubat. Note: x-axis is a retention time (min), y-axis represents abundance.

CONCLUSION

This study analysed amino acid and fatty acid compositions to differentiate between camel milk, milk powder and shubat. As a result, there were significant differences in amino acid and fatty acid content between raw milk, dried milk and shubat. Higher MUFA and low PUFA levels in our milk, dry milk and shubat were observed. Moreover, raw camel milk and shubat samples contained more essential amino acids than dried milk, indicating their biological value in digestibility and balanced amino acid composition. In addition, a higher content of essential amino acids was found in shubat samples compared to raw and dried milk. The results provide a theoretical basis for additional studies of camel milk composition of Bactrian camel in Kazakhstan.

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

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Physical and mathematical modelling of the massing process of marinated pork and beef preparation technology

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ABSTRACT

The main process in the technology of natural marinated semi-finished meat products is the marinating process, which depends on the marinating method, temperature conditions, and composition of the marinade mixture. To enhance the distribution of marinades within the meat, mechanical processing of the raw material is necessary, specifically using the massaging process, to achieve uniform distribution of curing agents, changes in structure, and increased activity of enzymatic systems utilized in marinades. The main goal of this study is to model the massaging process, which allows the processing of the obtained experimental data in the form of a criterion equation. For the research, 8 samples were used, including 3 experimental pork samples and 1 control, as well as 3 experimental beef samples and 1 control. The main components of the samples include pork meat, beef meat, canola oil, sunflower oil, olive oil, curing ingredients, spices and seasonings, bromelain enzyme, and yeast extract. To carry out the massaging process in the investigated procedure, a massager of the model MAL 50-1500 was employed. The research results indicate that the determined mass transfer equation demonstrates a predominant influence in the investigated massaging process, involving changes in the concentration of sunflower, canola, and olive oil in the product, affecting the diffusion coefficient, size of dispersed phase particles, and mass transfer coefficient within the load.

Keywords: marinating, massaging, beef, pork, mass exchange

INTRODUCTION

One of the most important issues in ensuring the quality of marinated pork and beef preparation is effectively structuring necessary components and mixing their ingredients, creating a consistency that meets the required standards **[1]**. The main process in the mentioned technology is the marination process, which depends on the marinating method, temperature conditions, and the composition of the marinade mixture itself **[2]**. To intensify brine distribution, mechanical treatment of raw materials is applied, namely tenderization, tumbling, or massaging of meat to distribute curing agents, change the structure, and increase the activity of enzymatic systems. As a result of this technological action, accelerated salting and meat ageing are expected, improvement of its organoleptic and physicomechanical indicators, particularly plasticity and shear strength. The projected technology involves a massaging operation, the essence of which lies in utilizing the energy from the fall of meat pieces from a certain height, impact, and friction of these pieces against each other and the inner surface of the rotating drum **[3]**.

The main factors of this process are the product density ρ, shear yield stress τ_r, variation in the concentration of sunflower, rapeseed, or olive oil in the product ΔC, the diffusion coefficient D, and the mass transfer coefficient in the technological mass β, processing time t the average size of meat pieces, and the rate of their advancement in the massage drum υ **[4]**. Considering a significant number of factors in the investigated process, it

is advisable to model it using the Buckingham π theorem, which allows processing the obtained experimental data in the form of a dimensionless equation **[5]**.

Considering the peculiarities of the studied massaging process, characterized by the action of centrifugal forces and fluid flows, which requires using Euler's number *Eu* to measure the ratio of pressure forces to velocity thrust in the model. The presence of the convective flow of technological media, leading to diffusion at the interface of interacting phases, necessitates applying Sherwood's number *Sh*. Local pulsations in non-stationary flows reveal the relevance of representing the heat and mass exchange in the sought equation by diffusion Fourier's number Fo_d **[6]**.

Scientific Hypothesis

The development of physical-mathematical modelling of the technological process (massaging or mixing) allows the processing of experimental data in the form of a criteria equation. Additionally, modelling the massaging process in the technology of natural marinated meat semi-finished products enables preliminary determination of the required equipment characteristics for this process, namely, the massager.

MATERIAL AND METHODOLOGY

Samples

In this article, 8 samples were examined: 4 samples using pork and 4 samples from beef.

- Sample 1 – pork without marinade.
- Sample 2 – pork with marinade based on rapeseed oil.
- Sample 3 – pork with marinade based on a blend of oils (sunflower: rapeseed = 70:30).
- Sample 4 – pork with marinade based on a blend of oils (sunflower: olive = 80:20).
- Sample 5 – beef without marinade.
- Sample 6 – beef with marinade based on rapeseed oil.
- Sample 7 – beef with marinade based on a blend of oils (sunflower: rapeseed = 70:30).
- Sample 8 – beef with marinade based on a blend of oils (sunflower: olive = 80:20).

Chemicals

Inorganic sulfuric acid salt ($C_6H_7NaO_6$, producer «Inter-Synthesis» Limited Liability Company, Ukraine, chemically pure for analysis).

Animals, Plants, and Biological Materials

For the researcher, we used beef grade II and pork grade II, with a high connective tissue content. Pork and beef meat were purchased from a retail network.

Instruments

The following tool was used to determine the moisture content in the prepared samples:

Analytical balance (Radwag AS 220/C, producer «Inter-Synthesis» Limited Liability Company, Ukraine).

Boxes (aluminium laboratory, producer «Inter-Synthesis» Limited Liability Company, Ukraine).

Ulab 3-31 M penetrometer (producer «Inter-Synthesis» Limited Liability Company, Ukraine).

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 authors used the following laboratory research methods:

The penetration degree was determined using the Ulab 3-31 M penetrometer (producer «Inter-Synthesis» Limited Liability Company, Ukraine). The sample's temperature was brought to 20 ±0.5 °C by placing it in an air bath at a constant temperature of 20 °C. The investigated sample was positioned steadily on the penetrometer table under the horizontal indenter. Penetration of natural marinated meat semi-finished products was determined using a needle indenter. Three measurements were taken on the open surface of the sample. Measurements were conducted at a distance of not less than 10 mm from the edge of the sample and at the maximum distance from the points of other measurements to avoid the deformed part of the surface entering the measurement zone. Air inclusions and other visible surface defects should be avoided. The penetration results are calculated as the arithmetic mean of the results of three parallel measurements, rounded according to standard СТ СЄВ 543. To convert the penetration value of elastic products (ready-to-eat food products, smoked products, carbonate, neck, bacon, and other whole-piece products) measured over 180 s into penetration stress θ, in Pa, with the indication of the used indenter, the following formula is applied:

$$\theta = Ph^{-2} = mgh^{-2}$$

Where:
P is the applied force in Newtons (N); h is the depth of penetration of the needle indenter in meters (m); g is the acceleration due to gravity (9.8 m/s²); m is the mass of the needle, rod, and additional load in kilograms (kg).

m rod = 47.3 g
m load = 50.0 g
m needle = 2.7 g.

The determination of product yield was carried out by weighing before and after thermal treatment, expressed as a percentage using the formula:

$$\text{Yield} = \frac{\text{A}}{\text{C}} \times 100$$

Where:
A is the mass of the product after thermal treatment in grams (g); C is the mass of the raw material for making the mince in grams (g).

Determining the water-holding capacity of natural marinated meat semi-finished products was carried out using the "Press Method," developed by Grau and Hamm in the modification of Volovinska and Kelman **[7]**. This method is based on pressing the test sample with a mass of 0.3 g under a load of 1 kg, sorption of the released moisture under pressure by filter paper, and determination of the amount of released moisture by the wet spot area on the filter paper using the method.

The determination of plasticity was carried out using the data obtained during the determination of water-holding capacity. The calculation was based on the wet spot area formed after pressing the filter paper and determining water-holding capacity **[7]**.

The water-retaining capacity was determined by the quantitative content of water retained by the test sample after thermal treatment **[7]**.

The fat-retaining capacity was determined as the difference between the fat content in the product and the amount of fat released during thermal processing **[7]**.

The pH was determined by the potentiometric method, based on measuring the electromotive force of an element consisting of a reference electrode with a known potential and an indicator (glass) electrode, the potential of which is determined by the concentration of hydrogen ions in the test solution **[8]**.

Description of the Experiment

Sample preparation: The preparation of samples for the mentioned experiments was carried out by DSTU 7963:2015 **[9]**. Sample collection was performed by DSTU 7992:2015 **[10]** and DSTU 8051:2015 **[11]**.

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: Experimental studies were conducted to obtain values that would serve as input data for the physical-mathematical modelling of the massaging process. Researchers determined penetration, product yield, water-holding capacity, plasticity, moisture-binding capacity, and pH. The results of these investigations clearly describe the meat's firmness and enable the conduct of physical-mathematical modelling of the process.

Statistical Analysis

Considering the peculiarities of the studied massaging process, characterized by the action of centrifugal forces and fluid flows, which requires using Euler's number *Eu* to measure the ratio of pressure forces to velocity thrust in the model. The presence of the convective flow of technological media, leading to diffusion at the interface of interacting phases, necessitates applying Sherwood's number *Sh*. Local pulsations in non-stationary flows reveal the relevance of representing the heat and mass exchange in the sought equation by diffusion Fourier's number Fo_d **[6]**. Statistical processing was performed in Microsoft Excel 2016. Values were estimated using mean and standard deviations and evaluated in the XL Stat statistical program.

RESULTS AND DISCUSSION

According to the classical technique, the marinade typically includes the following components **[12]**: table salt, ground black pepper, and bay leaf **[13]**. However, in the scientific study, it is suggested to add sunflower **[14]**, rapeseed **[15]**, or olive oil **[16]** to the marinade **[17]**, as presented in Table 1 and patented Patent no. 134474 **[18]**, Patent no. 134475 **[19]**, Patent no. 134476 **[20]**.

Table 1 Functional and technological indicators of control and experimental samples of natural marinated meat semi-finished products.

Sample	Content of rapeseed oil in the marinade	Content of sunflower (70%) + rapeseed (30%) oil in the marinade	Content of sunflower (80%) + olive (20%) oil in the marinade	Plasticity, cm²/g	Penetration, Pa
Pork semi-finished products					
1	6.64	-	-	9.45	5035.9
2	-	6.64	-	9.06	4355.5
3	-	-	6.64	7.74	1885.2
Control	-	-	-	6.75	1935.8
Beef semi-finished products					
1	6.64	-	-	6.88	8804.8
2	-	6.64	-	7.15	7038.2
3	-	-	6.64	6.72	4661.1
Control	-	-	-	7.17	4535.1

By employing the dimensional analysis theory as a mathematical method, the factors of the studied process **[21]** were initially represented in Table 2.

Table 2 Main calculated parameters of the massaging process.

No.	Process parameter	Dimension
1	Product density ρ, kg/m³	$kg.m^{-3}$
2	Processing time per load t, s	s
3	Shear yield stress $\tau_г$, N/m² (Pa)	$kg.s^{-2}.m^{-1}$
4	Diffusion coefficient D, m^2/s	$m^2.s^{-1}$
5	The average size of meat pieces ℓ, m	m
6	Meat piece advancement speed in the massager drum υ, m/s	$m.s^{-1}$
7	Mass transfer coefficient in technological mass load β, m/s	$m.s^{-1}$

The above similarity criteria were presented through the indicated physicomechanical and rheological factors of the studied massaging process **[22]** as follows:

Euler's criterion was represented as:

$$Eu = \frac{P}{\rho \cdot S \cdot \upsilon^2} = \frac{\tau}{\rho \cdot \upsilon^2} \quad (1)$$

Where:

P – is the resistance of the medium, which is equal to the load acting on the product during processing, H; S – is the area of force contact within the working volume, m²; $\tau = \frac{P}{s}$ - is the shear yield stress, Pa; υ – is the speed of meat piece movement in the massager drum: $\upsilon = \omega R_б = \frac{\pi \cdot n_б \cdot R_б}{30}$ $R_б$ – is the radius of the drum; $n_б$, ω – are the rotation frequency and angular velocity of the massager drum respectively. $n_б = \varphi \cdot n_{кр}$

Where:

φ – is the coefficient of the technological mass load inside the drum, which was taken within the range of φ = 0.8-0.85: was adopted as φ=0,82; $n_{кр}$ – is the critical drum rotation frequency, rev/min; which was adopted from the dependence $n_{кр} = \frac{12,3}{\sqrt{2R_б}}$

The Sherwood's criterion Sh is classically calculated as:

$$Sh = \beta \cdot \ell / D \quad (2)$$

Where:

ℓ – s is the characteristic size under the conditions of the investigated mass transfer, which can be identified with the average size of the dispersed phase particles, which for the investigated massaging process corresponds to the average size of meat pieces; D – is the diffusion coefficient **[23]**.

The mass transfer coefficient for the investigated process can be determined using the following relationship:

$$\beta = \frac{П_V}{\Delta C \cdot S} \quad (3)$$

Where:

P_V – volumetric productivity of the process.

The Fourier's number for diffusion was determined using the formula:

$$\mathrm{Fo}_д = \frac{D \cdot t}{\ell^2} \quad (4)$$

Using the experimental research data (Table 3) **[1]**, the main parameters of the massaging process were determined using the following methodology **[24]**.

Table 3 Functional and technological indicators of natural marinated meat semi-finished products.

Sample No.	pH	Moisture content, % of meat weight	Moisture content, % of total moisture	Plasticity, $10^6 \times cm^2/g$	Water holding capacity, %	Yield, %
Pork semi-finished products						
1	5.67	58.150	91.515	9.45	63.45	80.5
2	6.4	60.095	81.23	9.06	73.87	102.4
3	6.18	59.345	83.235	7.74	71.17	74.6
Control	6.15	58.515	81.475	6.75	71.77	62.6
Beef semi-finished products						
1	6.57	63.565	90.39	6.88	70.24	66.3
2	5.75	61.995	87.93	7.15	70.43	68.6
3	5.87	63.090	88.845	6.72	70.76	66.3
Control	6.04	63.755	85.65	7.17	74.39	65.8

Then, the speed of meat piece movement inside the massager drum is determined as follows:

$$\upsilon = \frac{\pi \cdot \varphi \cdot n_{кр} \cdot R_б}{30} = \frac{12.3 \cdot \pi \cdot \varphi \cdot \sqrt{R_б}}{30 \cdot \sqrt{2}} = \frac{12.3 \cdot 3.14 \cdot 0.82 \cdot \sqrt{0.6}}{30 \cdot \sqrt{2}} = 0.578 м/с$$

The investigated massaging process's characteristic size corresponds to the meat pieces' average size: $\ell = 0{,}04$ *m.*

The mass transfer coefficient for the investigated process can be determined using the following relationship:

$$\beta = \frac{П_v}{\Delta C \cdot S} = \frac{m}{t \cdot S \cdot \Delta C \cdot \rho} \quad (5)$$

Where:

t – is the processing time per product load **[25]**.

The massaging cycle in the developed marinating technology proceeds as follows. The rotary motion of the massager's executive elements for pork semi-finished products takes place for t = 30 minutes, while for beef semi-finished products, it's – t = 40 minutes. These cycles are repeated throughout 24 to 36 hours. The raw material and room temperature range from 4 to 6 °C **[26]**.

The change in the concentration of sunflower, rapeseed, or olive oil in the product is taken as ΔC = 0.664 (Table 1) **[27]**.

The product's density is assumed as follows: for pork semi-finished products, it is ρ = 1030 kg/m³; for beef semi-finished products of grade II, it is ρ = 1087 kg/m³ **[28]**.

Thus, the mass transfer coefficient β can be expressed as:

$$\beta = \frac{m \cdot (g + \frac{v^2}{R_б})}{t \cdot S \cdot \Delta C \cdot (g + \frac{v^2}{R_б}) \cdot \rho} = \frac{\tau}{t \cdot \Delta C \cdot (g + \frac{v^2}{R_б}) \cdot \rho} = \frac{\tau}{t \cdot \rho \cdot 0.664(9.81 + \frac{0.578^2}{0.6})}$$

Where g = 9,81 m/s² is the acceleration due to gravity, and the shear stress $\tau = \frac{P}{S} = \frac{m \cdot (g + \frac{v^2}{R_б})}{S}$; is represented by the magnitude $= m_{(g + \frac{v^2}{R_б})}$ which is the load in the investigated process resulting from the action of the force of gravity on the product mass and the centrifugal forces during its rotation in the working drum **[29]**.

The expression was then transformed

$$\beta = \frac{\tau}{t \cdot \rho \cdot 6.81} \quad (6)$$

The shear yield stress τ can be determined using the plasticity values X and the penetration number Q, obtained from the investigations of corresponding samples (Table 3).

Using the fact that the penetration index is proportional to the shear stress of the minced mass τ, the penetration coefficient Q was determined from the relationship:

$$Q = \frac{P}{h^2} = \tau \cdot k_{np} \quad (7)$$

where k_{np} – is the proportionality coefficient; h – is the depth of the indenter penetration into the minced mass under a certain load P, determined from experimental studies **[30]**.

Then, when using equation (7):

$$\tau = \frac{Q}{k_{np}} \quad (8)$$

When determining the plasticity, the following mathematical transformation method was used **[31]**.

$$X = \frac{S}{m} = \frac{S \cdot (g + \frac{v^2}{R_б})}{m \cdot (g + \frac{v^2}{R_б})} = \frac{S \cdot (g + \frac{v^2}{R_б})}{P} = \frac{(g + \frac{v^2}{R_б})}{\tau} \quad (9)$$

The magnitude $= m_{(g + \frac{v^2}{R_б})}$ represents the load in the investigated process due to the force of gravity acting on the product mass and the centrifugal forces during its rotation in the working drum **[32]**. Thus, when using equation (9), the sought shear stress takes the form:

$$\tau = \frac{(g + \frac{v^2}{R_б})}{X} = \frac{(9.81 + \frac{0.578^2}{0.6})}{X} = \frac{10.26}{X} \quad (10)$$

According to the research results, the diffusion coefficient of the marinade for the experimental sample of grade II beef is $D = 12.5 \cdot 10^{-8}$ m²/s, and for semi-fatty pork $D = 10.6 \cdot 10^{-8}$ m²/s.

Considering the substantial number of factors determining the process, we will replace the relationships between them with dependencies between the presented similarity criteria. To do this, we use a dimensional matrix **[33]**, which is formed using Table 4.

Table 4 Dimensional matrix of the investigated massaging process.

Parameters	ρ, kg/m³	υ, m/s	τ, H/m².kg/(m.s²)	t, s	ℓ, m	D, m²/s	β, m/s
M, kg	1		1				
L, m	-3	1	-1		1	2	1
T, s		-1	-2	1		-1	-1
Exponential coefficients	ε	δ	m	α	Θ	λ	

In the general form, the relationship between the presented parameters can be expressed as a function:

$$\beta = f(\rho, \upsilon, \tau, \ell, t, D) \quad (11)$$

Based on the dimensional matrix compiled in Table 4, the function takes the form of a power series:

$$\beta = K \cdot \tau^{m} \cdot \rho^{\varepsilon} \cdot \upsilon^{\delta} \cdot t^{\alpha} \cdot D^{\lambda} \cdot \ell^{\Theta} \quad (12)$$

Where:

K – is a constant coefficient.

The presented factor space with 6 variables and the number of dimensionless components determined by the π-theorem as 6 - 3 = 3 corresponds to the chosen similarity criteria, specifically the Sherwood, Fourier, and Euler numbers **[34]**.

We reproduce the dimensional matrix compiled in Table 3 in the following system of equations for the exponential coefficients of the mass transfer equation (12) **[35]**:

$$\begin{cases} m + \varepsilon = 0 & (13) \\ \delta - m - 3\varepsilon + \Theta + 2\lambda = 1 & (14) \\ -\delta - 2m + \alpha - \lambda = -1 & (15) \end{cases}$$

From equation (13), we obtain:

$$\varepsilon = -m \quad (16)$$

Substituting equation (16) into equation (14):

$$\delta + 2m + \Theta + 2\lambda = 1 \quad (17)$$

From the sum of equations (17) and (15):

$$\Theta + \alpha + \lambda = 0 \quad (18)$$

From equation (18):

$$\lambda = -\Theta - \alpha \quad (19)$$

Considering equations (12, 16, 19):

$$\beta = \rho^{-m} \cdot \upsilon^{\delta} \cdot t^{\alpha} \cdot \tau^{m} \cdot D^{(-\Theta-\alpha)} \cdot \ell^{\Theta} \quad (20)$$

Considering equation (20):

$$\frac{\beta \cdot \ell}{D} = \frac{\ell}{D} \cdot \left[\frac{\tau}{\rho \cdot \upsilon^2} \right]^{m} \cdot t^{\alpha} \cdot \upsilon^{(2m+\delta)} \cdot D^{(-\Theta-\alpha)} \cdot \ell^{\Theta} \quad (21)$$

Considering equations (21, 2):

$$Sh = Eu^{m} \cdot \left[\frac{\ell^2}{D \cdot t} \right]^{\Theta} \cdot \ell^{(1-\Theta)} \cdot D^{(-\alpha-1)} \cdot \upsilon^{(2m+\delta)} \cdot t^{(\alpha+\Theta)} \quad (22)$$

Considering equations (1, 22):

$$Sh = Eu^{m} \cdot Fo_{\partial}^{-\Theta} \cdot \left[\frac{\ell^2}{D \cdot t} \right]^{\alpha} \frac{\ell^{(1+\lambda-\alpha)}}{D} \cdot \upsilon^{(2m+\delta)} \cdot t^{\Theta)} \quad (23)$$

Considering equations (1, 23):

$$Sh = Eu^{m} \cdot Fo_{\partial}^{-\Theta} \cdot Fo_{\partial}^{\alpha} \cdot K \quad (24)$$

Then, the general expression for the mass transfer equation of the investigated process takes the form:

$$Sh = Eu^{m} \cdot \mathrm{Fo}_{\partial}^{(\alpha-\Theta)} \cdot K \quad (25)$$

$$f(K) = \frac{\ell^{(1+\lambda-\alpha)}}{D} \cdot \upsilon^{(2m+\delta)} \cdot t^{\Theta} \quad (26)$$

To obtain the initial data during the graphoanalytical analysis of the investigated process, we determined the values of the Fourier, Sherwood, and Euler numbers, and mass transfer coefficient using formulas (1, 2, 4) respectively, and experimental data obtained from the conducted research (Table 1, 3) **[36]**.

Using the data from Table 5.

Table 5 Similarity criteria values for the investigated massaging process.

Process parameter	Control	Experimental Sample No.1	Experimental Sample No.2	Experimental Sample No.3
Pork semi-finished products				
Plasticity X, cm²/g	6.75	9.45	9.06	7.74
Oil Content in the marinade, ΔC	0.664	0.664	0.664	0.664
Shear stress τ=10.26/X, MPa	1.52	1.08	1.132	1.326
Product density ρ, kg/m³	1030			
Particle size ℓ, m	0.04			
Processing time t, s	1800			
Flow velocity in the cutter bowl υ, m/s	0.578			
Diffusion coefficient D, $\times 10^{-8} m^2/s$	10.8	11.2	11.8	12.5
The mass transfer coefficient in the loading mass $\beta = \frac{\tau}{t \cdot \rho \cdot 6{,}81}$, m/s	0.12	0.0855	0.0897	0.105
Pork semi-finished products				
Sherwood's number is Sh×10⁴	4.45	3.05	3.04	3.36
Euler's number, Eu	0.00422	0.00314	0.00329	0.00385
Fourier's number, Fo_d	0.122	0.126	0.133	0.141
Beef semi-finished products				
Plasticity X, cm²/g	6.88	7.15	6.72	7.17
Oil content in the marinade, ΔC	0.664	0.664	0.664	0.664
Shear stress τ=10.26/X, MPa	1.49	1.445	1.527	1.431
Product density ρ, kg/m³	1087			
Particle size ℓ, m	0.04			
Processing time t, s	2400			
Flow velocity in the cutter bowl υ, m/s	0.578			
Diffusion coefficient D, $\times 10^{-8} m^2/s$	8.9	9.3	9.8	10.6
The mass transfer coefficient in the loading mass $\beta = \frac{\tau}{t \cdot \rho \cdot 6{,}81}$, m/s	0.0838	0.0816	0.0859	0.0805
Sherwood's number Sh, ×10⁴	3.77	3.51	3.51	3.04
Euler's number, Eu	0.0041	0.00398	0.0042	0.00394
Fourier's number, Fo_d	0.1335	0.1395	0.147	0.159

Additionally, a method for graph-analytical assessment of power functions was applied, and a graph of the function $Sh = f(Eu)$ was constructed. This function is linear, with its graph forming an angle φ with the abscissa axis in Figures 1 and 2 **[37]**.

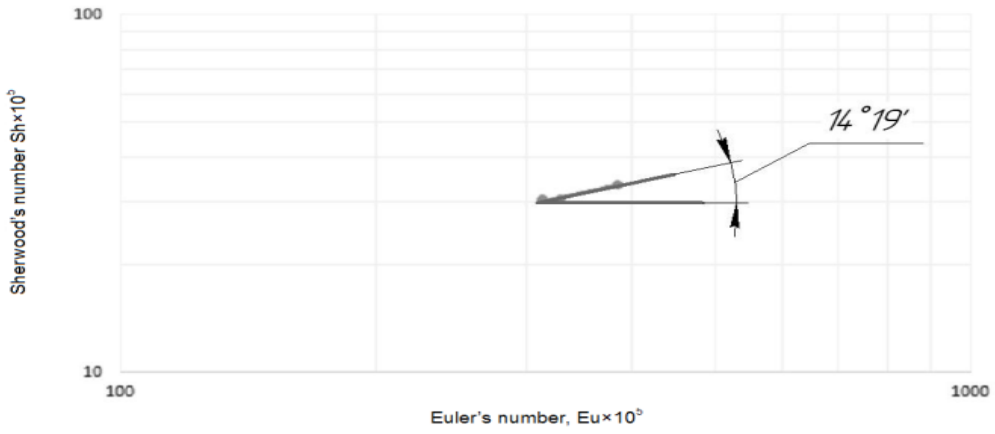

Figure 1 Graph of the function $Sh = f(Eu)$ for semi-finished pork products.

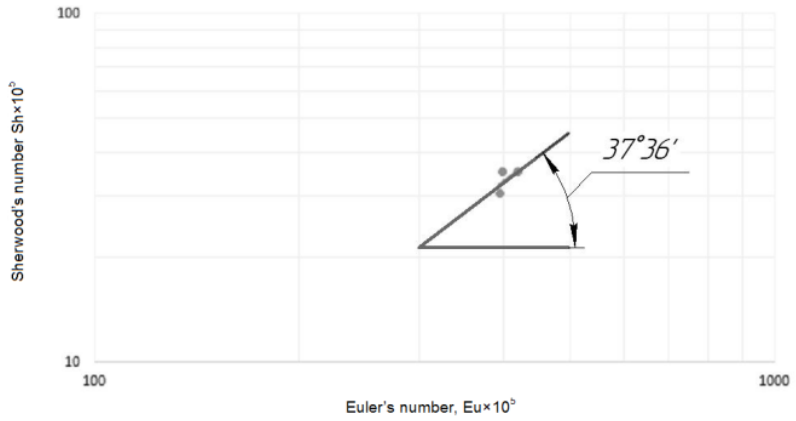

Figure 2 Graph of the function $Sh = f(Eu)$ for semi-finished beef products.

Then the value of the first power coefficient is determined.

$$m_c = tg = tg14.19° = 0.253$$
$$m_я = tg\varphi = 37.36° = 0.763 \quad (27)$$

Table 6 Calculated data for determining power coefficients.

Process parameter	Control	Experimental Sample No.1	Experimental Sample No.2	Experimental Sample No.3
Pork semi-finished products				
Dimensionless component $Fo_d^{-\Theta} \times 10^5$	7.851	7.906	8.001	8.104
Dimensionless component $Fo_d^{\alpha} \times 10^5$	133.324	135.579	139.445	143.745
Dimensionless component $Eu^m \times 10^5$	106.766	79.442	83.237	97.405
Dimensionless component $Sh / Eu^m \times 10^5$	9.642	7.122	7.015	7.451
Dimensionless component $\frac{Sh}{Eu^m \cdot Fo_\partial^{-\Theta}} \cdot 10^7$	5.309	4.856	4.565	4.257
Dimensionless component $\frac{Sh}{Eu^m \cdot Fo_\partial^{-\Theta} \cdot Fo_\partial^{\alpha}} \cdot 10^{11}$	398.205	358.159	327.364	296.132
$f(K) \times 10^{11}$	3.715	3.582	3.400	3.209
Beef semi-finished products				
Dimensionless component $Fo_d^{-\Theta} \times 10^5$	58.305	59.412	60.759	62.834
Dimensionless component $Fo_d^{\alpha} \times 10^5$	1816.060	1880.241	1959.660	2084.989
Dimensionless component $Eu^m \times 10^5$	312.830	303.674	320.460	300.622
Dimensionless component $Sh / Eu^m \times 10^5$	0.383	0.364	0.350	0.318
Dimensionless component $\frac{Sh}{Eu^m \cdot Fo_\partial^{-\Theta}} \cdot 10^7$	20.669	19.455	18.027	16.094
Dimensionless component $\frac{Sh}{Eu^m \cdot Fo_\partial^{-\Theta} \cdot Fo_\partial^{\alpha}} \cdot 10^{11}$	1.138	1.035	0.920	0.772
$f(K) \times 10^{11}$	1.691	1.691	1.691	1.691

Using the previous calculation method, a graph of the function $\frac{Sh}{Eu^m} = f(\mathrm{Fo}_\partial)$ was constructed using data from Table 6.

From this graph, the angle γ (Figure 3 and Figure 4) of its inclination to the abscissa axis was determined, and the value of the second power coefficient was found using the formula.

$$\Theta_c = -\mathrm{tg}\gamma = -\mathrm{tg}12.37° = -0.219 \quad (28)$$
$$\Theta_я = -\mathrm{tg}\gamma = -\mathrm{tg}23.2° = -0.428$$

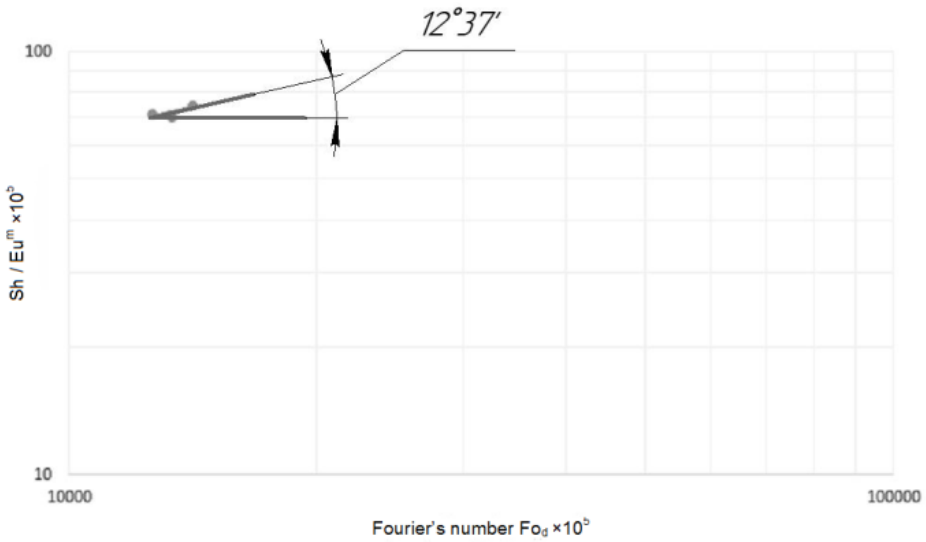

Figure 3 Graph of the function $\frac{Sh}{Eu^m} = f(\mathrm{Fo}_\partial)$ for semi-finished pork products.

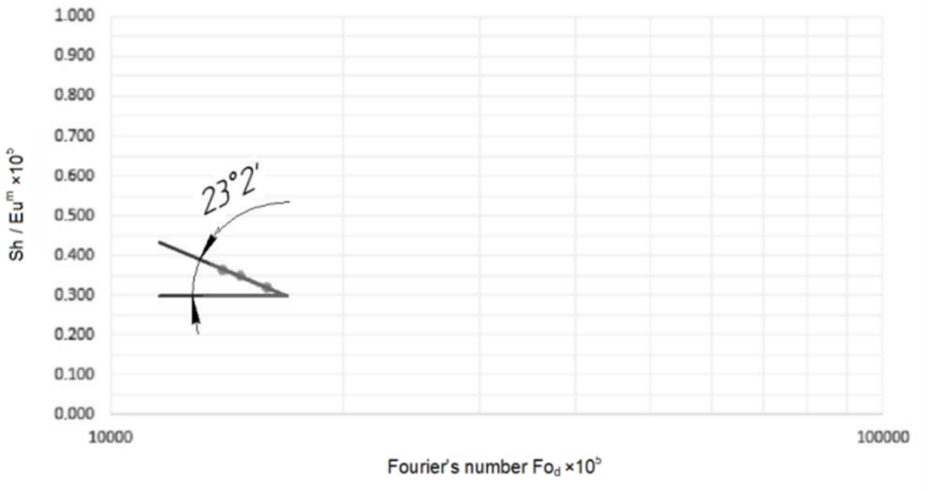

Figure 4 Graph of the function $\frac{Sh}{Eu^m} = f(\mathrm{Fo}_\partial)$ for semi-finished beef products.

Subsequently, a graph of the function was constructed $\frac{Sh}{Eu^m \cdot \mathrm{Fo}_\partial^{-\Theta}} = \mathrm{Fo}_\partial$ (Figure 5 and Figure 6) using the data from Table 6.

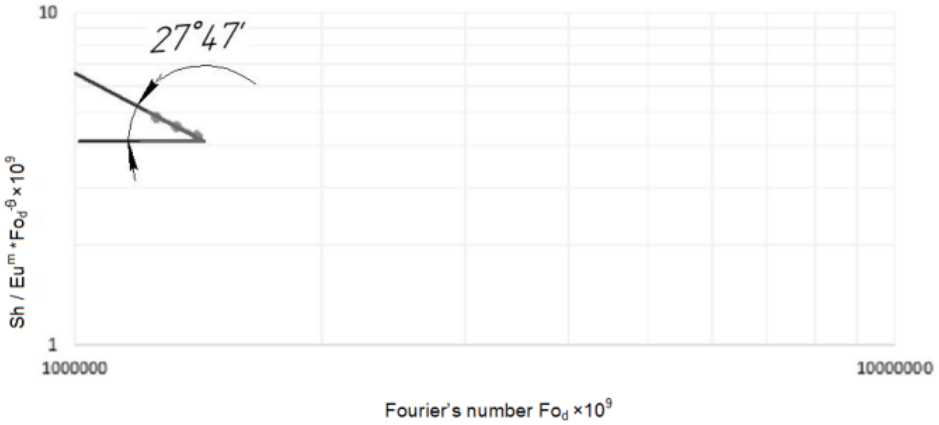

Figure 5 Function graph $\frac{Sh}{Eu^m \cdot \mathrm{Fo}_\partial^{-\Theta}} = f(\mathrm{Fo}_\partial)$ for semi-finished pork products.

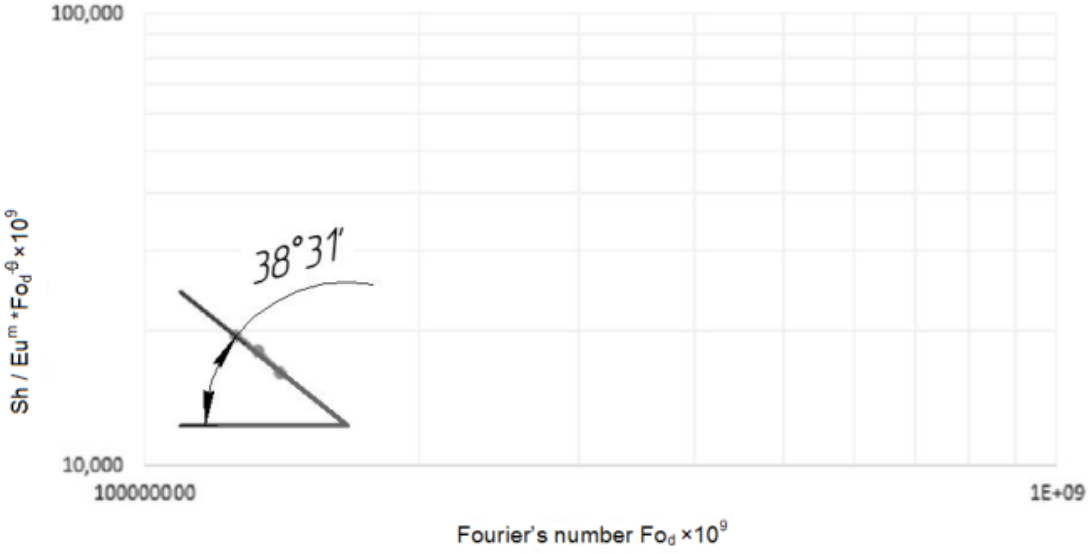

Figure 6 Function graph $\frac{Sh}{Eu^m \cdot \mathrm{Fo}_\partial^{-\Theta}} = f(\mathrm{Fo}_\partial)$ for semi-finished beef products.

From this graph, the angle γ (Figure 7 and Figure 8) of its inclination to the abscissa axis was determined, and the value of the second power coefficient was found using the formula.

$$a_c = tg\Theta_c = tg27.47° = 0.52 \quad (29)$$

$$a_я = tg\Theta_я = tg38.31° = 0.79$$

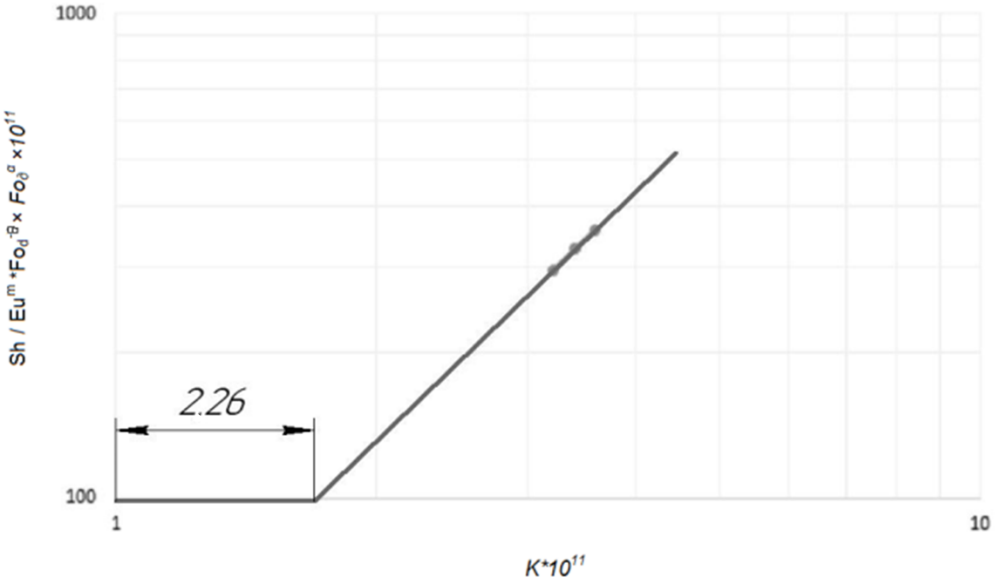

Figure 7 Function graph $\frac{Sh}{Eu^m \cdot \mathrm{Fo}_\partial^{-\Theta} \cdot \mathrm{Fo}_\partial^{\alpha}} = f(K)$ for semi-finished pork products.

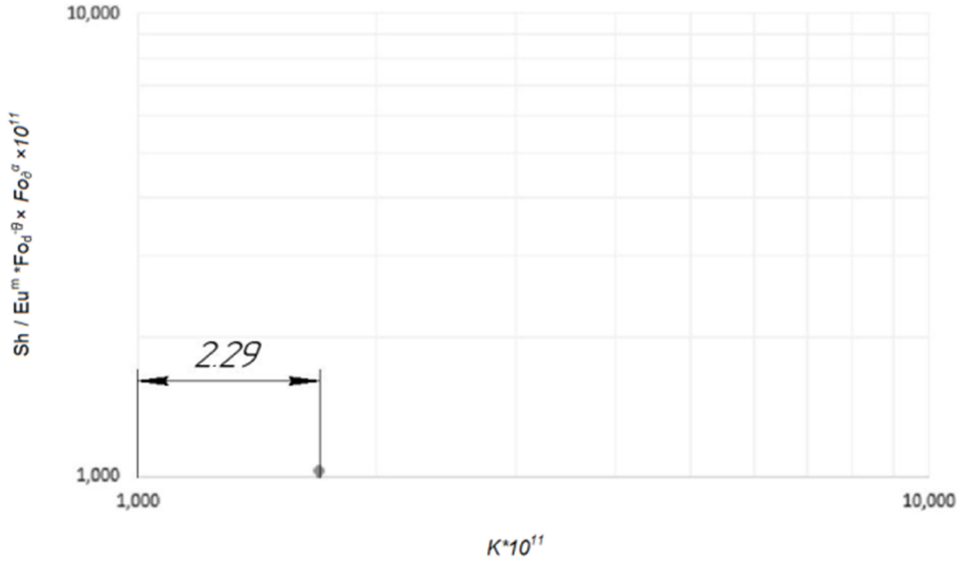

Figure 8 Function graph $\frac{Sh}{Eu^m \cdot \mathrm{Fo}_\partial^{-\Theta} \cdot \mathrm{Fo}_\partial^{\alpha}} = f(K)$ for semi-finished beef products.

Then, the sought difference was determined.

$$a_c\text{-}\Theta_c=0.52\text{-}(\text{-}0.219)=0.739 \quad (30)$$

$$a_я\text{-}\ \Theta_c=0.79\text{-}(\text{-}0.428)=1.218 \quad (31)$$

Using the data from Table 6, the process constant K was determined. In this case, the function on the left side of this equation consists of

$$f(K)=\frac{\ell^{(1+\lambda-\alpha)}}{D}\cdot\upsilon^{(2m+\delta)}\cdot t^{\Theta} \quad (32)$$

Thus, considering the obtained dependencies (28-31) – the final criterion equation of the vibrational mixing process was determined for pork ingredients (equation (33)) and beef ingredients (equation (34)) in the kneading process, which can be represented in the form of **[38]**.

$$Sh = Eu^{m_c} \cdot \mathrm{Fo}_\partial^{(\alpha_c - \Theta_c)} \cdot K_c = 2.26 \cdot Eu^{0.253} \cdot \mathrm{Fo}_\partial^{0.739} \quad (33)$$

$$Sh = Eu^{m_я} \cdot \mathrm{Fo}_\partial^{(\alpha_я - \Theta_я)} \cdot K_я = 2.29 \cdot Eu^{0.763} \cdot \mathrm{Fo}_\partial^{1.218} \quad (34)$$

The presented mass exchange equation illustrates the predominant influence in the studied kneading process of changes in the concentration of sunflower, rapeseed, or olive oil in product ΔC, on the value of the diffusion coefficient D, the size of dispersed phase particles, and the mass transfer coefficient in the load mass β. Using the derived equations (33) and (34) and the developed program, a recommended set of working regime parameters is found for the marinating operation of semi-finished pork and beef products under the conditions of the specified factors' influence. The analysis of the composed heat exchange equations allows the assessment of the dynamics of changing these parameters when altering the operational modes of the studied kneading process, respectively for pork (equation (35)) and beef (equation (36)).

$$\frac{\beta \cdot \ell}{D} = 2.26 \cdot \left[\frac{\tau}{\rho \cdot v^2}\right]^{0.253} \cdot \left[\frac{D \cdot t}{\ell^2}\right]^{0.739} \quad (35)$$

$$\frac{\beta \cdot \ell}{D} = 2.29 \cdot \left[\frac{\tau}{\rho \cdot v^2}\right]^{0.763} \cdot \left[\frac{D \cdot t}{\ell^2}\right]^{1.218} \quad (36)$$

CONCLUSION

The research results indicate that the derived mass exchange equation demonstrates a predominant influence on the studied kneading process, where changes in the concentration of sunflower, rapeseed, and olive oil in the product have a significant impact on the diffusion coefficient, size of dispersed phase particles, and mass transfer coefficient within the load mass. By using the complex equations and developed program, we determine the recommended set of operating parameters for the massaging operation in the marinating process of semi-finished products from pork and beef, considering the effects of the specified factors. Clearly, the analysis of the formulated heat exchange equations allows assessing the dynamics of these parameters as the operating conditions of the investigated massaging process change for pork and beef.

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

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Incorporation of gambir catechin crude extract in robusta instant coffee made from different coffee processing methods

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ABSTRACT

This research aimed to enhance the antioxidant properties of instant gambir coffee by adding gambir catechin crude extract during coffee processing using natural anaerobic, full wash, and honey methods. The experiment used a completely randomized non-factorial design (RALNF), with each treatment replicated five times. The treatments consisted of nine formulations (F), namely F1= natural anaerobic 87.5% (w/w): gambir catechin crude 5% (w/w), F2 = natural anaerobic 82.5% (w/w): gambir catechin crude 10% (w/w), F3 = natural anaerobic 77.5% (w/w), gambir catechin crude 15% (w/w), F4 = honey 87.5% (w/w): gambir catechin crude 5% (w/w), F5 = honey 82.5% (w/w): gambir catechin crude 10% (w/w); F6 = honey 77.5% (w/w): gambir catechin crude 15% (w/w) F7 = full wash 87.5% (w/w): gambir catechin crude 5% (w/w); F8 = honey 82.5% (w/w): gambir catechin crude 10% (w/w), and F9 = honey 77.5% (w/w): gambir catechin crude 15% (w/w). The results showed that the formulation treatments significantly affected the instant gambir coffee's water content, solubility percentage, acidity level (pH), total phenols, and IC50. The characteristics of the resulting instant gambir coffee included water content, solubility percentage, pH, total phenols, and IC50 with values of 6.56-7.02%, 94.35-96.55%, 5.25-5.75, 24.91-40.35 mgGAE/g, and 31.46-121.75 mg/mL, respectively.

Keywords: gambir, honey, full wash, coffee, natural anaerobic

INTRODUCTION

Coffee has become immensely popular worldwide, including in Indonesia, leading to a rapid growth of coffee shops and the increasing circulation of instant coffee packets. Generally, coffee served in cafes or marketed in packet form prioritizes flavor, with only a small proportion offering antioxidant properties, which naturally compounds such as chlorogenic acid. It is coffee's main bioactive compound with antioxidant properties **[1]**. Robusta coffee contains higher chlorogenic acid and caffeine levels than arabica coffee **[2]**. There is a decrease in chlorogenic acid content by 37-59% due to the high-temperature roasting process **[3]**.

The addition of gambir catechin crude to enhance the antioxidant properties of coffee was affected by caffeine levels and coffee acidity **[4]**. Adding gambir extract to the naturally processed robusta coffee powder resulted in a 45% increase in antioxidant properties **[5]**. Caffeine is associated with catechin compounds, affecting the free catechin in coffee. Therefore, the higher the caffeine content, the more catechins are bound, and the fewer free catechins in the coffee. Previous investigations showed that the amount of free catechin in a food system determines its antioxidant properties, indicating the more free catechins, the higher the antioxidant properties, and vice versa. In addition to caffeine levels, coffee pH affects antioxidant properties because catechin compounds are stable under acidic conditions. This indicated that adding crude catechin in coffee with a high pH level will produce more stable catechin compounds and enhance the coffee's antioxidant properties.

Generally, there are three commonly used coffee processing methods, natural anaerobic, full wash, and honey, which significantly affect caffeine content and pH. The full wash and honey methods are known as wet processing,

while natural anaerobic is a dry process. Natural anaerobic involves fermentation without going through the pulping process. The pulp's juice is used by acid bacteria during fermentation, resulting in a more acidic coffee with a sharper aroma (high caffeine). Coffee processed with the full wash method contains low caffeine content with a high pH level, while the honey method contains low caffeine and pH levels. Incorporating gambir catechin crude extract into instant robusta coffee processed with the natural anaerobic, full wash, and honey methods can produce gambir instant coffee with good physical, chemical, and antioxidant properties.

Scientific hypothesis

Incorporating gambir catechin crude extract into instant robusta coffee processed with the natural anaerobic, full wash, and honey methods can produce gambir instant coffee to increase the functional properties, especially its antioxidant activity.

MATERIAL AND METHODOLOGY

Samples

Instant coffee with a percentage of the processing method according to the treatment incorporated with gambir catechin extract.

Chemicals

The materials used consist of distilled water, tannic acid, 96% ethanol, 2,2-diphenyl-1-picrylhydrazil (DPPH), folin-ciocalteu, methanol, and Na_2CO_3 obtained from the Laboratory of Chemical Agricultural Products, Faculty of Agriculture, Sriwijaya University, Indonesia.

Biological Material

Gambier powder from Babat Toman Village, Musi Banyuasin Regency, South Sumatra, Indonesia. Robusta coffee powder from JagadRaye Coffee, a micro and small enterprise in Pagar Alam, South Sumatra, Indonesia.

Pasak bumi powder from the Laboratory of Chemical Agricultural Products, Faculty of Agriculture, Sriwijaya University, Indonesia.

Instruments

The tools used include an autoclave, blender (Philips, Holland), hot plate, incubator (Memmert, Germany), filter paper, laminar airflow (LAF), brand analytical balance (Kenko, Japan), drying oven (Memmert, Germany), pH meter (Eutech, Malaysia), micropipette (Dragon Lab, China), rotary vacuum evaporator, 80 mesh filter, spectrophotometer (A and E Lab, USA), and vortex (Digisystem, Taiwan).

Laboratory Methods

The parameters evaluated include water content **[6]**: measurement of water content using the gravimetric method. Soluble speed **[6]**: Dissolve 100 g of instant coffee in 200 mL of water. Then, the time instant coffee dissolves in water is calculated as the speed at which it dissolves in water using a stopwatch. Acidity Degree (pH) **[7]**: Total phenol **[8]**: Determination of total phenol content was carried out using a spectrophotometric method using Folin Ciocalteu reagent. Antioxidant activity **[9]**: Antioxidant testing using the DPPH method (2,2 diphenyl-1- picrylhydrazyl) was used.

Description of the Experiment

Sample preparation:

1. Preparation of gambir catechin crude extract. The dried gambir product was ground using a blender and sieved with a 60-mesh sieve. Subsequently, 100 g of gambir powder was macerated with ethanol solvent (1:3) for 24 hours. The crude extract of gambir catechin was filtered using filter paper and evaporated with a rotary evaporator at 60 °C until the ethanol evaporated (no ethanol aroma present). The crude extract of gambir catechin was dried using an oven dryer at 85 °C for ±15 hours. The dried sample was ground using a blender and sieved with a 60-mesh sieve, making it ready for application.
2. Preparation of instant robusta coffee powder Robusta coffee powder (natural, honey, full wash) was added to 100 °C water for 2 minutes with a coffee powder-to-water ratio of 1:4 and allowed to rest for 10 minutes. The coffee suspension was filtered using a filter cloth to obtain a filtrate. Maltodextrin (20% w/w) and Tween 80 (0.3% v/v) were added to the coffee filtrate and stirred using a mixer for 10 minutes at high speed to form foam. The foam was poured and levelled in an aluminium tray lined with polypropylene plastic with a thickness of 1 cm, dried using a food dehydrator at 60 °C for 8 hours, ground using a blender, and sieved with a 60-mesh sieve to obtain an instant coffee powder.
3. Preparation of instant coffee incorporated with gambir catechin crude extract. The ingredients used were prepared: instant coffee, crude extract of gambir catechin, and instant Javanese ginseng. According to the treatment, instant coffee with a percentage of the processing method was added with a crude extract of gambir catechin. Subsequently, instant Javanese ginseng at 7% (w/w) was added to each treatment combination. The

instant coffee incorporated with gambir catechin crude extract, weighing 30 g, was packed in an aluminium foil package and prepared for analysis.

Number of samples analyzed: We analyzed 9 samples.

Number of repeated analyses: All measurements of instrument readings were performed five times.

Design of the experiment: The samples This research used a wholly randomized non-factorial design (CRND) with coffee processing methods and gambir catechin crude extract treatments, which were repeated five times. The treatments consisted of nine formulations (F), namely F1 = natural anaerobic 87.5% (w/w): gambir catechin crude extract 5% (w/w), F2 = natural anaerobic 82.5% (w/w): gambir catechin crude extract 10% (w/w), F3 = natural anaerobic 77.5% (w/w): gambir catechin crude extract 15% (w/w), F4 = honey 87.5% (w/w): gambir catechin crude extract 5% (w/w), F5 = honey 82.5% (w/w): gambir catechin crude extract 10% (w/w), F6 = honey 77.5% (w/w): gambir catechin crude extract 15% (w/w), F7 = full wash 87.5% (w/w): gambir catechin crude extract 5% (w/w), F8 = honey 82.5% (w/w): gambir catechin crude extract 10% (w/w), and F9 = honey 77.5% (w/w): gambir catechin crude extract 15% (w/w).

Statistical Analysis

This study used a factorial, completely randomized design. The treatment with a significant effect was further tested using the honest real difference test (HSD) at = 5%. The data were analysed using the SAS software version of Windows 9 to analyse of variance.

RESULTS AND DISCUSSION

The water content of instant gambir coffee ranged from 6.56-7.02%. Among all treatments, natural anaerobic 77.5% (w/w): gambir catechin crude extract 15% (w/w) (F3) resulted in the highest water content, while honey 87.5% (w/w): gambir catechin crude extract 5% (w/w) (F4) had the lowest were presented in Figure 1.

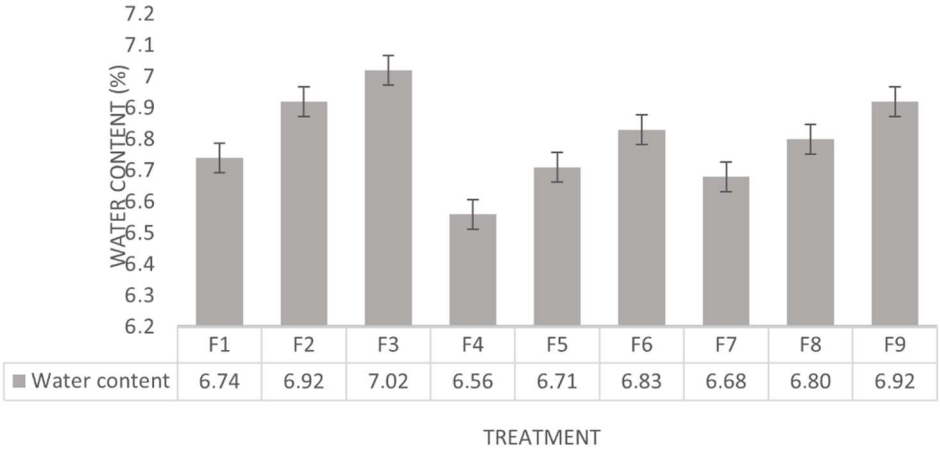

Figure 1 Water content of instant gambir coffee.

Analysis of variance showed that the coffee formulation treatment significantly influenced the water content. The results of the posthoc test BNJ 5% on water content, solubility percentage, and pH level of instant gambir coffee were presented in Table 1. Table 1 showed that treatment F3 had the highest water content compared to others and was not significantly different from treatments F2, F6, and F7. Theoretically, this occurred because naturally anaerobic-processed coffee contains more glucose, as pulping is not performed during the processing. The presence of glucose affected the amount of bound water in coffee. The gambir catechin crude extract also influenced the water content due to the several hydroxyl (OH) groups in catechin compounds. This indicated that the higher the gambir catechin crude extract content, the more OH groups that can bind water, thereby affecting the increase in water content. Haile and Kang **[10]** explained that coffee beans processed using dry processing methods such as natural anaerobic and wine have a heavier, finer, sweeter, and more complex character. The gambir catechin crude extract contained acidic catechin compounds despite having many OH groups **[11]**.

Table 1 The results of the test BNJ 5% on the water content, solubility percentage, and pH of instant gambir coffee produced.

Treatment	Water Content (%)	Solubility (%)	pH
F1 = 87.5% *natural*: Gambir 5%	6.74 ±0.02abc	95.85 ±0.22ab	5.75 ±0.02f
F2 = 82.5% *natural*: Gambir 10%	6.92 ±0.02cd	95.13 ±0.42ab	5.63 ±0.03e
F3 = 77.5% *natural*: Gambir 15%	7.02 ±0.08d	94.35 ±0.88a	5.56 ±0.02d
F4 = 87.5% *honey* : Gambir 5%	6.56 ±0.12a	97.55 ±0.59c	5.64 ±0.02e
F5 = 82.5% *honey*: Gambir 10%	6.71 ±0.11ab	95.68 ±0.62ab	5.53 ±0.01d
F6 = 77.5% *honey*: Gambir 15%	6.83 ±0.06bcd	94.95 ±0.08ab	5.45 ±0.02e
F7 = 87.5% *fullwash*: Gambir 5%	6.68 ±0.04ab	96.28 ±0.51bc	5.47 ±0.02e
F8 = 82.5% *fullwash*: Gambir 10%	6.80 ±0.07bc	95.47 ±0.44ab	5.33 ±0.03b
F9 = 77.5% *fullwash*: Gambir 15%	6.92 ±0.07cd	94.57 ±0.57a	5.25 ±0.02a

Note: Numbers followed by the same letter in the same column are not significantly different.

The water content of instant gambir coffee produced in this research was higher than the Indonesian National Standard (SNI) No 2983 of 2014, set at 5%. The value was also greater than **[4]** at 3.84-4.81%, instant coffee from Tungkal Jambi, which has a content of 1.57-1.61% **[12]**, and cold brewed instant coffee at 2.43% **[13]**.

The solubility percentage of instant gambir coffee produced ranged from 94.35-97.55%, where the lowest value was obtained in Treatment F3 (natural anaerobic 77.5%: gambir catechin crude extract 15%). In comparison, F4 (honey 87.5%: gambir catechin crude extract 5%) had the highest, as presented in Figure 2.

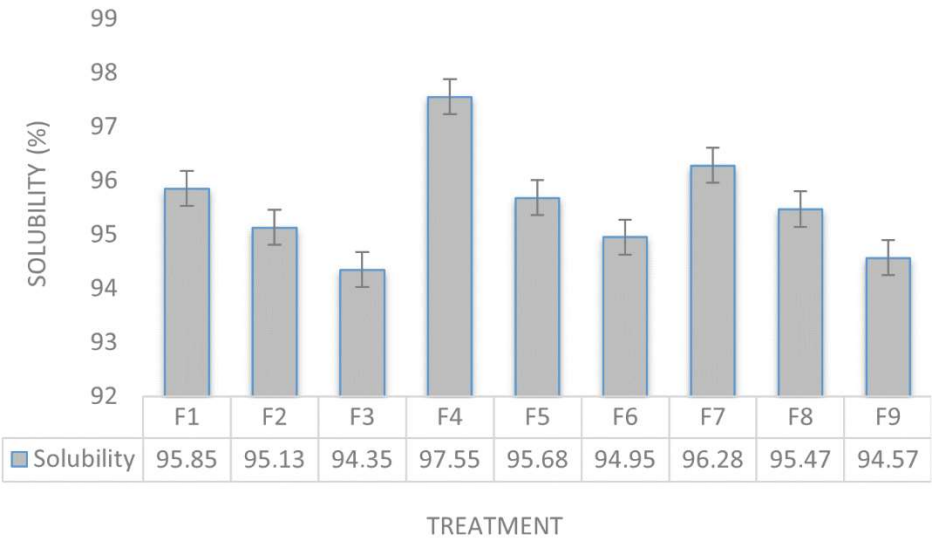

Figure 2 Solubility percentage of instant gambir coffee.

The formulation treatment significantly influences the solubility percentage of instant gambir coffee produced. The results of the BNJ 5% test are shown in Table 1. Table 1 shows that formulation F4 had the highest solubility percentage, 97.55%, but not significantly different from F7. This can be explained based on the principle of like dissolved likes, where non-polar compounds dissolve in non-polar solvents, and conversely, polar compounds are soluble in polar solvents. Previous investigations showed that the catechin crude extract of gambir contains semi-polar catechin compounds. Therefore, the solubility level decreases as the concentration of catechin crude extract of gambir increases and vice versa. Coffee processing methods also affected the solubility level of coffee because, according to Herawati **[14]**, honey coffee has the highest level of a polar compound **[15]**, namely chlorogenic acid, compared to other processing. The solubility percentage of instant gambir coffee produced was higher than the solubility level of 93.79% for instant coffee made from robusta beans processed using a vacuum dryer, as reported by Matanari **[16]**. However, the value was lower than Tungkal Jambi Liberika instant coffee, which had a content of 97.95-98.20% **[12]**.

The pH level of instant gambir coffee produced ranged from 5.25-5.75, with the lowest and highest levels found in formulations F9 (honey 77.5% (w/w): gambir catechin crude extract 15%) and F1 (natural anaerobic 87.5% (w/w): gambir catechin crude extract 5% (w/w), respectively were presented in Figure 3 .

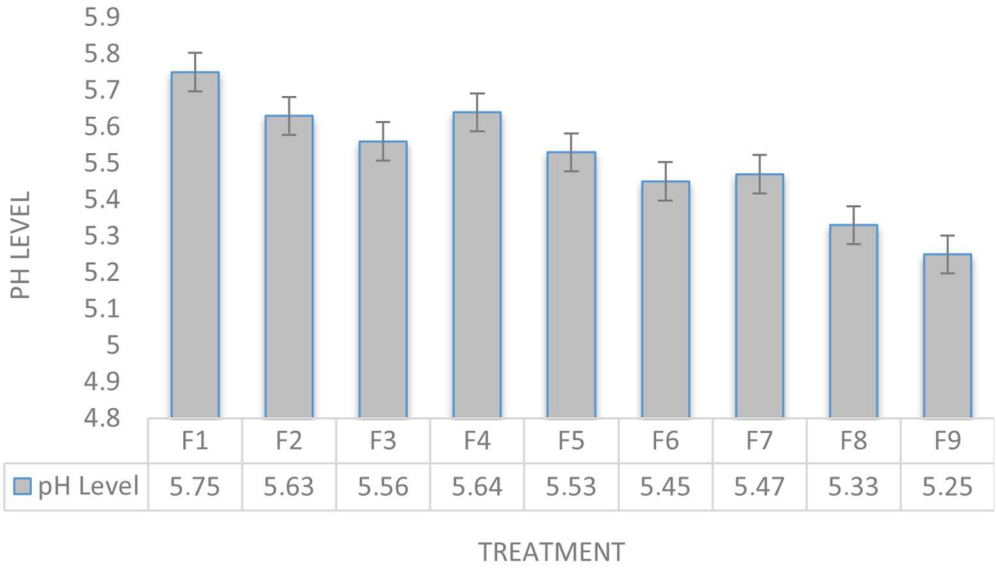

Figure 3 pH level of instant gambir coffee.

The analysis of variance showed that the formulation treatment significantly influenced the pH level of instant gambir coffee produced. The results of the LSD test at a 5% significance level for the pH level of instant gambir coffee are shown in Table 1. Formulation F9 produced the lowest pH level and significantly differed from other formulations as presented in Table 1 by the LSD test at a 5% significance level. This occurred because the full-wash coffee involved fermentation, during which aliphatic acids such as citric, malic, and quinic acids are formed **[17]**, thereby affecting the pH level of the coffee produced. The pH level of this instant coffee was also influenced by the crude extract content of gambir catechin, as catechin compounds are acidic, easily oxidized at neutral pH, and stable at low pH **[11]**. Therefore, the higher the concentration of crude extract of gambir catechin, the greater the pH level or the lower the pH value. The pH level of instant gambir coffee produced in this research is similar to robusta coffee at 5.47 **[18]**, instant mangosteen peel coffee at 5.26-5.63 **[19]**, brewed robusta coffee at 5.16-5.69 **[20]**, and fermented robusta coffee, which ranged from 5.25-5.37 **[21]**.

Table 2 The results of the 5% BNJ follow-up test on total phenol and IC_{50} of the resulting instant coffee.

Treatment	Total Phenol (mgGAE/g)	IC_{50}(ppm)	Clear zona (mm)
F1 = 87.5% *natural*: Gambir 5%	25.75 ±0.30b	86.02 ±0.17d	0.71 ±0.02a
F2 = 82.5% *natural*: Gambir 10%	30.53 ±0.73abc	44.54 ±0.19b	0.77 ±0.01a
F3 = 77.5% *natural*: Gambir 15%	34.38 ±0.38cd	42.23 ±0.23b	0.81 ±0.02b
F4 = 87.5% *honey*: Gambir 5%	24.91 ±4.30a	121.75 ±7.26e	0.71 ±0.01bc
F5 = 82.5% *honey*: Gambir 10%	27.01 ±3.80ab	65.10 ±0.13c	0.76 ±0.03bc
F6 = 77.5% *honey*: Gambir 15%	40.35 ±1.39d	31.46 ±0.39a	0.83 ±0.01cd
F7 = 87.5% *fullwash*: Gambir 5%	27.16 ±0.77ab	88.25 ±0.12d	0.77 ±0.02d
F8 = 82.5% *fullwash*: Gambir 10%	31.56 ±1.46bc	64.72 ±2.95c	0.83 ±0.02d
F9 = 77.5% *fullwash*: Gambir 15%	40.18 ±1.38d	49.89 ±3.78b	0.93 ±0.02e

Note: Numbers followed by the same letter in the same column are not significantly different.

The total phenol content of this instant gambir coffee ranged from 24.91-40.35 mgGAE/g, with the lowest and highest levels found in formulations F4 (honey 87.5% (w/w): gambir catechin crude extract 5% (w/w) and F6 (honey 77.5% (w/w): gambir catechin crude extract 15% (w/w), respectively were presented in Figure 4.

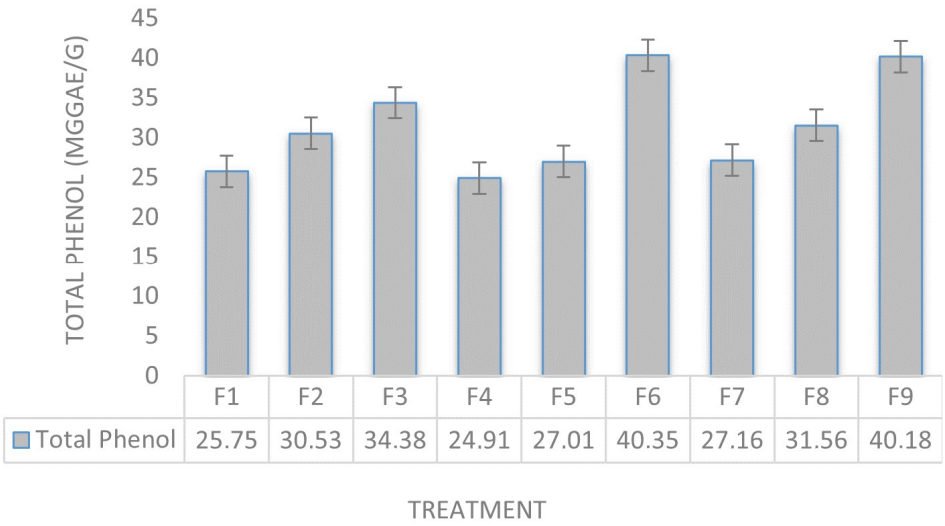

Figure 4 Total phenol of instant gambir coffee.

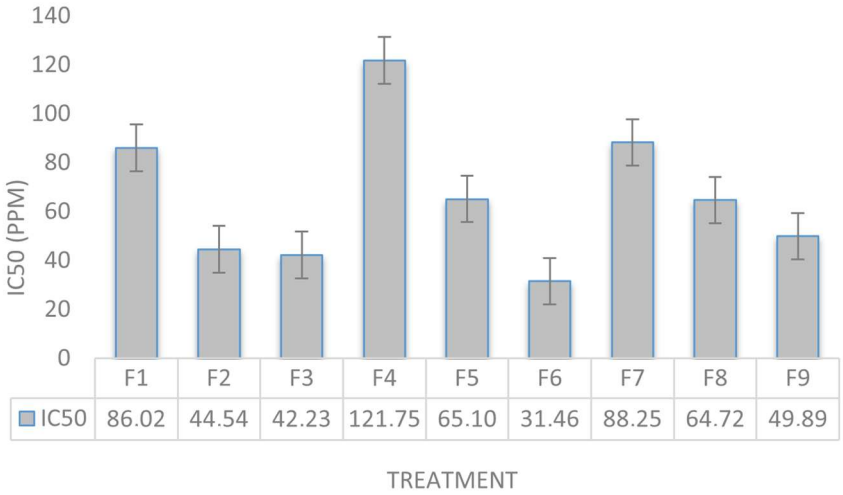

Figure 5 IC_{50} of instant gambir coffee.

The analysis of variance showed that the formulation treatment significantly affected the total phenol content of instant gambir coffee produced. The results of the LSD test at a 5% significance level for the effect of formulation treatment on total phenol content and IC_{50} of instant coffee produced were shown in Table 2. Table 2 showed that instant gambir coffee formulation F6 contained the highest total phenols and was not significantly different from formulations F3 and F9. This can be attributed to the coffee processing methods, which did not affect total phenols, but rather the concentration of coffee and gambir catechin crude extract. As the coffee concentration decreases, the caffeine content also reduces, while an increase in the gambir catechin crude extract, will lead to higher catechin compounds. Caffeine can bind to catechin compounds, however. due to the higher concentration of catechin compounds, the amount of free catechin compounds in instant gambir coffee is greater, causing an increase in total phenol values. Kamsina and Firdausni **[22]** reported that the addition of gambir catechin extract increased total phenols in wet noodles by 84%, while **[23]** added that gambir powder contains catechin compounds of 62.13%. The total phenol values of this instant gambir coffee are higher compared to baked coffee at 16.66 mg/mLGAE **[24]**, and cinnamon coffee of 34.46 mg/mLGAE **[25]**. However, the coffee produced from this research was lower compared to roasted arabica coffee, with a total phenol content of 49.90 mg/mLGAE **[26]**, raw and roasted robusta coffee with a value of 208.89 mg/mLGAE and 119.22 mg/mLGAE, respectively, as well as branded coffee in Indonesia at 46.27 mg/mLGAE **[27]**.

The antioxidant activity of the produced instant gambir coffee can be categorized as strong, as indicated by the IC_{50} value below 50 ppm, which ranged from 31.46-121.75 mg/mL. were presented in Figure 5. The analysis of variance showed that the formulation treatment significantly affected the IC_{50} value of the produced instant gambir coffee. The results of the 5% BNJ test in Table 2 showed that the honey coffee formulation with a concentration of 77.5% and 15% gambir catechin crude extract (F6) had the highest anti-oxidant activity, as indicated by the

lowest IC50 value. This formulation treatment differed significantly from others. The IC50 value was inversely proportional to the total phenol value, therefore, the lower the IC50 value, the higher the total phenol value, and vice versa. The IC_{50} value of the produced instant gambir coffee was similar to that of encapsulated green coffee extract **[28]**, cold-brewed green coffee **[13]**, and dried green coffee using a foam mat **[29]**, namely 87.65 ppm, 71.97-83.21 ppm, and 25.187 ppm, respectively. However, compared to green coffee from Ethiopia **[1]**, robusta coffee **[30]**, and non-instant gambir coffee **[4]**, at 167 ppm, 426-294 ppm, 710-2210 ppm, and 40.10-583.06 ppm, respectively, the IC_{50} value of the instant gambir coffee is lower.

The antibacterial test is carried out by measuring the diameter of the clear zone formed around the disc. The barrier zone formed in instant coffee ranges from 0.71 mm to 0.93 mm. The smallest resistance zone is in the F1 treatment (87.5% natural: Gambir 5%) while the largest resistance zone is in the F9 treatment (77.5% fullwash: Gambir 15%). The results of measuring the antibacterial activity value of functional instant coffee can be seen in Figure 6.

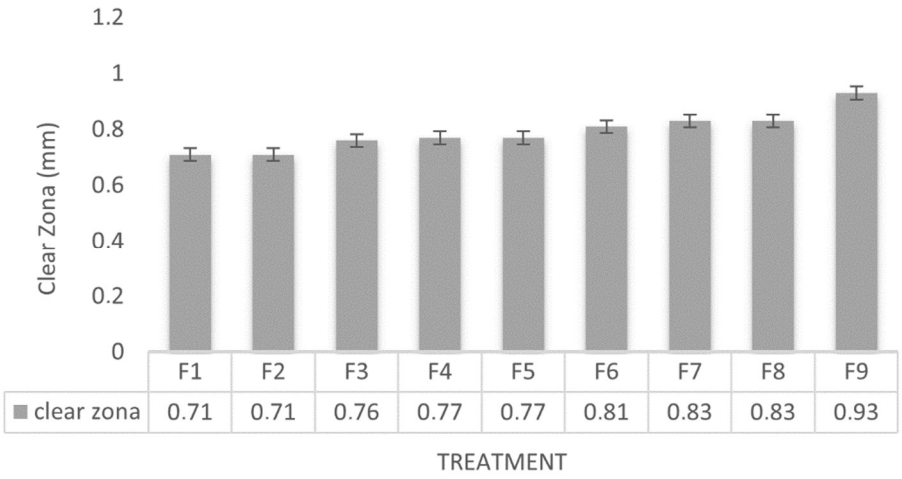

Figure 6 Clear zona of instant gambir coffee.

The results of the analysis of the diversity of antibacterial activity show that instant coffee formulation and catechin extract from gambir have a significant effect on the antibacterial activity of functional instant coffee.The results of the BNJ 5% further test showed that the F1 treatment (87.5% natural: Gambir 5%) was significantly different from the F9 treatment (77.5% fullwash: Gambir 15%). This shows that the addition of gambier catechin extract can increase the antibacterial activity of instant coffee. The flavonoid content in gambier catechin extract is able to inhibit the growth of gram-positive bacteria (*Staphylococcus aureus*). This is because the flavonoids and peptidoglycan layer in gram-positive bacteria are polar so that flavonoids will more easily penetrate the cell wall layer which can cause lysis of bacterial cells **[31]**. The more concentration of gambier catechin added, the antibacterial activity of instant coffee will increase. Robusta coffee also contains compounds that have antibacterial properties, namely caffeine. Caffeine is an alkaloid compound. Alkaloid compounds work by inhibiting cell wall synthesis which can cause lysis. Chlorogenic acid also acts as an antibacterial. The mechanism of this compound is to enter the bacterial cell nucleus and damage the cell wall structure. Apart from that, phenolic compounds in the form of flavonoids in coffee are also able to inhibit bacterial growth **[32]**.

CONCLUSION

The addition of crude gambir catechin extract to coffee processed using all the methods used can increase the antioxidant properties. Reducing the concentration of coffee in each processing method and increasing the concentration of crude gambir catechin extract had a significant effect on increasing the antioxidant properties.The formulation treatment significantly affected the water content, solubility percentage, pH, total phenol, and IC50 of the produced instant gambir coffee. The characteristics of the produced instant gambir coffee included water content, solubility percentage, pH degree, total phenol, and IC50 with values of 6.56-7.02%, 94.35-96.55%, 5.25-5.75, 24.91-40.35 mgGAE/g, and 31.46-121.75 mg/mL, respectively.

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Chicken combs as a raw material in the manufacturing of chopped semi-finished horse meat products

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ABSTRACT

Food safety stands at the forefront of social policies across nations. Secondary meat raw materials present a potential source for meat production. Our research objective was to analyze the nutritional value and functional-technological properties of horsemeat and protein additives of animal origin, particularly secondary meat raw materials such as chicken combs. We also sought to empirically determine their optimal quantities for developing chopped semi-finished horsemeat products, which are protein-enriched and have high consumer appeal. We aimed to establish efficient methods for utilizing collagen-rich materials like chicken combs. Additionally, we assessed the feasibility of using slaughter by-products and the potential of minced meat and pastry products as valuable raw material sources. Based on our findings, we deduce that chicken combs, a lower-value secondary raw material, possess notable physical, chemical, and safety properties. These traits underline its high biological worth and environmental benignity, qualifying it as a viable ingredient for protein enrichment and as an additive in minced meat and paste product manufacturing.

Keywords: qualimetry forecasting, food security, government strategy, combined food product, chicken combs, chopped semi-finished horse sausage

INTRODUCTION

In 2019, K.-Zh. Tokayev, the President of the Republic of Kazakhstan, in his address "Constructive Public Dialogue: The Foundation for Kazakhstan's Stability and Prosperity," outlined five priority development areas for the nation. One such area was the "Developed Agro-Industrial Complex."

While agriculture remains a pivotal resource for Kazakhstan, its potential is yet to be fully realized. The nation holds considerable prospects for producing organic and eco-friendly products demanded both domestically and internationally **[1]**. The strategic plan for sustainable growth in the Agro-Industrial Complex (AIC) emphasizes expanding local food production and ensuring food safety, thus addressing numerous socio-economic challenges facing the Commonwealth of Independent States (CIS), Kazakhstan included **[2]**, **[3]**.

In recent years, the food safety landscape has evolved with heightened competition and surging consumer expectations, making product quality and safety paramount **[4]**. Horse meat, integral to Kazakh culture and cuisine due to its unique dietary attributes, can be enhanced when amalgamated with supplementary ingredients. This combination augments the meat's amino acid profile, its vitamin and mineral content, and the functional and technological characteristics of the final product. Furthermore, a core goal of Kazakhstan's agricultural sector is to bolster local production, focusing on high-quality and safe food raw materials, including meat **[5]**, **[6]**.

Today's competitive AIC market necessitates that processing enterprises craft products meeting rigorous standards of quality and safety, aligning with consumer preferences **[7]**, **[8]**. Current industrial food production trends pivot towards curating functional products that support and enhance health. These are achieved through

regulating and normalizing bodily functions, either holistically or targeted towards specific organs. Secondary products from livestock and poultry processing, rich in proteins, essential amino acids, polyunsaturated fatty acids, and other vital nutrients, play a crucial role in this **[8]**. However, developers and producers of these innovative agricultural products face challenges. They grapple with bottlenecks such as gauging consumer satisfaction with particular product attributes, understanding product quality expectations, and ascertaining the significance of certain quality metrics for both internal and external consumers **[9]**, **[10]**, **[11]**.

An efficacious approach to navigate these challenges is adopting key Total Quality Management principles like "customer focus" and "data-driven decision-making" using qualimetry techniques, inclusive of qualimetry forecasting **[12]**. Although qualimetry-based product quality forecasting is an emergent scientific discipline, its application in the AIC is sporadic. Qualimetry forecasting encompasses methods that anticipate shifts in consumer requirements pertaining to product quality and leverage these insights to enhance future product competitiveness **[8]**, **[10]**.

The directives of the Concept of State Policy in the Domain of Healthy Nutrition have delineated primary objectives for healthy dietary practices **[5]**, **[7]**. However, not all countries can consistently supply the recommended dietary meat intake, leading to meat imports that might not always meet desired safety and quality standards. Harnessing the potential of indigenous livestock breeding can offer a solution **[13]**, **[14]**. That said, innovating and integrating new food technologies might introduce new dietary risks. Therefore, ensuring food safety by identifying and mitigating potential contamination risks becomes imperative for maintaining product quality standards.

Given this backdrop, there arises a pertinent need to undertake focused research aimed at innovating and refining the techniques of employing poultry by-products in meat product manufacturing, minimizing waste, and diversifying and enhancing product quality **[11]**, **[15]**.

Scientific Hypothesis

With escalating prices and a scarcity of prime raw materials for Kazakh production, there's an urgency to maximize the use of livestock and poultry slaughter by-products. Chicken combs represent one such underutilized resource. Incorporating them as protein enhancers in chopped semi-finished products can bolster protein utilization and reduce the end product's cost. However, the feasibility of utilizing chicken combs in Kazakhstan's food production remains under-explored, highlighting the need to investigate their biological attributes. The primary objective of our research was a holistic evaluation of the safety of protein supplements derived from chicken comb processing, intended for the production of semi-finished horse meat products.

MATERIAL AND METHODOLOGY

Samples

The primary subject of our experimental research was chicken combs. Combs from 42-day-old broiler chickens, raised under the care of Ardager LLP in Semey city, EKR, were utilized for the experiments. Experimental evaluations of the nutritional and biological worth of raw meat (encompassing chicken combs and horse meat), protein complexes, and finished products were conducted as per the following sequence:

1. Marketing research
2. Physicochemical and organoleptic evaluations
3. Determination of the mass fraction of total ash
4. Mass fraction of protein, ascertained using the Kjeldahl method
5. Quantification of fat and fatty acid content
6. Measurement of meat's moisture binding capacity (MBC)
7. Analysis of water-holding capacity (WHC)
8. Determination of fat-holding capacity (FHC, %)
9. Evaluation of structural and mechanical properties (SMP)
10. Analysis of macro- and microelement content.

Chemicals

All employed reagents were of U.S.P. grade or higher. Solvents, inclusive of water, bore the LC/MS distinction.

Animals, Plants and Biological Materials

Chicken combs, integrated into chopped meat products, constituted the main focus of our experimental inquiries. The study specifically leveraged combs from 42-day-old broiler chickens, reared at LLP Ardager, Semey city, EKR.

A staggering 18,000 chickens are processed daily at the facility, yielding approximately 108 kg of combs. Individual combs typically weigh between 5-6 grams.

For this study, chicken combs from the Cobb-500 chicken cross (meat variety) were chosen. These chickens were raised under industrial conditions, nourished with industrial dry supplement feed, received disinfection and deworming (with the latter administered on the 14th day), and were last vaccinated on their 20th day of life. The last vitamin supplement was administered on the 30th day. The typical slaughter age, by the 40-42nd day, sees chickens reaching a weight of around 2.5 kg. This period ensures that remnants of disinfectants and antibiotic drugs have been eradicated, and stable immune reactivity is observed 14 days post the last vaccine administration.

For the marketing research segment, 312 Semey residents were consulted: 152 males and 160 females, aged between 18 to 60 years. It's imperative to highlight the deep-seated meat-centric Kazakh culinary traditions, given the region's nomadic history. The Kazakh ethos emphasizes robust hospitality, with a strong inclination towards satiating guests with meat. As such, regional inhabitants exhibit a pronounced preference for meat products, where factors like taste, aroma, color, and presentation significantly influence their choices **[16]**.

Instruments

Our investigative arsenal comprised an array of equipment, including:

- DK6 automated incinerator and distillation device
- Soxhlet extractor
- "Kristal-4000" gas-liquid chromatograph, complemented with a flame ionization detector and the "NetChrom" software suite
- Milk butyrometer
- Heppler consistometer
- "Dropping 105M" capillary electrophoresis system

Capillary zone electrophoresis, one of the contemporary methodologies at our disposal, is geared towards the accurate quantification of water-soluble vitamins. This technique hinges on the migration and separation of ionic forms of analyzed constituents, propelled by an electric field, and subsequently records their electrophoretic mobility at a 200 nm wavelength.

It's pertinent to note the distinct advantages of capillary electrophoresis over methods like fluometry and photometry. Comparable to HPLC, capillary electrophoresis permits the simultaneous assessment of all components under scrutiny. Its benefits, when juxtaposed with chromatographic techniques, include enhanced efficiency, minimized reagent consumption, and the non-necessity of chromatographic columns. Given their excellent solubility in aqueous and aqueous-organic solutions, water-soluble vitamins emerge as optimal candidates for separation and analysis via capillary electrophoresis.

All instruments deployed in this study underwent meticulous calibration and certification, as endorsed by the Kazakh Metrology Center.

Laboratory Methods

The mass fraction of moisture was determined according to GOST 33319-2015 **[17]**. According to GOST 9793-74 **[18]** and GOST R 51479-99 **[19]**, the moisture content was calculated following (1):

$$X1 = (m1 - m2) \cdot 100/(m1 - m) \quad (1)$$

Where:

X1 – moisture content, %; m1 – the mass of the sample with the weighing bottle before drying, g; m2 - the weight of the sample with the weighing bottle after drying, g; m – the mass of the weighing bottle, g.

Determination of the mass fraction of total ash was determined according to GOST 31127-2012 **[20]**.

Mass Fraction of Protein via the Kjeldahl Method: Using the DK6 and UDK129 automated incinerator and distillation apparatus, protein's mass fraction determination was executed in line with GOST 25011-2017 **[21]**. The procedure involves adjusting the DK6 (VELP SCIENTIFICA, Italy) to heat the test samples either at 420 °C for 20 minutes or at 370 °C for 60 minutes. Once heated, the samples are cooled to 50-60 °C, after which 50 ml of ammonium salt-free distilled water is added to each. A receiver, an Erlenmeyer flask, contains 25 ml of a 4% boric acid solution. Within the distillation apparatus, a tube housing a prototype is situated. To the sample, 50 ml of an alkali (35% sodium hydroxide solution) is introduced, and subsequently, 100 ml of distillate is collected. The ensuing step is titration: 10 drops of an indicator are incorporated, followed by titration with a 0.2N HCl solution. For titrating 25 mg of nitrogen present in ammonia, 8.92 ml of a 0.2N HCl solution is requisite (where 1 ml of 0.2N HCl corresponds to 2.803 mg of nitrogen).

Determination of Fat and Fatty Acid Content: This determination abided by the Soxhlet method and conformed to GOST R 55483-2013 **[22]**. For discerning the fatty acid composition, lipids were segregated from the experimental samples via hexane extraction using a Soxhlet apparatus. The resultant extract was then evaporated to dryness in a round-bottom flask, facilitated by a rotary evaporator at temperatures ranging between

30-40°C. To the dry extract, 10 ml of hexane, 400 µl of 0.5M sodium ethylate in ethanol, and 50 µl of acetic acid were added. After a vigorous 2-minute stir, the reaction mixture was allowed to settle for 5 minutes. Post-settling, it was filtered through a paper filter, rendering the solution ready for analysis. Upon procuring the ethyl esters, the fatty acid composition was ascertained through gas chromatography, utilizing the "Kristal-4000" gas-liquid chromatograph, supplemented with a flame ionization detector and the "NetChrom" software suite **[22]**, **[23]**.

Chromatography Conditions:

- Injector temperature: 188 °C
- Detector temperature: 230 °C
- Furnace temperature: 188 °C
- Analysis duration: 2 hours
- Column content: polyethylene glycol adipate (20%) on celite 545

Other analyses involved determining the:

- Mass fraction of minerals
- Mass fraction of sodium chloride
- Organoleptic evaluation of the end products, gauged on a five-point quality scale for meat products

Functional and Technological Properties of Minced Meat: The pH value was determined using the potentiometric method, adhering to ST RK ISO 2917-2009 **[24]**. In the Moisture Binding Capacity (MBC) study, designated meat samples were placed on an ashless filter situated atop a glass plate, ensuring the samples were centered. This setup was subsequently covered with a plate of identical dimensions, upon which a 1 kg weight was placed and retained for 10 minutes. Post this duration, the filter, now devoid of the weight and bottom plate, had the periphery of the moisture spot surrounding the compressed minced meat delineated with a pencil. Upon drying in air, the outer contour of the spot was discerned. The areas of the spots engendered by both the pressed meat and absorbed moisture were gauged using a planimeter. The wet spot's magnitude was computed from the difference between the aggregate area of the spot and the area crafted by the minced meat. To give a quantitative perspective, 1 cm^2 of the wet spot's area on the filter equates to 8.4 mg of water.
The formulas calculate the content of bound moisture (2) and (3):

$$x1 = (A\text{-}8.4\ B) \cdot 100/\ m0 \quad (2)$$

$$x2 = (A\text{-}8.4\ B) \cdot 100\ /\ A \quad (3)$$

Where:
x1 – the content of bound moisture, % to minced meat; A – the total moisture content in the sample, mg; B – wet spot area, cm2; m0 – weight of the sample, mg; x2 – content of bound moisture, % to total moisture.

Determination of WHC (%), a sample of 5.00 ±0.01 g was evenly applied with a glass rod on the inner surface of a wide part of the milk butyrometer. The butyrometer was tightly closed with a stopper and placed in a water bath at the boiling point with the narrow part down for 15 min. The mass of released moisture was determined by calculation of the number of divisions on the butyrometer scale (4):

$$WHC = V\text{-}VVS,$$
$$VVS = a\text{-}p\text{-}t\text{-}'\text{-}W, \quad (4)$$

Where:
B – the total mass fraction of moisture in the sample, %; a – division price of the butyrometer; a = 0,01 cm; n – number of divisions; t – mass of the sample, g.

FHC (%) determination was determined by the method of sequential determination of the main functional properties of minced meat from one sample, developed by the employees of VNIIMP Salavatulin et al. **[25]**.
SMP properties was checked according the determination of the ultimate shear stress of minced meat **[26]** by the Heppler consistometer. The container for the product is filled with the test sample, the surface is leveled with a spatula or knife, setting its level relative to the zero division of the instrument scale. The scale determines the cone's immersion depth in the product (in mm), setting and selecting a certain weight.
The ultimate shear stress is determined by the formula (5):

Θ_i=K M/h^2, (5)

Where:
– the ultimate shear stress, Pa; K – cone constant, for a = 60°, K = 2.1 m/kg; m – cone mass with the bar and additional weight, kg; h – immersion depth of the cone, m.

Mass fraction of amino acids on the system of capillary electrophoresis "Dropping 105M" **[27]**.The content of macro- and microelements was determined according to GOST R 55484-2013 by atomic absorption methods similar to the determination of heavy metals **[22]**, **[28]**, **[29]**, **[30]**, **[31]**, **[32]**. Vitamin content in raw materials and food products by capillary zone electrophoresis was checked according to GOST R 55482-2013 **[33]**, **[34]**. Standard solutions of the following vitamins were used as a control sample: thiamine chloride; riboflavin; nicotinamide; and ascorbic acid. The technique is designed to measure the mass fraction of free forms of vitamins in prefixes, vitamin supplements, concentrates and mixtures, in this regard, work was carried out to select the mass of the weighed portions of the analyzed sample:

- conditions of analysis;
- buffer: for the determination of vitamins in the CZE variant (sodium tetraborate + boric acid);
- capillary: Leff/Lobsch = 65/75 cm, ID = 50 mkm;
- voltage: +25 kV;
- temperature: +30 °C;
- pressure: 0 mbar, 50 mbar;
- detection:
- stage 1. Time 899 sec, e.g. 25 kV, press.0

Microbiological Studies: Microbiological examinations adhered to ST RK GOST R 51448-2010 **[35]**, titled "Meat and Meat Products: Sample Preparation Methods for Microbiological Research." In the exploration of the microflora in long-term storage products, time-tested microbiological techniques were employed, encompassing sampling methods, sample preparation for microbiological analyses, and microorganism cultivation methods.

The studies pursued the determination of various microbial quantities: mesophilic aerobic and facultative anaerobic microorganisms following GOST 10444.15-94; E. coli bacteria group (coliforms) per GOST 31747-2012; and mold, CFU/g as per GOST 10444.12-2013 **[29]**. Other focus areas included sulfite-reducing clostridia and S. aureus **[36]**, **[37]**.

Determination of Toxic Elements Content: This assessment was carried out following GOST 26929-94 **[38]** using Atomic Absorption Spectroscopy (AAS) on the "KVANT-Z.ETA-T" spectrometer complemented by its associated software. AAS, as a method, hinges on measuring the selective absorption of optical radiation of a designated wavelength by the element's neutral atoms in question. It stands as one of the most precise and efficacious physicochemical analysis techniques.

Modern devices facilitate control and data processing via a personal computer, employing the "QUANT" software. The AAS's analytical signal is the optical density of the atomic vapor, ascertained on one of the resonance lines of the targeted element, which correlates with the element's concentration. This relationship is described by the Bouguer-Lambert-Beer law (6).

$$A_\lambda = K_\lambda \cdot C \cdot L \; A_\lambda = K_\lambda \cdot C \cdot L \quad (6)$$

Where:
K_λ is the absorption index at the wavelength of the analytical line; is the thickness of the absorbing layer in the atomizer.

The methodology encompasses measuring the absorption (optical density) of the atomic vapor of the element under examination, achieved via electrothermal atomization in the spectrometer's graphite furnace. These optical density measurements occur at the element's resonant spectral line, emitted by the corresponding hollow cathode lamp.

Preparation and implementation of atomic absorption measurements of heavy metals were carried out according to GOST 30178-96 **[39]**:

- determination of the content of toxic elements by AAS on a spectrometer with electrical atomization "KVANT-Z.ETA-T" with software;

- the content of pesticides by thin layer chromatography;
- the content of pesticides in raw materials (chicken combs, horse meat) and in finished products by gas-liquid chromatography using an analytical stationary gas chromatograph "Crystallux-4000M" with an electron capture detector and software "NetChrom";

The content of radionuclides by thin-layer chromatography accoding to GOST R 56931-2016 **[40]**.

Description of the Experiment

Sample preparation: Samples for the physicochemical and organoleptic testing were prepared as a weighed sample of twice crushed product weighing 2-3 g, taken with an accuracy of 0.001 g was dried in a metal bottle with a glass rod in a drying oven at a temperature of 105 °C during an hour.

For the detection of the mass fraction of protein (according to the Kieldal method) 2g of the product was sieved on a 2 mm sieve and it was dried at 105 °C until constant weight. The sample has been weighed and transferred quantitatively to a combustion tube.

For each sample, add to the test tube reagents for wet combustion: 7g of potassium sulfate (K_2SO_4), 5 mg of selenium (Se) in powder form (or two tablets of K_2SO_4 with selenium – 3.5 g), 7 ml of concentrated sulfuric acid (98%), 5 ml of hydrogen peroxide (H_2O_2) with a concentration of 35%.

For MBC study, samples of minced meat (0.3 g) were weighed on a polyethylene mug with a diameter of 15-20 mm.

For the vitamins determination, samples were prepared according using the extraction of vitamins with an aqueous solution of sodium tetraborate in the presence of sulfite ion. The extract was centrifuged (5000-6000 rpm for 5 min.) and, if necessary, filtered through a membrane filter.

Number of samples analyzed: We had 4 experimental samples and a control one in our study. A marketing study has been conducted in a form of survey in 312 residents.

Number of repeated analyses: All instrumental measurements were performed in five times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was two times.

Design of the experiment: Our first step involved conducting marketing research, gathering sociological insights using internet technologies. Aimed at discerning the potential demand and benefits of a novel product, we devised questions pertinent to the population's inclination towards semi-finished products. From November 2018 to May 2019, a questionnaire survey engaged 312 residents of Samey city, encompassing 152 males and 160 females spanning diverse age groups and professions. Post this phase, we evaluated the quality and safety properties of minced meat that incorporates chicken comb elements.

Organoleptic evaluations of the end product were conducted by tasting commissions using a five-point scale. This assessment verified the product's alignment with primary quality indicators, such as appearance, sectional view, aroma, taste, and texture, relative to standard requirements **[41]**.

Safety assessments encompassed examinations of microbiological indicators and determinations of toxic and radioactive element content.

Statistical Analysis

Data analysis was executed using Microsoft Excel and Statistica 15. All experiments were replicated thrice, with reported outcomes representing the average of these determinations, accompanied by standard deviations. The Student's t-test facilitated the statistical evaluation of the derived data. All data is articulated as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

Food Food plays a pivotal role in influencing human health **[42]**. Presently, humankind grapples with a scarcity of food resources. Nevertheless, through the deployment of avant-garde technologies and high-yield breed crossings, we possess the means to tackle this challenge **[43]**. An avenue worth exploring is the utilization of secondary raw materials from the animal breeding industry for food production **[44]**. A method to extract protein from less-valued raw materials is through its hydrolysis. This process enables the extraction of isolated collagen proteins of exceptional purity, suitable for the fabrication of sausages and minced semi-finished products **[45]**. These proteins are renowned for their impressive solubility and fat-retention properties and find frequent application in the crafting of pâtés and assorted sausages. Given that hydrolysates are rich in collagen degradation products, they enhance pâtés with dietary fibers and augment the bound moisture content in minced meat concoctions **[46]**, **[47]**. Motivated by these attributes, our research delved into assessing poultry's secondary raw material (chicken combs) – a treasure trove of proteins. We approached this through the lens of modern combinatorics, nutrition science, and food engineering. This strategy empowers us to refine food technology and conjure novel food compositions with superior nutritional and biological worth **[48]**.

Application of Qualimetry Prediction in Quality Assessment

As we venture into the realm of new meat product development, it becomes imperative to align with consumer anticipations and adhere to sanctioned quality and safety benchmarks of food ingredients and end products. Miles and Frewer **[49]** have recently articulated apprehensions regarding the diminishing caliber of meat products, particularly in their organoleptic facets. Put succinctly, many products on the market exhibit indistinguishable taste and aroma profiles. In this backdrop, echoing the voice of consumers during product development emerges as a priority. The doctrine of qualimetry prediction is instrumental here. Qualimetry forecasting of product quality indices rooted in consumer preferences is an emergent paradigm **[50]**, **[29]**. Encompassed within are the product quality attribute tree, holistic qualimetric evaluation, qualimetric scaling, expert qualimetry, and prognostic qualimetry **[51]**. The qualimetry blueprint for product quality prognostication aids in delineating the array of product quality and safety markers aligned with consumer expectations. It ensures the harmonization of organoleptic attributes of nascent products with consumer tastes right from the conception phase **[29]**. Essentially, qualimetry prediction equips us to steer product and service quality, meeting imminent demands and ensuring robust market competitiveness **[52]**. By leveraging the qualimetry modus operandi, we have enhanced the efficiency and adaptability of our quality management system, both for pioneering and established products **[51]**.

To gauge consumer predilections for minced semi-finished items, we orchestrated a questionnaire survey from November 2018 to May 2019, engaging 312 residents of Samey, consisting of 152 male and 160 female participants spanning varied age brackets and professions.

The qualitative benchmarks for these semi-finished products were ascertained based on organoleptic factors such as appearance, aroma, hue, form, and sectional view. Our selection algorithm utilized information detailing the interrelations among pairs of objects, emphasizing the existence of stringent preference dynamics between them. For this purpose, we introduced a relational variable.

This novel approach grants us the capability to embed performance metrics into the holistic assessment of our target products. The relational matrix, illustrating the alternative solutions based on the organoleptic attributes of minced horsemeat products containing BO, is presented herewith (7):

$$a_{ij} = \begin{cases} 1, if\ i\ variant\ equivalent\ to\ j \\ 3, if\ i\ variant\ \ exceeds\ moderately\ to\ j \\ 5, if\ i\ variant\ \ greatly\ exceeds\ to\ j \end{cases} \quad (7)$$

A square matrix ||a|| of the relationships in the decision alternatives (8) was constructed from the obtained data:

$$a_{ji} = \frac{1}{a_{ij}}, a_{ii} = 1, i, j = \overline{1,n} \quad (8)$$

Using the ||a|| matrix, the priority vector was calculated (9). We calculated the sum of the columns.

$$Xj = \sum_{i=1}^{n} a(ij),\ j = 1,...,n \quad (9)$$

of the matrix ||a|| in the form of a vector-string {2.283; 7.500; 9.000; 6.600; 8.866} and divided each column element by this sum. As a result, a new matrix of ||a *|| values were obtained, which allows to estimate the importance of each individual indicator in the overall assessment of product perception (9).

View – 1, Smell – 4, Taste – 2, Consistency – 3, View on the cut – 5.

Finding the average value of each i-j string allowed us to obtain a vector-column of priorities {0.442; 0.151; 0.109; 0.150; 0.147}.

Within the distribution of priorities between the indicators of organoleptic evaluation appearance – 44.2%, then smell follows – 15.1%, then the view on the cut – 14.7%, consistency – 15.0%, taste – 10.9% is characterized by the greatest weight coefficient.

In the formulation of chopped semi-finished product technologies, it's vital to integrate consumers' perspectives. These insights underpin the construction of a qualimetry model, ensuring product quality with optimal organoleptic attributes. The merit of mathematical methodologies in processing expert evaluations of food product quality is that they yield objective outcomes. This assertion finds resonance in our findings and is further corroborated by Bezerra et al. **[53]**, who presented evidence that chicken combs are a promising source of animal protein. Furthermore, aspartic and glutamic acids, stemming from chicken comb hydrolyzation, possess chelate properties with antioxidative effects, thus influencing the flavor and texture of the end product. Studies by Zinina et al. **[6]** and Srisantisaeng et al. **[54]** align with our data, attesting that incorporating chicken combs

into meat products augments the quality attributes, given the pronounced proteolytic characteristics of chicken comb hydrolysates.

Identification of Critical Control Points in Chopped Semi-finished Product Technology

From the vantage point of the content and utilization of biologically active components in meat substrates, the processing of secondary poultry products – namely chicken legs and combs – proves intriguing **[48]**, **[55]**, **[56]**. A paramount directive in pioneering a new meat product is the assurance of utmost wholesomeness and unwavering safety standards. The product's safety hinges on the presence or absence of pathogenic and non-pathogenic microbes, toxins they might produce, chemical contaminants such as heavy metal salts, disinfectants, pesticides, antibiotics, hormones, antiparasitic agents, and radionuclides, as well as mechanical pollutants like metal fragments, bone shards, or glass **[37]**, **[38]**, **[39]**, **[40]**.

Quality, in the context of a product, is an ensemble of attributes determining its aptness to fulfill specific needs. Chiefly, meat and its derivatives' quality is gauged by: constituents utilized by our physiology for biological synthesis and energy requisites; organoleptic facets like appearance, hue, texture, and aroma; and the absence of noxious substances and harmful microbes. Equally pivotal is the stability of these properties and the retention of their quality markers throughout storage and transit **[4]**, **[50]**, **[57]**, **[58]**.

The quality metrics of meat and its products are influenced by the nature and properties of raw materials, applied recipes, technological processing conditions, and storage dynamics. A holistic and objective assessment of these interdependencies forms the bedrock for discerning factors that sway product quality and safety. Ensuring superior quality necessitates meticulous raw material selection, rigorous adherence to production, storage protocols, and sanitation standards.

All produced items undergo scrutiny to ascertain alignment with quality standards, be it the TC regulations of national and transnational standards or technical specifications (TS) for emerging semi-finished products **[4]**, **[59]**. Figure 1 elucidates a block diagram delineating the production pathway for semi-finished meat items, pinpointing the critical control junctures for the fabrication of such products.

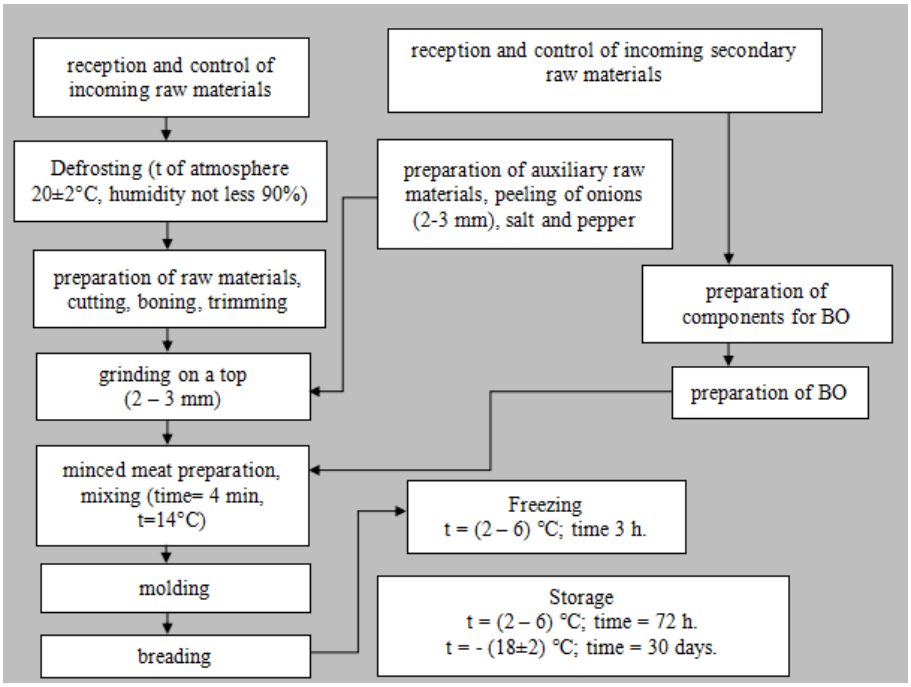

Figure 1 Block diagram of meat semi-finished product production.

The deployment of detection algorithms emerges as a sound approach to manage and effectively oversee facets including the acceptance and preliminary scrutiny of meat and supplementary raw materials, freezing protocols, and periodic evaluations, all integral to preserving quality and safety.

Our challenge was centered around engineering a technology for chopped semi-finished products via protein augmentation. This entailed validating the adoption of secondary food resources (namely, chicken combs) for protein enhancement, which would offer superior food and biological value metrics, and orchestrating a strategy for managing pivotal control junctures throughout the fabrication of a novel meat item **[60]**. The realm of semi-finished meat product manufacturing constitutes a substantial, niche industry, poised for promising expansion given the contemporary societal dynamics and burgeoning consumption patterns **[61]**. The refinement of emerging

methodologies amplifies the operational efficacy of meat enterprises. Bazhenova **[5]** underscores that the integration of hydrolysates can bolster the product's digestibility by 2.0 to 2.5-fold, compared to solely harnessing the foundational raw material. Insights from Smith **[9]**, as well as from Zhumanova and Rebezov **[62]**, have unearthed promising results from their endeavors to produce hydrolysates derived from the heads and limbs of terrestrial birds. Accelerating the momentum of scientific exploration in the domain of multifaceted meat products is buoyed by the following elements: a deficit in local meat resources, a significant influx of sub-par imported meat, and its ever-escalating price point.

The surging consumer appetite for processed meat commodities spurs producers to amplify production capacities and diversify their product portfolios. A myriad of nutritional studies have accentuated the pressing imperative to birth functional foods, replete with vital micronutrients. Presently, meticulously crafted recipes and methodologies have been established, permitting the confluence of meat semi-products under varied thermal regimes, leveraging both animal and vegetal raw materials. The fusion of both animal and plant-derived proteins in semi-finished products not only diversifies the product spectrum but also champions the judicious exploitation of raw material assets, satiating the populace with quality nourishments, optimizing both nutrition and practicality. The trajectory of meat and its derivatives production and consumption is on an upward curve. Projections posit that the meat market will burgeon at an annual clip of 10% over the impending triennial period. The chilled meat product segment is witnessing the most brisk expansion, roping in not just meat processors but also retail entities. This momentum owes to the irreplaceable nutritional essence of meat. However, one must remain cognizant that meat, apart from potentially being substandard, can also manifest as a vector for foodborne illnesses. For instance, muscles from debilitated or fatigued animals exhibit depleted glycogen levels and diminished enzymatic activity, culminating in compromised meat quality. Additionally, a surge in muscle acid content and heightened levels of protein hydrolysis products foster environments conducive for pathogenic microflora proliferation, truncating shelf-life. Muscle tissue from critically unwell animals is notably more taxing to digest and less palatable for human consumption compared to that from hale specimens. It is also acknowledged that refrigerated semi-finished items possess a rather constrained shelf-life, and any dereliction in adherence to storage protocols adversely affects product quality. It's imperative to note that semi-finished meat items are designed for subsequent culinary applications.

Research on the Chemical Composition and Safety Metrics of Chicken Combs as a Protein Augmentation Raw Material

Analyzing from the perspective of biologically active constituent content in raw meat, secondary poultry products emerge as a domain of intrigue **[55]**. Their chemical makeup boasts a rich ensemble of proteins, polyunsaturated fats, enzymes, vitamins, carbohydrates, and mineral salts, making them apt for not just conventional meat products but also specialized items **[48]**.

The outcomes of our investigative pursuits suggest that chicken combs, a relatively untapped meat industry by-product for culinary applications, hold potential **[63]**, **[64]**. These combs are repositories of connective tissue proteins, notably collagen, which exhibits commendable biological and functional attributes such as superior water-retention and texture-forming capacities. Moreover, their mineral content renders them versatile across various culinary paradigms.

To validate the feasibility of employing chicken combs in the formulation of horse-meat culinary items, a comprehensive gamut of evaluations was undertaken: safety metrics of raw materials were ascertained, overarching chemical compositions were delineated, and mineral content was gauged (Table 1).

The raw material (chicken combs) was tested for radiological safety. The total specific activity of β- emitting nuclides and the content of cesium-137 (Cs-137) isotopes were determined. Cs-137 content has been calculated by the following formula (10):

$$\mathrm{A}_{\mathrm{Cs-137}} = \frac{n_0}{\text{Х.В.}} \frac{k_{\text{св}} k_{\text{оз}}}{pm} 1000 \tag{10}$$

Where:

n is the preparation count without background, imp./min; k_{CB} is the coefficient of communication set by the standard reference; k_{o3} is the ashing coefficient of the sample; m is an ash sample taken for analysis, g; *C.E.* is a chemical yield of the carrier; P is a correction for self-absorption in the sample.

Table 1 Content of mineral elements in chicken combs.

Mineral substances	Content, mg/kg
Macro elements	
Magnesium	46.8
Potassium	393.7
Calcium	102.3
Sodium	2500.9
Microelements	
Iron	46.3
Copper	0.39
Zinc	1.90
Chrome	5.60
Manganese	0.19
Nickel	<0.1
Cobalt	<0.1
Macro elements	
Magnesium	46.8
Potassium	393.7
Calcium	102.3
Sodium	2500.9
Microelements	
Iron	46.3
Copper	0.39
Zinc	1.90
Chrome	5.60
Manganese	0.19
Nickel	<0.1
Cobalt	<0.1

Note: Based on the test report.

Table 2 demonstrates the results of the experimental checking combs. We have found that the total specific activity of β-emitting radionuclides in chicken combs is within permissible norms (Table 2) which allows using the raw material in the production of protein enrichment.

Table 2 Content of toxic elements in chicken combs.

Name of indicators, units of measurement	Normative documents	Norms according the normative document	Actually received
Toxic elements, mg/kg (not more)			
Lead	GOST 30178-98	0.5	0.077
Arsenic	GOST 31266-2004	0.1	0.022
Cadmium	GOST 30178-96	0.05	not detected
Mercury	MUK 4.1.1472-03	0.03	not detected
Antibiotics, mg/kg,			
Levomycetin	STRK ISO 13493-07	not allowed	not detected
Tetracycline group	STRK 1505-2006	not allowed	not detected
Pesticides mg/kg, (not more)			
Hexachlorocyclohexane, (α, β,γ-isomer)	MI 2142-80	0.1	not detected
DDT and its metabolites	MI 2142-80	0.1	not detected
Radionuclides, Bq/kg (not more)			
Cesium-137	GOST 32161-2013	200	7.9
Strontium-90	GOST 32163-2013	-	6.7

Table 2 Cont.

Name of indicators, units of measurement	Normative documents	Norms according the normative document	Actually received
Toxic compounds, mg/kg (not more)			
Lead	GOST 30178-98	0.5	0.077
Arsenic	GOST 31266-2004	0.1	0.022
Cadmium	GOST 30178-96	0.05	not detected
Mercury	MUK 4.1.1472-03	0.03	not detected
Antibiotics, mg/kg (not more)			
Levomycetin	STRK ISO 13493-07	not allowed	not detected
Tetracycline group	STRK 1505-2006	not allowed	not detected
Pesticides mg/kg (not more)			
Hexachlorocyclohexane (α, β,γ -	MI 2142-80	0.1	not detected
isomer)	MI 2142-80	0.1	not detected
DDT and its metabolites			
Radionuclides, Bq/kg (not more)			
Cesium-137	GOST 32161-2013	200	7.9
Strontium-90	GOST 32163-2013	-	6.7

Note: Based on the test report.

Microbial purity is an essential safety indicator and critical checkpoint in food manufacturing. So, raw materials were studied by the microbiological analysis for the content of QMAFAnM, CFU/g, and ECGB, and fungal contamination. The checking has been conducted for the 0.0001 g of product in CFU/g (Table 3).

Table 3 Microbiological parameters of chicken combs.

Name of indicators, units of measurement	Norm according to the ND	Actual results
Microbiological indicators:		
- QMAFAnM, CFU/ g, not more	5×10^6	1×10^6
- ECGB in 0,0001 g of a product	not allowed	not detected
- mold, CFU/ g, not more	500	3

Note: Based on the test report.

In analyzing of the presented in the Table 3 data, it can concluded that the raw materials under investigation comply with the requirements of TR/CU 034/2013 regarding microbiological safety indicators. At the next step, comb‘s physical and chemical parameters were tested (Table 4).

Table 4 Chicken combs physical and chemical indicators studied in the experiment.

Indicators	Content
Protein, %	10.20
Moisture, %	87.65
Fat,%	1.25
Ash, %	0.90
pH	6.4
Water-holding capacity, % to total moisture	70.28

Note: Based on the test report.

From the data presented in Table 4, it is evident that the combs retain a substantial moisture quotient, with over 70% existing in a bound state, and exhibit a nearly neutral pH of 6.4. Further, an examination of the mineral constituents of the raw material under our research purview (as depicted in Table 1) revealed an appreciable concentration of both Cr and Zn. Chromium plays a pivotal role in modulating carbohydrate metabolism and blood glucose levels. A dearth of Cr in the organism might manifest as heightened fatigue and pronounced anxiety **[58]**. Zinc's biological significance is underscored by its salutary impact on the endocrine, immune, and nervous systems. Intriguingly, an adequate Zn content in products can mitigate the levels of toxic elements and heavy metals by as much as 30% **[64]**.

Thus, having meticulously appraised the raw materials in terms of their physicochemical attributes and safety benchmarks, it can be posited that seemingly undervalued secondary raw materials, like chicken combs – distinguished by their elevated biological merit and ecological benignity – can be seamlessly integrated into the realm of protein augmentation, serving as additives in the fabrication of minced meat and paste-based offerings.

CONCLUSION

An exhaustive perusal of existing literature underscores the latent potential of collagen-rich resources in both the meat and pharmaceutical sectors. In the quest to evolve the methodologies of chopped semi-finished product production, it becomes paramount to resonate with consumer sentiments. These insights then become the bedrock upon which a qualimetry model is architected, striving for optimal consumer-centric organoleptic attributes. Such a qualimetry paradigm for a nascent product is holistic; it's not merely an exercise in crafting quality and safety benchmarks, but rather a composite ensemble of quantitative stratagems aimed at gauging quality. This ensures alignment with anticipated consumer aspirations, alongside formulating recommendations to perpetuate this envisioned quality. Resorting to mathematical nuances while interpreting expert critiques on the consumer quality of edibles yields an unbiased, definitive outcome. The hierarchy of priorities, in terms of organoleptic appraisal, unfolds as follows: appearance assumes paramount significance at 44.2%, succeeded by aroma at 15.1%, visual appeal upon slicing at 14.7%, texture at 15.0%, and flavor at 10.9%. Concomitant with our investigative revelations, combs were found to harbor an abundant moisture content, with the majority (over 70%) being bound. The pH profile of this entity hovered close to neutrality, registering a value of 6.4. A detailed mineralogical exploration spotlighted the pronounced presence of both Cr and Zn. It is imperative to accentuate that despite their perceived modest value, chicken combs – when gauged on physicochemical and safety metrics — emerged as repositories of profound biological value and environmental congruence. Consequently, they stand poised to be harnessed in protein enhancement ventures, especially as fortifying agents in minced meat and pasty concoctions.

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The impact of the application of the economic value-added method in the food company

Radoslav Bajus

ABSTRACT

One of the most popular methods to measure the performance and success of the company has become the economic value added. EVA supports strategic planning, and management can measure and evaluate performance at the division level. Through it, you can find results that are important not only for the company's management and the owners but also for the company's shareholders. For businesses in the market, it is essential to measure and evaluate the performance of the business, as they are exposed to the risk of competition and, of course, pressure from the environment. However, many companies are underestimating this measurement due to time-consuming and evaluating business performance based on financial statements. Therefore, they can only assess traditional indicators, often insufficient to determine the company's performance. The paper aims to inform readers about all necessary information related to the EVA method and draw the calculation on a specific example. We have determined the application of the method to a particular company as our primary goal. Therefore, we can get information on the performance of the selected company. The applied values and data are further applied and interpreted, which results in how the company manages its assets and how it can continue to improve its performance.

Keywords: company performance, financial analysis, economic value added, weighted average cost of capital, EVA

INTRODUCTION

At the same time, with globalization and the development of capital markets, there is an increasing intensification of investment capital, which results in the sale of businesses and joint ventures. The negative consequence is the emerging problem related to the company's market valuation **[1]**. In the business world, the market environment is rapidly evolving and changing, which is often the result of changes in corporate governance **[2]**. Therefore, companies must continuously assess the results of their business activities and observe trends recorded through indicators. Business valuation methods can be divided into three primary groups: the company's yield methods, market methods, and asset valuation **[3]**. Over time, modern financial analysis methods have been launched to consider many financial aspects affecting business performance. One of the most popular methods to measure the performance and success of the company has become the economic value added **[4]**.

The EVA supports strategic planning, and management can measure and evaluate performance at the division level. Through it, you can find important results for the company's management, owners, and shareholders **[1]**.

For businesses in the market, it is very important to measure and evaluate their performance, as they are exposed to the risk of competition and, of course, pressure from the environment **[5]**.

However, many companies are underestimating this measurement due to time-consuming and evaluating business performance based on financial statements. Therefore, they can only assess traditional indicators that are often insufficient to assess the company's performance.

Scientific Hypothesis

The scientific goal of the paper is to find out which factors significantly impact the EVA performance indicator and which are insignificant based on the study of literary sources, own research, and the information declared in the financial statements of the investigated company. The scientific hypothesis is that the most significant impact on the EVA performance indicator in a given company is a change in short-term financial assets, long-term financial assets, and change in receivables. An insignificant impact of the average cost factor is expected.

MATERIAL AND METHODOLOGY

In this thesis, on the topic of the application of the EVA method in the company, we are focusing on the EVA method, which is one of the yield methods. We will select all published theoretical facts about the EVA method to highlight the major approaches to the topic. We will analyse chosen most essential facts out of many that can best approach the subject.

One of the company's main activities is to buy and sell dairy products and provide accompanying activities. Initially, the company traded solely on foreign markets, initially in Hungary and later expanded to the Czech Republic. It expanded to Slovakia in 2006. The company does not manufacture dairy products, it only provides trading. Their main vendors are from Poland and since 2015 also from Slovakia. Certificates IFS and BRC ensure high quality and safety demands on products of Polish manufacturers. Manufacturers established their private brands under which products are offered. The company aims to gain recognition on the domestic market based on high quality of its products, which would increase the customer base. The company's long-term strategy is to establish its trademark. It is intensely focused on investing in strengthening the brand in the domestic market. The company also tries to expand its network of retailers. Mentioned activities and efforts to promote its dairy products lead to increased revenues of the mentioned brand, which may, in turn, lead to positive economic results and an expansion of the company as a whole. The analyzed company will be Limited Liability Company, which will provide us with necessary information.

The analyzed company achieved a relatively high net turnover over the last 4 years, permanently increasing in individual years since 2019. In 2019, the company achieved a net turnover of € 109537693; in 2020, it was € 110192056; in 2021 € 117190996; and in 2022, it was € 118684961.

When calculating the company's performance, we will use the base of the financial statements for the last 5 years, i.e., 2018-2022. It is the period in which pre-Covid, Covid and post-Covid years alternate. We were interested in the development and results achieved in individual monitored indicators in the monitored period, alternating between years without external influences and years marked by COVID-19 and post-COVID-19 years.

Collecting necessary data will be the first step to start the practical part. In the practical part, we will use the comparison; we will compare the collected data and information over the last five years. We will also apply the analysis, using only selected data necessary for calculations by reducing a large data set.

Based on the results we will use the induction. We will use induction because we will reach a general conclusion from the calculated results of the indicators, which will be considered as our starting assumptions, and we will be able to evaluate the performance. We will also use the prediction method to predict future business performance.

The main purpose of the thesis is to apply the method to the company, by which we obtain the performance data of the selected company. The applied values and data are further applied and interpreted, which leads to the conclusion of how the company manages its assets and how it can continue to improve its performance.

The status of the investigated topic

In addition to financial analysis, we use several other methods to determine the company's financial situation. These multidimensional models work with several criteria and are also assigned a specific weight. Subsequent acquisition of an aggregate statement with one number identifies the state of the company that evaluates the degree of financial health of the business. These aggregate indices are aimed at determining the performance of a business in terms of value creation, which serves the company's owners and investors. These are credit indicators. On the other hand, we can also evaluate the company's ability to repay its commitments based on these indicators. This is a prediction that the company will not be approaching bankruptcy shortly. In this case, they are bankruptcy indicators **[5]**. Performance is commonly used. It is used in several fields. The performance characterizes the course or manner in which the investigated or observed object realizes a certain activity based on the similarity with the reference approach of the course of the given activity. Explaining this characteristic requires comparing the reference and the investigated phenomenon from the aspect of the established criterion scale **[6]**. Great importance is currently attached to determining the company's value; therefore, if the company's management needs to know the company's value or a certain part, it must request an expert opinion, which will be prepared exclusively by the expert organization. An expert organization or institute uses one method to determine a

company's general value when making an expert opinion. There are the property method, yield (business) method, combined method, liquidation method, comparative method, and others **[7]**.

The thesis focuses on newer, more modern methods, applying market characteristics and the EVA (Economic Value Added) indicator. Internal financial and external factors were applied when determining the analyzed company's performance. Internal factors were primarily focused on the company's ability to repay obligations, the efficiency of asset utilization, the optimization of the capital structure and its impact on the company's stability, the company's activity and the ability to manage its resources, and the ability to maintain an optimal turnover cycle of funds.

EVA method (economic value added)

The company's value and its increase over a period are determined by changes in expectations regarding the growth of the company's cash flows and changes in the company's risks that lead to changes in the discount rate. Accounting reflects only the history of the company **[8]**. The Profit and Loss Statement reflects what happened during the year, and the balance sheet reflects the assets and liabilities of the company at a certain time, which is also historical data. Consequently, it is impossible to identify and measure value creation solely based on the accounting statements. However, it is easy to verify with a quantitative point of view when it is necessary to analyze the relationship between the creation of a shareholder's value or the value added of the shareholder and EVA, economic gain, and value added **[9]**.

We can use the EVA method in several situations, such as:

- setting business goals,
- measuring the performance of separate units,
- communicating with investors and shareholders,
- motivating managers,
- evaluating the business,
- capital budgets,
- or the analysis of capital itself **[10]**.

Stern Stewart & Co. has declared that the EVA method is a tool that correctly takes into account the creation or, on the contrary, the destruction of the company value. It is proven that increasing the value of EVA is key to increasing company value creation. Therefore, EVA is an indicator directly linked to generating wealth for shareholders. Coca-Cola's CEO also said that the EVA method is a way of controlling the company **[9]**.

In the business world, therefore, in the last few years, great attention has been paid to the Economic Value Added – the EVA method. In 1991, the consulting company Stern, Stewart & Co developed and published its concept of Economic Value Added as an operating profit that is reduced by the cost of the capital used to produce this given profit. Therefore, we can say that the main stimuli and motives came from the USA at the beginning of the 1990s.

EVA is a concept that is expressed simply and defined as a method of assessing real profitability. EVA is a strict financial model. Therefore, it intends to analyze and propose how to increase the value of shareholders, for which a financial culture of value is needed, which conceptually assists all those involved in decision-making in the company, aligning strategies and goals that implicitly create value **[11]**.

The actual calculation of the EVA indicator depends on the availability of information and data, the method of determining the cost of capital. An important question is also whether our goal is to establish relative or, on the contrary, absolute value. The basic formula for calculating this indicator, "economic value added," consists of three values:

$$EVA = NOPAT - C \cdot WACC \tag{1}$$

Where:

EVA – is economic value added, $NOPAT$ (Net Operating Profit After Tax) represents net profit after tax, C is the total capital invested (Capital), which is tied in assets for operational activities of the company, the capital is expressed by the sum of equity capital (equity) with interest-bearing foreign resources, $WACC$ represents the weighted average cost of invested capital (Weighted Average Cost of Capital) **[12]**.

There is also a second method for expressing the EVA value, which is based on the accounting data; however, the results of this calculation may be less accurate than those of the previous calculation **[13]**. The advantage is that we can link the EVA indicator to the internal accounting. It is about economic profitability, and Value Spread does this EVA expression. This method is calculated as follows **[6]**:

$$E = (ROE - WACC) \times C \text{ or } EVA = ((NOPAT/C) - WACC) \times C \quad (2)$$

Market value added (MVA) is another value closely related to the economic value added. We use this indicator to measure business performance and the effectiveness of the managerial work **[5]**. Only those companies whose shares are traded on the stock exchange may use the MVA indicator. It reflects the wealth of the owners or shareholders. EVA indicator is directly connected with the MVA. MVA represents the present value of the amount that investors expect. Therefore, if we subtract the invested capital from the market value of the share, we get the result of the MVA indicator. According to the definition, MVA is the present value of the expected future value of the EVA. Market value added is calculated as follows **[14]**:

$$MVA = \text{market value of the share–invested capital} \quad (3) \text{ or}$$
$$MVA = \text{value of a company – total invested capital} \quad (4)$$

EVA equity

The Value Spread method is associated with the EVA equity variant and compares the cost-effectiveness (re) and return of equity (ROE). The difference between these two variables (ROE-re) makes the Value Spread or value range. The EVA equity is calculated by multiplying the Value Spred (the difference between the return on equity and cost-effectiveness of equity capital) and equity. One of the biggest advantages is that the EVA equity indicator can be estimated from publicly available accounting data. On the other hand, the EVA estimate may be distorted because we consider an accounting model that is not adapted to the economic model. The indicator is calculated as follows:

$$(ROE - \text{re}) \times VI = EVA\ Equity \quad (5)$$

EVA entity

The Capital Charge method is associated with the EVA entity variant, and to calculate the EVA indicator using this method, it is necessary to quantify the amount of NOPAT (net operating profit), NOA (net operational assets), and the amount of the average cost of capital **[15]**. In the conditions of the Slovak Republic, however, this is where the problem, or more precisely, mismatch, between the accounting information available and the accounting information required, occurs. The EVA entity variant is calculated as the difference between the net operating profit (before the interest payment) and the cost of capital by which the profit was achieved. The EVA entity indicator is calculated as follows **[11]**:

$$NOPAT - WACC \times NOA = EVA\ Entity \quad (6)$$

On the territory of the Slovak Republic, and under the terms of the Slovak legal order, we consider the Value Spread method to be the most appropriate, even though the Capital Charge method is more accurate. Its disadvantage is the necessity of several modifications that are too complex even for experts **[16]**, **[17]**. Relating Value Spread method, we beg to leave to state that it does not require modifications to the accounting data, which is time-consuming, and, at the same time, the method of basic accounting is preserved and respected **[18]**. For the reason mentioned above, the Value Spread method is considered more suitable and often used in practice than Capital Charge in SR conditions.

RESULTS AND DISCUSSION

Stern Stewart Corporation developed EVA method as a synthetic measure of financial performance. According to Stern Stewart Corporation (2002), EVA is a financial performance metric most directly linked to creating shareholder value over time **[19]**.

As the benchmark for measuring business performance, the EVA indicator is the subject of many scientific studies **[20]**. Business success depends on the quality of methods and techniques used for performance measurement, as well as on the ability of managers to manage the internal state and results of a company **[21]**.

Although increasingly complex methods have been developed, they failed to fully integrate (scientifically and practically) the 'multidimensional' feature of performance **[22]**. The concept of EVA is not new. However, due to EVA's heavy reliance on Capital Invested, it is best used for asset-rich companies rather than companies dominated by intangible assets such as technology businesses **[23]**.

Despite EVA's straightforward formula and advantages over other earnings measures in terms of performance management and superior relationship with market value, in practice, it is riddled with the same challenges associated with corrections and adjustments that users believe are needed to mitigate distortions by accounting

standards **[24].** Economic Value Added improves firms' efficiency and value production. EVA uses accounting statement data to calculate the value growth of a company **[25]**. EVA combines the familiar concept of residual income and the principle of modern corporate finance **[26]**. The EVA is independent of capital or equity level, and the relative company's performance is measured **[27]**. EVA is a key method and has a significant impact on business performance. The use of EVA necessitates various approaches depending on the company´s specifics **[28]**. Most scholars and practitioners do not question that businesses exist to create profit for their owners; they argue that economic value added (EVA) is the best metric available. EVA measures residual income, which means it measures the difference between a firm's return and cost on capital **[29]**. The ideological basis of this indicator can be found in microeconomics, which states that the purpose of business is to maximise profits **[30]**. However, it is not accounting but economic profit created only when its range exceeds the so-called normal profit derived from the average cost of capital incurred by creditors (interest cost) and the owners. In these shareholders, it is the opportunity cost **[31]**.

Economic Value Added (EVA) is an internal management performance measure that compares net operating profit to total cost of capital **[32]**. Economic value added (EVA) is also called economic profit **[33]**. Economic value added (EVA) is a kind of residual income that takes into account the cost of capital in the course of operations **[34]**. According to the theory of EVA, the value of economic added value created by a corporation is the net operating profit after tax minus the total cost of capital **[35]**. The EVA model informs us that profitability, size, growth ability, and intangible business activities are substantially and positively linked. In contrast, the opposite is true concerning the capital structures of a business **[36]**. Prosperous businesses tend to employ the EVA methodology less than those in a defensive situation. Under such circumstances and with the help of commercial processes, it adds value, which is generally one of the main motivational factors for conducting business **[37]**. A business has value-added where EVA is positive, whereas if it is negative, it does not. The reason for this may lie in expected high investment costs in the future. There is a relationship with a leverage effect; if it does not function positively, it negatively influences business performance **[38]**. Through the Economic-Value-Added (EVA) valuation model, the expected market value of equity can be determined by adding the book value of equity with the present value of expected EVAs under the assumption of constant required return and constant return on equity **[39]**. EVA is recognized as an important tool for performance measurement and management worldwide, particularly as a component of corporate strategy in advanced economies **[40]**. Concepts such as EVA or ROIC permanently become an element of measuring performance **[41]**. Economic added value or economic value added (EVA) is a financial method to estimate the economic profit of a business. It is the value created by shareholders' required return and is closely linked to the return on capital employed **[42]**. The EVA indicator is also a significant part of bankruptcy models or indicators **[43]**.

One of the company's main activities is buying and selling dairy products and providing accompanying activities. Initially, the company traded solely on foreign markets, initially in Hungary, and later expanded to the Czech Republic. It expanded to Slovakia in 2006.

Calculation of EBIT

In this section, based on financial statements and internal data from the company's management, we will describe the procedure for calculating all the components needed to calculate the EVA. As mentioned above, this analysis will cover the last 5 years, i.e., from 2018 to 2022.

The economic result from ordinary activities before interests and taxes is EBIT. We have calculated it as the sum of the cost of the interest and profit or loss for the accounting period, as you can see in the following Table 1.

Table 1 shows that EBIT is negative only in the year 2021. In other years, EBIT is positive, i.e., the company makes a profit. At the beginning of the analyzed period (year 2018), EBIT had a high value, which decreased by 75% later. Therefore, this indicator's evolution is variable and changes every year.

Table 1 Calculation of the EBIT.

	YEAR				
	2018 (€)	**2019 (€)**	**2020 (€)**	**2021 (€)**	**2022 (€)**
The economic result before taxes	292706	71407	304866	- 336096	276230
Interest expense	550	0	0	219	934
EBIT	293256	71407	304866	- 335877	277164

Note: EBIT – Earnings Before Interest and Taxes. Source: Author´s own elaboration.

Calculation of the NOA component

The balance sheet is the starting point for calculating the capital invested. We have determined the amount of capital invested based on the assets reported in the balance sheet. We had to activate individual items that were not in the balance sheet, allocate assets of a non-operative character, and finally set aside assets with non-interest-bearing foreign capital. When activating items, we have included items that are not assets. However, the company uses them for its core business.

Marketing

We should take into account also marketing when calculating the EVA method. As far as marketing is concerned, costs also include advertising costs.

Table 2 Marketing.

	YEAR				
	2018 (€)	**2019 (€)**	**2020 (€)**	**2021 (€)**	**2022 (€)**
Annual Marketing Expenses (costs)	399655	398511	400611	402360	406583
Straight-line depreciation of expenses from 2018	79931	79931	79931	79931	79931
Straight-line depreciation of expenses from 2019	-	79702	79702	79702	79702
Straight-line depreciation of expenses from 2020	-	-	80122	80122	80122
Straight-line depreciation of expenses from 2021	-	-	-	80472	80472
Straight-line depreciation of expenses from 2022	-	-	-	-	81317
Total depreciation	79931	159633	239755	320227	401544
Accumulated expenses	399655	798166	1198777	1601137	2007720
Accumulated depreciation	79931	239564	479320	799547	1201091
The residual value of marketing expenses as of 31.12. (accumulated expenses - depreciation)	319724	558602	719457	801590	806629

Note: Source: Author´s own elaboration.

The EVA method aims to capitalize advertising costs, set as long-term intangible assets and depreciated. In Table 2, we can see that the advertising costs are approximately € 400,000 every year during the five analyzed years. For the calculation procedure, we performed a 5-year straight-line depreciation by dividing each year's annual marketing expenses (costs) by the 5 years.

Leasing

Leasing is another indicator that needs to be included in these calculations. Leasing does not exist in the original accounting statements. In the case of the EVA method, leasing is taken into account only if the company owns the property acquired through leasing. For this reason, we cannot use leasing at this company in the EVA method, and the values related to leasing in further calculations will be zero.

Calculation of the non-operating assets

When calculating non-operating assets, it is necessary to determine which assets are operative and are, therefore, necessary for the company's main earning activity. In addition to the the balance sheet adjustment, including marketing costs, working capital, and leasing, we had to exclude non-operating assets. In the following Table 3, the modified balance sheet on the asset side can be seen. We included the marketing costs and working capital determined by deducting excess funds and foreign capital from current assets.

Table 3 Adjustment of assets.

Assets	YEAR				
	2018 (€)	**2019 (€)**	**2020 (€)**	**2021 (€)**	**2022 (€)**
Long-term assets (accounting)	767778	700908	686386	924831	971282
Activated marketing	319724	558602	719457	801590	806629
Non-operating assets	- 6006	- 5971	- 5971	- 5971	- 5971
Working capital	- 1389728	- 851790	- 1703997	- 2164673	- 445808
Total	- 308232	401749	- 304124	- 444223	1326132

Note: Source: Author´s own elaboration.

Table 4 Adjustment of liabilities.

LIABILITIES	YEAR				
	2018 (€)	**2019 (€)**	**2020 (€)**	**2021 (€)**	**2022 (€)**
Equity (accounting)	351126	57752	294540	29838	2216408
Reserves	-	-	-	-	-
Equivalents (activated marketing costs)	659358	343 997	- 598664	- 474061	- 890276
Interest-bearing foreign capital from the balance sheet	-	-	-	-	-
Total	- 308232	401749	- 304124	- 444223	1326132

Note: Source: Author´s own elaboration.

Subsequently, we made adjustments to the balance sheet on the liabilities side. Assets and liabilities must be equal even after adjustments, as shown in Tables 3 and 4.

We acquire the non-operating assets through the following components (Table 5):

- Operationally necessary amount of funds – the liquidity should be from 0.2 to 0.6. We want the liquidity to be 0.4; then, the operational amount of the funds is calculated as the product of liquidity (0.4) and short-term liabilities from the balance sheet.
- Surplus cash – we deduct the operationally necessary amount of cash from short-term financial assets. The company has a high value.
- Other assets that are not necessary for operational activities – long-term financial assets that the company has in the same value for 4 years, and by approximately € 35 more in 2018.

After entering the values in Table 6, we enumerated the NOA.

Table 5 Calculation of non-operating assets

	YEAR				
	2018 (€)	**2019 (€)**	**2020 (€)**	**2021 (€)**	**2022 (€)**
The operationally necessary amount of funds	1920972	2007414	1914581	1568463	787920
Surplus cash (excess funds)	969170	204486	1310008	1267959	1689759
Other assets that are not necessary for operational activities (non-operating assets)	6006	5971	5971	5971	5971
Non-operating assets Total	975176	210457	1315979	1273930	1695730

Note: Source: Author´s own elaboration based on the financial statements.

Table 6 NOA enumeration.

	YEAR				
	2018 (€)	**2019 (€)**	**2020 (€)**	**2021 (€)**	**2022 (€)**
Total assets	6237109	6220015	6229049	5687784	5827228
+ leasing	0	0	0	0	0
+ activation of marketing costs (reduced by depreciation)	319724	558602	719457	801590	806629
- Non-operating assets	6006	5971	5971	5971	5971
- Non-interest-bearing foreign capital	5885969	6162230	5934488	5657928	3610820
- Surplus funds	969170	204486	1310008	1267959	1689759
- **Accruals**	3920	4181	2164	1739	1175
NOA	**- 308232**	**401749**	**- 304124**	**- 444223**	**1326132**

Note: NOA – Net Operating Assets. Source: Author´s elaboration.

Calculation of the NOPAT Component

The net operating profit after taxes deduction is NOPAT. When calculating NOPAT, it is important to follow the same procedure as it is set for the NOA. Therefore, if we included the company´s activities and assets corresponding to these activities in the NOA, we have to include their revenues and costs in the NOPAT calculation. When determining the value of the NOPAT, we decided to work with the economic result from ordinary activities, which is before taxes and is adjusted by non-deductible and deductible items (Table 7).

Table 7 Calculation of the NOPAT Component.

Tax 21%	YEAR				
	2018 (€)	2019 (€)	2020 (€)	2021 (€)	2022 (€)
the economic result from ordinary activities	292706	71407	304866	-336096	276230
marketing – deduct expenses	399655	398511	400611	402360	406583
marketing – add depreciation	-79931	-159633	-239755	-320227	-401544
NOPBT	612430	310285	465722	-253963	281269
tax	61468	14995	64022	0	58008,3
NOPAT	550962	295290	401700	-253963	223261

Note: NOPAT – Net Operating Profit After Taxes; NOPBT – Net Operating Profit Before Taxb. Source: Author´s own elaboration.

Calculation of the WACC component

The last component for calculating the economic value added is the WACC. When calculating the WACC component, it is necessary to express the costs of the foreign capital and the costs of the equity. To quantify the cost of foreign capital, the company must have a bank loan, an overdraft loan, or a lease. However, the company does not have foreign capital of any kind. The company has financial assistance that we could use in the calculation. However, this financial assistance is in the form of non-interest-bearing loans; we would not be able to identify the market value of the debt; in that case, it is impossible to express the cost of foreign capital (Table 8).

Table 8 The economic result after distribution.

	YEAR				
	2018 (€)	2019 (€)	2020 (€)	2021 (€)	2022 (€)
The economic result (after taxes)	232741	50449	236788	- 264703	216068
Allocation to the reserve fund	664	664	664	0	664
Allocation to the social fund	108.62	58.97	47.27	0	48.64
The economic result after the distribution	231968	49726	236077	-264703	215355

Note: Source: Author´s elaboration based on the financial statements.

The equity costs in Table 9 are expressed by dividing the economic result after distribution by equity.

Table 9 Enumeration of the costs of equity capital

	YEAR				
	2018 (€)	2019 (€)	2020 (€)	2021 (€)	2022 (€)
E	351126	57752	294540	29838	2216408
C	6237109	6220015	6229049	5687784	5827228
Re	66.06%	86.10%	80.15%	-889.52%	9.72%

Note: E – Own Capital; C – Total Capital; Re – Costs of Equity Capital. Source: Author´s elaboration based on the financial statements.

Table 10 Enumeration of the WACC.

	Percentage				
	2018 (%)	2019 (%)	2020 (%)	2021 (%)	2022 (%)
WACC	3.72	0.80	3.79	-4.67	3.7

Note: WACC – Weighted Average Cost of Capital. Source: Author´s elaboration.

Calculation of the EVA

In the previous sections, we have calculated all necessary EVA components. In this section, there is a concrete calculation of economic value added. In Table 11, the EVA entity is calculated. There are two ways to calculate this indicator. If we chose the calculation method through the sum of equity and foreign interest-bearing resources, we would choose a formula with a C value, which represents capital invested in the long term. However, we have decided to calculate it by the value of the NOA, which is the sum of net working capital and non-current assets. We have expressed the EVA entity using the NOPAT, NOA, and WACC values. This calculation is also the basic calculation for the EVA method. It is calculated as a deduction NOA from NOPAT multiplied by WACC. The EVA Equity is an indicator in which the ROE value must be calculated first. We determined it as the share of profit and equity. Subsequently, we subtracted the cost of equity capital from the ROE value and multiplied the result by equity, thus obtaining the EVA Equity value. Table 13 shows this calculation.

Table 11 Calculation of the EVA ENTITY indicator.

	YEAR				
	2018 (€)	**2019 (€)**	**2020 (€)**	**2021 (€)**	**2022 (€)**
NOPAT	550962	295290	401700	-253963	223261
NOA	-308232	401749	-304124	-444223	1326132
WACC	3.72%	0.80%	3.79%	-4.67%	3.70%
EVA Entity	562428	292076	413226	-274708	174194

Note: EVA – Economic Value Added; NOA – Net Operating Assets; NOPAT – Net Operating Profit After Taxes; WACC – Weighted Average Cost of Capital. Source: Author´s elaboration based on the financial statements.

Table 12 Enumeration of the ROE indicator.

	YEAR				
	2018 (€)	**2019 (€)**	**2020 (€)**	**2021 (€)**	**2022 (€)**
profit	232741	50449	236788	- 264703	216068
E	351126	57752	294540	29838	2216408
ROE	0.6628	0.8735	0.8039	-8.8713	0.0975

Note: E **–** Own Capital; ROE – Return on Equity. Source**:** Author´s elaboration based on the financial statements.

Table 13 shows the final calculation of the EVA equity indicator using return on equity.

The EVA equity had a slightly decreasing trend since 2018. The highest value was € 772 in 2018, and the lowest € 665 in 2022.

Table 13 The EVA Equity.

	YEAR				
	2018 (€)	**2019 (€)**	**2020 (€)**	**2021 (€)**	**2022 (€)**
ROE	0.6628	0.8735	0.8039	-8.8713	0.0975
E	351126	57752	294540	29838	2216408
Re	0.6606	0.861	0.8015	-8.8952	0.0972
EVA Equity	772	722	707	713	665

Note: ROE - Return on Equity; Re – Costs of Equity Capital; EVA - Economic Value Added; E – Own Capital. Source: Author´s elaboration.

Sensitivity analysis

By sensitivity analysis, we can find out how sensitive, in our case, the EVA indicator is related to changes in the individual factors. The factors represent this top indicator. In the sensitivity analysis, we change the original value or factor and determine its effect on the EVA. All factor values are changed by 10%. In Table 14, this sensitivity analysis is shown. Factors with low impact on the EVA indicator, i.e., the EVA value change is not very significant, we will consider less significant. We can also conclude that the sensitivity of the EVA indicator to changes in these less significant factors is low. We will consider factors whose same 10% change causes a significant impact on the EVA value to be significant factors.

Table 14 Sensitivity analysis.

	YEAR				
	original values of the indicator (year 2022)	Increase in value by 10%	Original EVA	New EVA	Change in the EVA
Long-term tangible assets	965311	1061842	174194	184895.92	10702
Long-term intangible assets	806629	887292	174194	182843.92	8650
Long-term financial assets	5971	6568	174194	175200	1006
Inventory	234756	258232	174194	390592.72	216399
Receivables	2122266	2334493	174194	415001.08	240807
Short-term financial assets	2477679	2725447	174194	419597.11	245403
Short-term liabilities	3610820	3971902	174194	340863.59	166670
Additional Value/ sales	1.98%	2.178%	174194	200868.02	26674
Personal costs/sales	0.09%	0.099%	174194	169481.7	-4712
Depreciation/sales	0.57%	0.627%	174194	172846.33	-1348
Other revenues-other costs/ sales	0.13%	0.143%	174194	172817.45	-1377
Sales	15162471	16678718	174194	193538.74	19345
Costs of OC	9.72%	10.692%	174194	166587.95	-7606
WACC	3.70%	4.07%	174194	166587.95	-7606
OC/C	38.04%	41.84%	174194	166587.95	-7606
NOA	1326132	1458745	174194	188637.79	14444
RONA	16.63%	18.29%	174194	193538.74	19345

Note: EVA – Economic Value Added; WACC – Weighted Average Cost of Capital; OC – Own Capital; C – Capital; NOA – Net Operating Assets; RONA – Return on Net Assets. Source: Author´s elaboration.

The sensitivity analysis aims to find out how the value of the EVA changes in 2022 due to changes in individual factors by 10%. Table 14 shows that the EVA is the most sensitive to the change in short-term financial assets when its value increases by € 245403, and to the change in receivables, where the EVA is increased by € 240807. On the contrary, the most significant factor that affects the EVA negatively is the WACC factor, i.e., the weighted average cost of capital, whose 10% change caused the EVA to decline by € 7606 in 2022.

As we can see, all factors impacted the EVA, but the least significant factor was the long-term financial asset, which in 2022 caused the increase of the EVA only by € 1006. Therefore, we can conclude that this factor is the least significant. The smallest change in the EVA related to the decline in value added is due to the write-off of revenues, which would reduce the EVA by € 1348.

Based on an analysis of modern indicators, we found out that the company created an economic value added for its owners during the four years of the analyzed period. In the following Table 15, there is an analysis of two basic modern indicators to determine whether the company was creating value for its owners or not. In 2021, the company did not create value for its owners.

Table 15 presents modern indicators for the monitored period.

	YEAR				
	2018 (€)	2019 (€)	2020 (€)	2021 (€)	2022 (€)
EVA Entity	562428	292076	413226	-204128	174194
RONA	76.85%	73.15%	31.08%	40.53%	16.63%

Note: EVA – Economic Value Added; RONA – Return on Net Assets. Source: Author´s elaboration.

The core objective of the company should be to increase its value. It is precisely to achieve this goal that an analysis of the economic added value is appropriate. The company created added value for its owners in 2022. However, it has been the lowest among the previous years. We would suggest setting the increase of the economic value added by at least € 200000 as the main goal for the year 2023. In this case, the goal is to have the EVA indicator of at least € 350000 in 2023.

By improving the other factors affecting the EVA, the company will be able to reach this goal again.

Table 16 Suggested Company Goals.

	YEAR	
	2022 (€)	**Goal by 2023 (€)**
Economic value added	174194	350000
Sales	15162471	17000000
Costs/sales	105.25%	88.24%
Advertising (marketing)	406583	206583
Non-interest-bearing foreign capital	3610820	1772819
NOA	1326131	1927306
Work productivity	134823	148305

Note: NOA - Net Operating Assets, **Source:** Author´s elaboration

Another factor contributing to the increase in value added is the indicator of net operating assets, which should be higher in the future. For example, this could be achieved in the company by reducing non-interest-bearing foreign capital by at least 17%, which represents a reduction of € 600000 after calculation. In this case, the NOA value would increase to 1927306 €. By not investing in advertising and services in a proportion just like in previous years, their profits will increase, and the amount of free money will also increase. Therefore, loans can be repaid in the form of non-interest-bearing foreign capital.

In the case of the net working capital, reducing liabilities by paying invoices faster, for example, before the due date, would positively affect the company, improving working capital. The company should strive for a decrease in high receivables, which would mean faster repayment of invoices from customers, thus returning money for products to them faster than before.

To support the growth of sales and the decrease of the company's costs, the productivity of the company's employees should also increase. The company can achieve this goal through added value and the number of employees. In 2022, this share was for €134823, and to achieve better employee productivity, we can set this target value at, for example, €148305, which represents an increase of 10%. The company will achieve this goal by improving its premises' equipment and renewing computer systems for employees, which was implemented in 2021. It is also expected to increase the motivation and qualification of employees.

CONCLUSION

In this thesis, we worked with the EVA method. The benefit was the evaluation of the company's performance. In addition to competitive pressures and other negative influences on the market, the company needs to perform this analysis. With internal data, we proceeded further in the analysis by applying them to the sub-indicator calculations necessary to enumerate the top indicator, specifically the economic value added. Through the analysis, we discovered the company's problems during the monitored period. It was positive for the company that the economic result was positive in almost all years, i.e., it was in profit. In 2019, the economic result was negative. This did not only mean a loss but also affected the EVA indicator negatively, by which we found out that the company did not create value for the owners during that year. In recent years, the company has created value. After the evaluation, we proposed the EVA concept for the next period of the company's operation on the market. We proposed how the company should continue in the future using the method of economic value added, implement and realize the results of the analysis in the company, pay attention to reducing costs, and increase the value of the company. When evaluating the EVA, we found out that there is a common problem with measuring performance based on accounting data only. If we applied only these data to the EVA, the resulting values would be distorted, and thus, it would not be possible to determine the economic value added. If the company wants to succeed, it is necessary to include in the analysis components not included in the financial statements. Therefore, it must be activated as an asset. The method we used is a true image of the company. It shows the process of creating a company's value and managing business effectively.

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Acidification effects of starfruit (*Averrhoa Bilimbi* L.) on soy milk-based cottage cheese: A physicochemical and organoleptic assessment

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ABSTRACT

Using organic acids from citrus plants such as lemon and lime as a coagulant in soft cheese has been widely practiced. However, Wuluh starfruit (*Averrhoa Bilimbi* L.) is rarely used, especially in making cottage cheese from soy milk. Wuluh starfruit, which has a distinctive taste and aroma and is not shared by other citrus fruits, has the potential to be utilized in making cottage cheese. This study aimed to determine and study the effect of using a natural coagulating agent, Wuluh starfruit juice, as a coagulant in making cottage cheese from soy milk. A completely Randomized Design with six levels of treatment and three replications was used as the research design. The treatments were variations in the addition of Wuluh starfruit juice, namely as follows: SKA0 = control, citric acid 0.4%, SKA1 = 10%, SKA2 = 20%, SKA3 = 30%, SKA4 = 40%, and SKA5 = 50%. Cottage cheese from treatment SKA3 was the most preferred by the panelists based on the organoleptic evaluation with taste value (6.16 ±0.94%), aroma value (6.16 ±0.94%), texture value (5.24 ±1.20%), colour value (5.32 ± 0.85%), and acceptability value (5.72 ±0.51). SKA3 treatment was also the most preferred on the physicochemical properties of yield (26.43 ±1.13%), moisture (62.21 ±0.20%), ash (1.70 ±0.03%), protein (16.36 ±0.25%), fat (18.28 ±0.19%), pH (3.66 ±0.02), vitamin C (224.36 ±0.01 mg/kg), antioxidant activity (69.44 ±1.60%) and salt (50.33 ±0.58 ppm).

Keywords: starfruit, cheese, storage, viability, acidification

INTRODUCTION

Cheese is made through an enzyme addition to the milk **[1]**. Cheese is made most commonly from pasteurized cow milk, but the milk of other mammals may be used. Cheese production is common to households in many developing countries, which provides a helpful service in increasing the shelf-life of valuable human foodstuff like milk **[2]**. Cheese is widely known as a nutritious food that is an excellent source of calcium, vitamin A, riboflavin, and vitamin B12 **[3]**.

Cottage cheese is a low-calorie cheese with a minimal fraction of fat **[4]**. Cottage cheese is a highly regarded dairy product. There has been increased interest in specialty cheese, including additives like herbs, spices, or vegetables. The popularity of these cheese products is increasing due to their better biological value and improved flavour. Herbs and spices are used in different forms in food and traditional medicine because of their beneficial impact on health **[5]**.

One type of milk that can be used as the main ingredient for making cheese is soy milk. Milk can be extracted from soybeans and other legumes, which offer very cheap sources of vegetable milk, and could be used as substitutes for whole milk from animal sources in the production of cheese curds **[6]**. Soy milk, sometimes called soy drink or soy beverage, is a white emulsion resembling cow milk (conventional milk) in appearance and consistency. Soybean milk provides an alternative to malnourished infants and individuals who suffer from cow

milk-associated allergies **[7]**. Soy milk, one type of milk, has gained economic prominence and worldwide popularity due to its use in the cheese industry. Fermented soy milk products, particularly yoghurt, buttermilk, and cheese, are trending in the world market because they are driven by medical needs and healthy food labels **[8]**.

Generally, cheese is divided into three types, namely hard cheese, semi-hard cheese, and soft cheese, with a moisture content of not more than 30-40% for hard cheeses, 35-45% for semi-hard cheese, and 45-75% for soft cheese **[9]**. Soft cheeses made without ripening are called fresh cheeses. Fresh cheese is cheese made from fresh milk coagulated with enzymes or acids. Cottage cheese is an example of soft cheese **[10]**. Cottage cheese is classified as cheese that is made in a short time because it does not undergo ripening and does not use renin as a coagulant so that it can be consumed immediately after production.

Coagulation of milk into cheese is not limited to using starter bacterial cultures that produce lactic acid but can also use several types of acids such as acetic acid, citric acid, and lactic acid **[10]**. Natural sources of citric acid, such as starfruit, can be an alternative as a coagulant in cheese making. Natural ingredients are increasingly preferred because they are considered safer and healthier as antioxidants that prevent cancer.

Wuluh starfruit (*Averrhoa bilimbi* L.) is a green fruit whose utilization is still limited. Wuluh starfruit has a reasonably sour taste and is usually used as a cooking spice or herbal medicine It contains a lot of citric acids, oxalic acid, tannins, saponins, glucose, sulfur, formic acid, peroxides, flavonoids, triterpenoids **[11]**. Previous studies used Wuluh starfruit juice to make cottage cheese from cow milk. However, the process still uses rennet, and the effect of adding Wuluh starfruit juice as a coagulant has yet to be studied **[12]**. Thus, we were interested in studying the effect of adding starfruit juice on the physicochemical and organoleptic properties of cottage cheese from soy milk.

Scientific Hypothesis

Using a natural coagulating agent, such as Wuluh starfruit juice, in making cottage cheese from soy milk can improve the physicochemical and organoleptic properties of cottage cheese from soy milk. We expect an increase in the physicochemical properties of cottage cheese on taste value, aroma, texture, colour, and acceptability values, and also in the organoleptic evaluation of cottage cheese in yield, moisture, ash, protein, fat, pH, vitamin C, antioxidant activity, and salt.

MATERIAL AND METHODOLOGY

Samples

The main raw material in this study was fresh soy milk obtained from Marapi Dairy Milk, Padang City, West Sumatra Province, Indonesia. Other raw materials used in the processing of cottage cheese are starfruit juice, citric acid (food grade), and salt (food grade) were purchased from the local market.

Chemicals

The chemicals used in the analysis consisted of aqua DM (Bratachem), selenium mix (Merck), boric acid (Pudak Scientific), sodium hydroxide (Merck), Tashiro indicator (Merck), hydrochloric acid (Merck), hexane (Bratachem), sulfuric acid (Smart Lab), DPPH (Sigma-Aldrich) and Methanol (Merck).

Instruments

The tools used in this research are a mixer, oven, digital scale, filter cloth, plastic utensils, knife, pH meter, and glassware. The instruments used for parameter testing are a UV-Vis spectrophotometer (Thermo scientific Genesys 150), Kjeldahl testing device, soxhlet extraction device, laboratory oven (Memmert UN 110), furnace (Carbolite AAF 1100), pH meter (Hanna Instrument HI 2211) and saltmeter (Lutron YK-31SA) and other tools.

Laboratory Methods

First, in coagulant solution preparation (Wuluh starfruit juice), Wuluh starfruit weighed as much as 1 kg and was washed with running water and mashed using a blender. Separation of pulp and juice was done using a sieve. According to the treatment variation, Wuluh starfruit juice can be added directly to milk. The 0.4% citric acid coagulant solution was prepared by dissolving synthetic citric acid in aqua DM (w/v) and stirring thoroughly.

Second, fresh soy milk was pasteurized at 72 °C for 20-30 seconds while stirring in the cottage cheese-making process. The pasteurized milk was then cooled to a temperature of 35 °C. Wuluh starfruit juice with five treatments was added gradually so the pH level would not drop too low. As a control, 0.4% citric acid solution was used as a coagulant to replace starfruit juice. The curd formation occurs for ±30 minutes after adding a coagulant **[13]**.

The curd is then filtered with a filter cloth consisting of 4 layers for 1 hour until the whey is no longer dripping. The curd that had been separated from the whey was then pressed for 15 minutes to remove the remaining water content. Cottage cheese is placed in packs and stored in a refrigerator at 7 °C. Then, pH measurements were carried out using a pH meter electrode to determine the acidity of starfruit at the five stages of adding wuluh juice to the cottage cheese.

Description of the Experiment

Sample preparation: In this study, cottage cheese from soy milk was produced by acidification method using juice extracted from Wuluh starfuit.

Figure 1 Wuluh starfruit (*Averrhoa Bilimbi* L.).

The treatments used were variations in the addition or concentration of Wuluh starfruit juice, namely as follows:

SKA0 : Control, citric acid 0.4%
SKA1 : Wuluh starfruit juice 10%
SKA2 : Wuluh starfruit juice 20%
SKA3 : Wuluh starfruit juice 30%
SKA4 : Wuluh starfruit juice 40%
SKA5 : Wuluh starfruit juice 50%

Pure citric acid was used as a comparison or control of citric acid, which is the dominant organic acid compound contained in Wuluh starfruit, which is 92.6-133.8 meq/100g of total solids, far exceeding the content of oxalic acid, acetic acid, and other organic acids **[14]**. The addition of starfruit juice and pure citric acid was based on the amount of milk used. The product of the six treatments can be seen in Figure 2.

Number of samples analyzed: We analyzed 6 samples.

Number of repeated analyses: All measurements of instrument readings were performed six 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 physicochemical properties analysis, the proximate analysis included moisture content using the gravimetry method with a laboratory oven, ash content using the dry ashing method with the furnace, fat content using the soxhlet extraction method, and protein content using the micro-Kjeldahl method **[15]**.

The cheese pH was measured using an electrode pH meter. Vitamin C levels were measured using UV-VIS spectrophotometry by adjusting the wavelength range from 265 nm to 271 nm. The radical 1.1-diphenyl-2-picryl hydrazyl (DPPH) was used to determine the antioxidant activity of the cheese **[16]**. Samples from all treatments were put in equal volumes into DPPH, which had been dissolved in methanol (100 µM). After 15 min at room temperature, absorbance was measured at 517 nm by UV-Vis spectrophotometer. The cheese salt content was measured using a saltmeter/salinometer.

Then, in the organoleptic evaluation it is a method to determine panellists' response to cottage cheese products. The organoleptic evaluation was carried out with four parameters: colour, aroma, texture, and taste, because the level of consumer preference for a product is influenced by taste, aroma, texture, and colour **[16]**. The evaluation was identified using a 7-point hedonic scale: 1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, and 7 = like extremely.

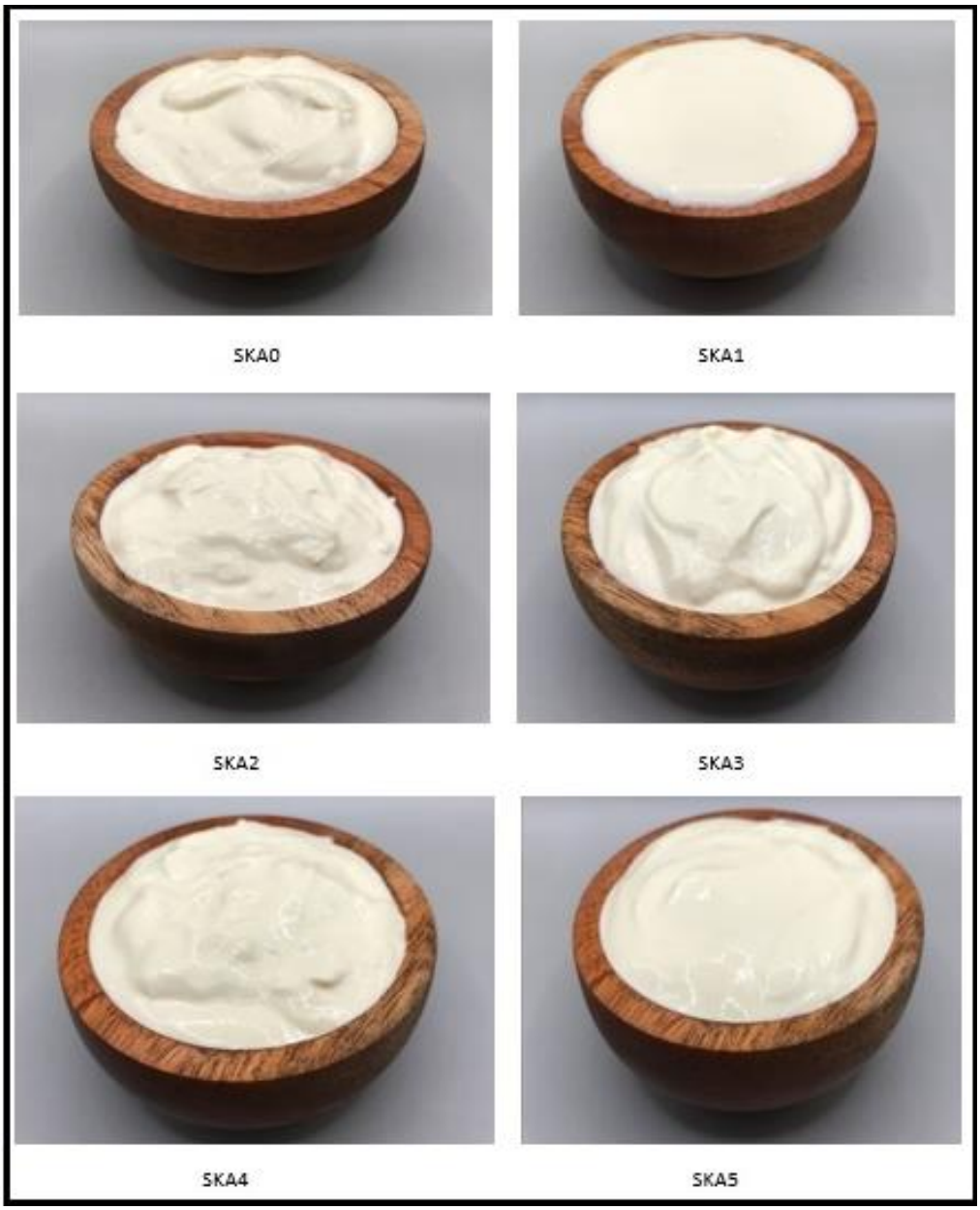

Figure 2 Cottage cheese from soy milk variations within the addition of Wuluh starfruit juice.

Statistical Analysis

Microsoft Excel and Statistica 15 produced the statistical data analysis. All experiments were carried out in triplicate, and the results reported are the results of those replicate determinations with standard deviations. The research design used was a one-level, Completely Randomized Design (CRD) with 6 levels of treatment and 3 replications. The data were analyzed using analysis of variance (ANOVA) with the F test and Duncan's New Multiple Range Test (DNMRT) advanced test at a 5% significance level. The ANOVA test is used because it can test differences in means of more than two groups and treatments, as this research wants to test whether there are significant differences between the treatments. Meanwhile, the Duncan test is used because it is more thorough and can be used to compare the effect of significantly different treatments with a large number of treatments.

RESULTS AND DISCUSSION

Physiochemical Properties

Regarding physicochemical properties, cottage cheese from soy milk was produced by acidification method using starfruit juice. The treatments used were variations in the addition or concentration of Wuluh starfruit juice: SKA0 (Control, citric acid 0.4%), SKA1 (Wuluh starfruit juice 10%), SKA2 (Wuluh starfruit juice 20%), SKA3 (Wuluh starfruit juice 30%), SKA4 (Wuluh starfruit juice 40%), and SKA5 (Wuluh starfruit juice 50%). The physicochemical properties of cheese produced from the six treatments are shown in Table 1.

Table 1 Physicochemical properties of cottage cheese from soy milk with variations in the addition of Wuluh starfruit juice.

Parameters	Treatments					
	SKA0	SKA1	SKA2	SKA3	SKA4	SKA5
Yield (%)	22.33 ±0.35	7.82 ±0.09	21.82 ±0.41	31.01 ±0.05	19.12 ±0.09	21.68 ±0.63
Moisture (%)	59.38^{a} ±0.43	61.77^{b} ±0.3	64.57^{c} ±0.34	65.24^{c} ±0.14	69.86^{d} ±0.06	75.41^{e} ±0.27
Ash (%)	2.21^{a} ±0.05	2.50^{ab} ±0.27	2.86^{bc} ±0.04	3.10^{cd} ±0.05	3.25^{cd} ±0.07	3.70^{d} ±0.22
Protein (%)	17.60^{f} ±0.13	16.35^{e} ±0.18	14.34^{d} ±0.21	12.51^{c} ±0.18	11.31^{b} ±0.22	10.22^{a} ±0.26
Fat (%)	17.67^{f} ±0.01	16.50^{e} ±0.39	14.73^{d} ±0.16	11.18^{c} ±0.12	8.38^{b} ±0.37	6.32^{a} ±0.30
pH	4.89^{d} ±0.01	5.62^{e} ±0.02	4.99^{d} ±0.02	4.79^{c} ±0.01	4.28^{b} ±0.00	3.11^{a} ±0.01
Vitamin C (mg/kg)	150.46^{a} ±0.00	153.12^{b} ±0.78	190.65^{c} ±0.25	243.50^{d} ±0.21	247.14^{e} ±0.11	268.57^{f} ±0.21
Antioxidant Activity (%)	4.05^{a} ±0.90	21.17^{b} ±0.78	27.33^{c} ±1.13	31.53^{d} ±0.78	44.44^{e} ±1.14	45.65^{e} ±0.69
Salt (ppm)	57.00^{d} ±0.00	39.00^{e} ±1.00	46.00^{c} ±1.15	50.33^{a} ±1.73	58.33^{d} ±1.15	68.00^{b} ±0.00

Note: a, b, c, d, e, f means within a row with different superscript letters are significantly different between treatments.

Yield is the ratio of the dry weight of the extract to the number of raw materials **[17]**. The cheese yield was obtained by weighing the weight of the resulting cheese and dividing it by the weight of the fresh milk used as a percentage **[18]**. It can be concluded that the more the addition of Wuluh starfruit juice, the higher the yield of cheese produced from soy milk.

The curd results from precipitation or coagulation of casein contained in milk. Protein coagulation and the decrease in pH are maximized, which is directly proportional to the increased concentration of added starfruit juice. Wuluh starfruit includes an organic acid (citric acid) with a low pH to precipitate casein in soy milk. Coagulation under optimum acid conditions will increase enzyme performance in forming a compact and sturdy curd **[19]**. Under these optimum acidic conditions, while the curd is filtered and chopped, less fat and casein are lost with the whey, so more fat can be retained for higher cheese yields **[20]**.

The cheese yield obtained from the control treatment, SKA0 (citric acid 0.4%), was 21.10%. This value is the average yield of cheese obtained by the acidification method for a pure citric acid coagulant agent. This value is under the treatment of SKA3, SKA4, and SKA5. The increase in the concentration of starfruit juice in each treatment was directly proportional to the yield of curd produced. The most curd obtained was with the addition of 50% starfruit juice (SKA5) because the acidity level was closer to the isoelectric point of milk casein **[21]**. The increase in cheese yield is also influenced by the moisture content bound to the casein network in the resulting product.

The moisture content is an important quality parameter that determines the water-holding capacity of the casein tissue to maintain a good cheese texture **[22]**. The moisture content of cottage cheese from soy milk is the addition of the Wuluh starfruit juice in the making of cottage cheese from soy milk, which influenced the increase of moisture content in each treatment. Each treatment increased with differences in adding Wuluh starfruit juice to soy milk. Wuluh starfruit is a fruit that contains a lot of moisture. Wuluh starfruit has a moisture content of 94.78% **[23]**. The high moisture content is influenced by the number of single water molecules or groups of water bound to the pectin surface through hydrogen bonds between -OH groups on pectin molecules and H atoms of water molecules.

Ash is a mineral element or inorganic substance that does not burn during combustion. An increase in water content accompanied the decrease in ash content. The higher the water content, the lower the ash content, and vice versa **[24]**. The more addition of Wuluh starfruit juice in soy milk, the lower the ash content produced in the cottage cheese. The more addition of Wuluh starfruit juice, the lower the ash content. The ash content value in the control (SKA0) was almost as high as the addition of starfruit juice in the SKA3 treatment.

Protein is a nutrient that is very important for the body because this substance functions as a building block and regulator. The more Wuluh starfruit juice was added to soy milk, the more the value of protein content in cottage cheese decreased **[25]**, **[26]**. The value of protein content decreased compared to the control (SKA0). The weak hydrogen bonds are broken when a protein is subjected to external stress, such as being heated or exposed to an acid (e.g., citric acid). This condition causes the protein to change. Proteins that are defective due to denaturation have a looser structure, are more random, and are mainly insoluble. The protein contained in cheese is easily digestible. This is because the protein breakdown process in cheese occurs appropriately **[27]**.

Fat content in cottage cheese decreased with the addition of Wuluh starfruit juice in soy milk. The value of fat content decreased in all treatments compared to the control (SKA0). An increase in the value of the moisture content accompanies the decrease in fat content in cottage cheese. This follows the statement that the factors that play a role in accelerating fat breakdown are air, light, temperature, and moisture content. The higher the moisture content of the cheese, the lower the fat content in the cheese.

The decreased quantity of fat was presumed to be due to heating, causing fat oxidation. This results in reduced fat content in cottage cheese **[28]**, **[29]**, **[30]**. The use of acid also affects the low fat because the acid can cause hydrolysis of fat. This can reduce the fat content in cheese. The proximate composition of cottage cheese from soy milk with six treatments can be observed in Figure 3.

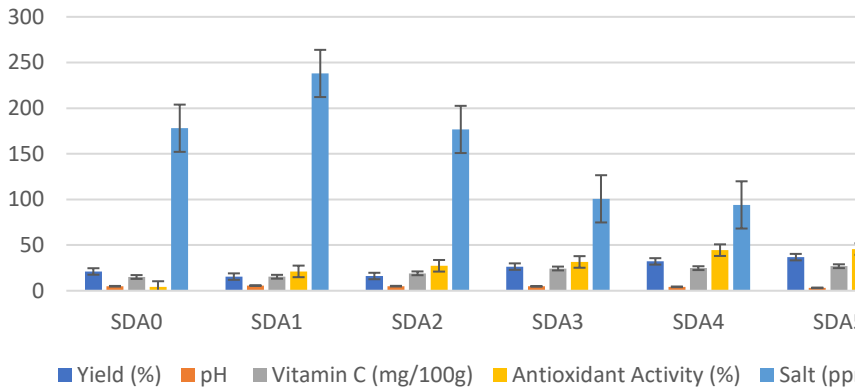

Figure 3 The proximate composition of cottage cheese from soy milk with six treatments of Wuluh starfruit juice addition.

Figure 3 shows the proximate composition of cottage cheese from soy milk from six treatments of Wuluh starfruit juice addition. pH is the highest in SKA1 (with 10% Wuluh starfruits), which was 5.62 and the lowest in SKA5 (with the addition of 50% Wuluh starfruits), which was 3.11. Vitamin C is the highest in SKA5 (with the addition of 50% Wuluh starfruits), which was 268.57 and the lowest in SKA0 (with the addition of 0.4% Wuluh starfruits), which was 150.46. Antioxidant Activity is the highest in SKA5 (with the addition of 50% Wuluh starfruits), which was 45.65, and the lowest in SKA0 (with the addition of 0.4% Wuluh starfruits) which was 4.05. Salt is the highest in SKA5 (with the addition of 50% Wuluh starfruits), 68.00, and the lowest in SKA1 (with 10% Wuluh starfruits), 39.00.

Organoleptic evaluation

Taste is the most important parameter in consumer acceptance of a product. Taste differs from the aroma and involves the five senses of the tongue. Tal factors can influence taste, such as chemical compounds, temperature, concentration, and interaction with other flavor components **[31]**, **[32]**. The taste of a product is a combination of aroma and taste, if the senses of taste detect food, panellists can distinguish one different type of food **[33]**. The resulting cottage cheese product had a characteristic sour taste of organic acids (citric acid). The increase in the concentration of Wuluh starfruit juice caused the cheese to become sourer. Adding salt can reduce the taste of too sour and create a savoury taste typical of cheese. The savoury taste was obtained by adding table salt to each treatment **[34]**.

The data showed that the higher the addition of Wuluh starfruit juice, the panellist acceptance rate. This is because Wuluh starfruit has a sour taste that affects the taste of the cheese. However, from the panellists' acceptance data, it can be concluded that the addition of Wuluh starfruit has been accepted by the panellists on a scale of 5.24 to 4.32, which means that the panellists already like the taste of the cheese.

The panellists' highest evaluation of the taste was found in the SKA3 treatment (with the addition of 30% Wuluh starfruits), which was 6.56 (moderately) with a balanced combination of sour and salty flavours. The panellists' lowest evaluation was the SKA5 treatment (adding 50% Wuluh starfruits), which was 4.32 (neither like nor dislike) with a taste that was too sour. For comparison, cottage cheese produced with 0.4% citric acid coagulant (SKA0) has a value of 5.24 (slightly).

The sense of smell influences aroma. In general, the nose can receive four types of odours can be received by the nose: fragrant, sour, rancid, and charred **[35]**, **[36]**. Aroma is also caused by chemical stimulation, which is responded to by the olfactory nerves in the nasal cavity. The aroma test was carried out by giving an assessment using the sense of smell; then the panellists gave a value to the aroma aspect of the questionnaire.

The data showed that the higher the addition of Wuluh starfruit juice, the panellist acceptance rate. This is because Wuluh starfruit has a sour taste that affects the taste of the cheese. However, from the panellists' acceptance data, it can be concluded that the addition of Wuluh starfruit has been accepted by the panellists on a scale of 5.24 to 4.32, which means that the panellists already like the taste of the cheese.

The panellists' highest evaluation of the taste was found in the SKA3 treatment (with the addition of 30% Wuluh starfruits), which was 6.56 (like moderately) with a balanced combination of sour and salty flavours. The panellists' lowest evaluation was the SKA5 treatment (adding 50% Wuluh starfruits), which was 4.32 (neither like nor dislike) with a taste that was too sour. For comparison, cottage cheese produced with 0.4% citric acid coagulant (SKA0) has a value of 5.24 (slightly).

The sense of smell influences aroma. In general, the nose can receive four types of odours: fragrant, sour, rancid, and charred. Aroma is also caused by chemical stimulation, which is responded to by the olfactory nerves in the nasal cavity **[37]**. The aroma test was carried out by giving an assessment using the sense of smell. Then the panellists gave a value to the aroma aspect of the questionnaire.

The appearance of food is largely determined by moisture and fat content. The texture changes can be caused by loss of moisture or fat content, breaking of emulsions, and hydrolysis of proteins. The texture of the cheese was strongly influenced by the fat in soy milk, which was the main ingredient of this cottage cheese **[38]**. The high-fat content of milk will cause the cheese texture to become soft. So, the fat coagulated by organic acids in Wuluh starfruit juice determines the softness of the cheese texture. The higher the addition of Wuluh starfruit juice, the cottage cheese product produced is softer, and the impact on the higher level of panellist acceptance **[39]**, **[40]**.

The highest value of texture was found in the SKA5 treatment (addition of 50% Wuluh starfruits) with a value of 6.44 (like moderately). In comparison, the lowest texture value was found in the SKA1 treatment (addition of 10% starfruit Wuluh), which was 4.64 (like slightly). This means that the panellists' acceptance rate is normal to very like. As a comparison, the SKA0 treatment (addition of 0.4% citric acid) had a value of 5.08 (slightly).

Colour is an evaluation that uses the sense of sight. Colour factor determines whether food is delicious or nutritious because it is considered and affects the evaluation **[41]**. Variations in the concentration of Wuluh starfruit juice affected the colour of the cottage cheese. The colour of the cheese produced was dominated by the colour of the main ingredient, soy milk. In addition, adding Wuluh starfruit juice as a coagulant also affected the cheese **[42]**. The chlorophyll pigment in the fruit causes the resulting cheese product to be greenish-white (55.56).

The highest evaluation of the colour was found in the SKA1 treatment (addition of 30% Wuluh starfruit juice), which is 6.04 (like moderately) because the cheese had an almost pure white colour. The lowest value was found in the SKA5 treatment (addition of 50% Wuluh starfruit juice), which was 3.76 (neither like nor dislike) because the cottage cheese had a colour that was dominated by green. As a comparison, the SKA0 treatment (addition of 0.4% citric acid) had a higher value than the SKA1 treatment due to the absence of the addition of Wuluh starfruit, so there was no greenish colour in the resulting cottage cheese.

Overall organoleptic evaluation (colour, aroma, texture, and colour) of soy milk cheese was conducted by 25 semi-trained panellists. The highest value was found in the SKA3 treatment (addition of star fruit Wuluh 30%) with a scale of 5.66 (like moderately). Organoleptic properties of cottage cheese from soy milk with variations in the addition of Wuluh starfruit juice can be observed in Table 2.

In the Table 2, organoleptic evaluation is used to determine the response of panelists to cottage cheese products on the level of consumer preference for a product. In the taste preference, the higher the addition of Wuluh starfruit juice, the panelist acceptance rate decreased. The panelists' highest evaluation of the taste was found in the SKA3 treatment (6.56), while the panelists' lowest evaluation was the SKA5 treatment (4.32). In the aroma preference, the higher the addition of Wuluh starfruit juice, the panelist acceptance rate decreased. The panelists' highest evaluation of the taste was found in the SKA3 treatment (6.56), while the panelists' lowest evaluation was the SKA5 treatment (4.32).

Table 2 Organoleptic properties of cottage cheese from soy milk with variations in the addition of Wuluh starfruit juice.

Parameters	Parameters					
	SKA0	SKA1	SKA2	SKA3	SKA4	SKA5
Taste	4.68±0.75	4.84±1.28	5.44±0.92	6.16±0.94	6.76±0.44	4.48±0.77
Aroma	4.92±1.29	5.12±1.39	5.76±1.13	6.16±0.94	6.84±0.37	4.48±0.96
Texture	5.08±1.19	4.48±1.19	5.00±0.76	5.24±1.20	5.76±1.16	6.68±0.48
Color	4.24±1.23	4.36±1.44	5.28±0.94	5.32±0.85	6.68±0.48	3.84±0.80
Acceptability	4.73±0.37	4.70±0.35	5.37±0.32	5.72±0.51	6.51±0.50	4.96±1.22

Note: The evaluation was identified using a 7-point hedonic scale (1 = dislike extremely, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, and 7 = like extremely).

In the texture preference, the higher the addition of Wuluh starfruit juice, the cottage cheese product produced is softer, and the impact on the higher level of panelist acceptance. The highest value of texture was found in the SKA5 treatment (6.44) while the lowest texture value was found in the SKA1 treatment (4.64). In the color preference, variations in the concentration of Wuluh starfruit juice affected the color of the cottage cheese. The highest evaluation of the color was found in the SKA1 treatment (6.04), while the lowest value was found in the SKA5 treatment (3.76). In the total of four preferences acceptability, overall organoleptic evaluation (color, aroma, texture, and color) of soy milk cheese was carried out by 25 semi-trained panelists. The highest value was found in the SKA3 treatment (addition of star fruit Wuluh 30%) with a scale of 5.66 (like moderately).

CONCLUSION

Using Wuluh starfruit juice as a coagulant showed effects on the physicochemical properties and organoleptic evaluation of cottage cheese from soy milk. It can be concluded that the cottage cheese product from the SKA3 treatment (the addition of 30% Wuluh starfruit juice) was the most preferred by the panellists from organoleptic properties on taste value (6.16 ±0.94%), aroma value (6.16 ±0.94%), texture value (5.24 ±1.20%), color value (5.32 ± 0.85%), and acceptability value (5.72 ±0.51). The cottage cheese product from the SKA3 treatment was also the most preferred by the panellists from physicochemical properties on yield (26.43 ±1.13%), moisture (62.21 ±0.20%), ash (1.70 ±0.03%), protein (16.36 ±0.25), fat (18.28 ±0.19%), pH (3.66 ±0.02), vitamin C (224.36d ±0.01 mg/kg), antioxidant activity (69.44 ±1,60%) and salt (50.33 ±0.58 ppm). This product's microbiological properties, heavy metals, and shelf life can be carried out for further research.

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Quality of bull beef of the Ukrainian black and white dairy breed in dependence on the development of subcutaneous adipose tissue

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ABSTRACT

Determining the compliance of the quantitative and qualitative characteristics of the domestic cattle breed beef by the EUROP carcass standards is of great importance during Ukraine's accession to the European Union. The beef quality of a 21-month-old bull of the Ukrainian black and white dairy breed dependent on the subcutaneous adipose tissue development was evaluated at "Zhuravushka" FG in Kyiv region. From birth to 4 months of age, they were kept in groups of 25 heads. Growth and fattening were carried out at a feeding platform. For slaughter, the cattle were formed by a method of analogous groups. Following the EUROP system, the coverage of the carcasses with the subcutaneous fat was visually evaluated in five classes. The colour of the muscular and adipose tissue was determined by a scale of 1 to 7. The marbling of the muscular tissue was evaluated on a scale of 1 to 12, and the thickness of the carcass fat was measured between the 12th and 13th ribs as per the JMGA method. For chemical analysis to be conducted, 300 g of m. longissimus dorsi were taken from each cattle. The minced meat from that place was analyzed for total fat content – according to DSTU ISO1443:2005, mass, total ash - according to DSTU ISO 936-2008, moisture – according to DSTU ISO 1442-2005, pH – according to DSTU ISO 2917-2001 with the use of the laboratory ionometer (I-160M), penetration with the use of the automatic penetrometer PM DH in the laboratory of the department of meat, fish and seafood technologies of the National University of bioresources and nature management of Ukraine (NUBNMU). The beef's moisture-retaining capacity, broth tasting, and cooked meat were carried out in the "Meat Quality" laboratory of the Department of Milk and Meat Production Technologies of the NUBNMU. As the amount of subcutaneous fat increases, the marbling class of the bull beef does not increase. The development of the subcutaneous adipose tissue has no impact on the colour, pH, boiling, and transverse cut force of the beef. Due to better subcutaneous adipose tissue development, the meat has a higher moisture-retaining capacity than beef with its smaller amount. The development of the adipose tissue on the carcasses of the 21-month-old bull beef of the Ukrainian black and white dairy breed by the EUROP standard does not permit the prediction of the qualitative characteristics of the beef.

Keywords: meat productivity, bulls, fat (subcutaneous fat), intramuscular adipose tissue, Ukrainian black and white dairy breed

INTRODUCTION

There are certain differences between the evaluation criteria for beef in different countries. The European Union classifies beef carcasses by the EUROP system **[1]**. Following this system, when evaluating the carcasses, the class of the fat- subcutaneous fat is one of the main features determining their value. DSTU 4673-2006 "Cattle for slaughter. Technical conditions", developed in Ukraine **[2]**, provides for the evaluation of cattle only by live weight and carcass weight and does not consider subcutaneous fat thickness on them. For consumers' decision-making about beef purchase, the most important factor is the amount of visible fat (36%), followed by the price (25%), then its color (19%) **[3]**. But when there is a lot of subcutaneous fat, the yield of the carcasses and the proportion of their edible parts decreases **[4]**. It is not used to improve the meat quality **[5]**. Therefore, to adapt the Ukrainian standards to the requirements of the European Union by the EUROP system and to determine the need for their introduction into production, an urgent issue is to determine the impact of the development of the subcutaneous adipose tissue on the quality (technological, chemical and tasting properties) of the beef of the widely-spread Ukrainian black and white dairy breed.

The adipose tissue development in cattle depends on the breed and productivity **[6]**, age, and growth rate **[7]**. The development of the subcutaneous adipose tissue impacts beef quality by protecting the muscles in a refrigerating chamber against drying out when the carcasses are cooled, which might increase their stiffness **[8]**. This is a problem for the meat obtained from cattle with less adipose tissue **[9]**. Deposition of a large amount of the subcutaneous fat increases the feed consumption by the cattle during their growth **[10]**.

The beef fat has a low nutritional value in the processing industry. For healthy eating, people are forced to partially replace fatty raw materials with dietary ones **[11]**. The biological value of the meat is improved **[12]** by a fermentation method, and its human consumption and healthful properties are improved by the addition of rosemary extract **[13]**, iodine compounds **[14]**, citrus honey **[15]**, and protein-wheat texture, which contains a balanced set of amino acids **[16]**.

The beef source in Ukraine for the meat industry is the cattle of meat, dairy, combined breeds, and breeds obtained from their crossing. A great genetic diversity of the cattle produces different quality meat **[17]**. Consumers are interested in its nutritional value, sensory characteristics, and taste **[18]**.

The problem of meat quality formation in cattle with the different development of subcutaneous fat has not been sufficiently covered. Thus, the beef obtained from Limousin bulls at the ages of 25 to 27 months with the classes of the subcutaneous fat (by the EUROP requirements) "2" and "3" does not differ in tenderness **[19]**. Since fat distribution by fat depots is also the accounting subject of waste generation, disclosing the formation features of the qualitative characteristics of cattle beef is necessary to produce its components effectively. The article is aimed at determining the relationship between the qualitative characteristics (pH, moisture-retaining capacity and penetration of the longest back muscle, sensory properties) of the beef and the development of the subcutaneous adipose tissue, which characterizes the quality of the bull carcasses of the Ukrainian black and white dairy breed.

Scientific Hypothesis

Previous studies have shown that the better development of the adipose tissue on the bull carcass negatively correlates to their growth rate and breeding value. Its impact on the formation of the qualitative characteristics of beef has not been confirmed. It is expected that the development of the subcutaneous adipose tissue of the cattle correlates to the fat content inside the muscles, which impacts certain sensory and technological properties of the beef. The deposition of various amounts of subcutaneous fat and its impact on the meat quality of the Ukrainian black and white dairy breed cattle can vary from the general trend of its formation in the cattle.

MATERIAL AND METHODOLOGY

13 Ukrainian black and white dairy breed bulls were studied at “Zhuravushka” FG in the Kyiv region. From birth to 4 months of age, they were kept in groups of 25 heads. Further, they were fed with the home-produced feed at the feeding platform by the rations adopted by the farm. The bulls were slaughtered at 21 months in the farm's slaughterhouse (Kalynivka village). The difference between the bulls in the group in terms of age and live weight was up to 5 % at the time of slaughter.

Samples

The coverage degree of the carcasses with the subcutaneous fat was classified into five classes (Figure 1): 1st (low) – the subcutaneous fat is almost absent; 2nd (slight) – a small amount of the subcutaneous fat, the muscles shows through almost the entire carcass; 3rd (average) – almost the entire carcass is covered with the fat, it is accumulated in the chest and shoulder parts; 5th (very high) – the entire carcass is covered with the fat without gaps, it is largely accumulated in the chest.

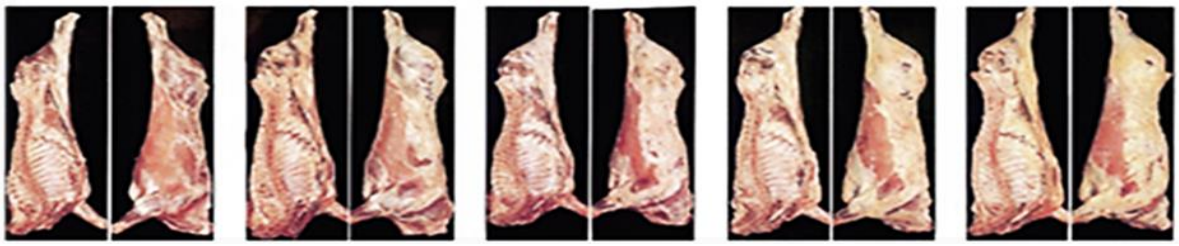

Figure 1 Evaluation scale of subcutaneous fat **[1]**.

Under the JMGA method **[20]**, the colour of the muscular and fat tissue was evaluated with the use of a colour scale of 1 to 7, and the marbling of m. longissimus dorsi - between the 12th and 13th ribs. After slaughter, the meat piece (300 g) was selected from m. longissimus dorsi for chemical analysis to be conducted.

Chemicals

Solution of hydrochloric acid, 1.5 %, ("Khimlaborreaktyv" LLC, Ukraine)
Solution of sulfuric acid, 5 % ("Khimlaborreaktyv" LLC, Ukraine).
Chloroform ("Khimlaborreaktyv" LLC, Ukraine).

Animals, Plants, and Biological Materials

21-month-old bull of the Ukrainian black and white dairy breed, which belonged to "Zhuravushka" FG, Brovary district, Kyiv region.

Instruments

Stationary weighing scales 4BDU-15X-P ("Axis", Ukraine). Weight unit >0.5 kg, weighing range from 10 to 1500 kg. Weighing of the bulls before slaughter.
Gas chromatographer (Kupol_55, "Shimadzu Corporation", Japan).
Drying cabinet (SNOL, "Khimlaborreaktiv" LLC, Ukraine).
Distiller for steam distillation (Velp Scientifical UDK 129, "Khimlaborreaktiv" LLC, Italy).
Laboratory ionometer I-160M. Determination of beef pH.
Automatic penetrometer PMDP. Determination of beef penetration.

Laboratory Methods

The total fat content in m. longissimus dorsi was determined by DSTU ISO1443:2005 **[21]**, total ash mass – by DSTU ISO 936:2008 **[22]**, and moisture content – by DSTU ISO 1442:2005 **[23]**. The following physical and technological characteristics of the beef were also studied: pH – by DSTU ISO 2917-2001 **[24]**. The boiled beef (aroma, juiciness, tenderness, easy chewing) and broth (colour, taste, strength) were tasted by the requirements given in the article **[25]**. The bulls were formed into a group before slaughter by age, which did not exceed 5%.

For the development of the carcass fat to be determined, the bulls were slaughtered in the slaughterhouse (Kalynivka village). Up to that moment, their pre-slaughter live weight was determined by weighing before and after 24 hours of fasting with free access to water. After the cattle were slaughtered and skinned, their carcasses were weighed. After that, the development of the subcutaneous adipose tissue was evaluated by the EUROP classification **[1]**.

Description of the Experiment

Sample preparation: There are rules for antemortem inspection and veterinary-sanitary inspection of meat and meat products (2002) **[26]**. An hour after slaughter, 21-month-old cattle were examined for subcutaneous adipose tissue development. During the second calendar day, after boning and veining the slaughtered cattle, 13 samples of 300 g from m. longissimus dorsi were taken from each cattle.

Number of samples analyzed: From the conducted experiment for chemical analysis, 13 meat samples from m. longissimus dorsi of each 21-month-old cattle were used by one sample. In the minced meat from m. longissimus dorsi of the slaughtered bulls, the values of the qualitative characteristics were determined once at the age of 21 months**.**

Number of repeated analyses: The study was conducted 3 times.

Number of experiment replications: The number of repeats of each experiment to determine one value was also 3 times.

Design of the experiment: At the first stage, the bulls of the Ukrainian black and white dairy breed were kept in groups of 25 heads from birth to 4 months of age. After that, they were fed with the home-produced feed at the feeding platform. In the second stage, the bulls were slaughtered in the slaughterhouse (Kalynivka village) and the coverage degree of the carcasses with the subcutaneous fat was determined after slaughter. In the third

stage, the colour of the muscular and fat tissues, the marbling of m. longissimus dorsi, the chemical composition of the beef, and the technological and tasting properties were evaluated. At the last stage, the correlation relationship between the development of the subcutaneous fat and the sensory and technological properties was determined.

Statistical Analysis

The data was statistically processed using Microsoft Excel 2016 in combination with XLSTAT. The indicators were evaluated by the correlation coefficients, which were calculated by appropriate methods **[27]**.

RESULTS AND DISCUSSION

Indicators of the commercial classification of the carcasses of 21-month-old Ukrainian black-spotted dairy cows according to the development of fat were in the range from "weak" to "medium". The majority (61.1%) of them are assigned to class 2.5. Subcutaneous fat contributes to the trend towards the highest negative correlation with beef technological properties such as muscle eye area (r = -0.495) and acidity (r = -0.252) (Table 1). Therefore, the better development of fat under the skin, reducing the muscle cell area, simultaneously also leads to a decrease in the number of valuable edible parts in the carcass **[5]**. A decrease in the pH of muscle tissue occurs as a result of glycolysis, during which lactic acid is formed in the meat, and it is microbiologically more stable.

Table 1 Correlation between the development of fat on the carcass and technological properties of beef.

Feature	r
pH	-0.252
Moisture-retaining capacity	0.093
Penetration	0.137
Marbling	0.010
Fat thickness	-0.034
Loin eye area	-0.495

With the increase in the development of subcutaneous adipose tissue, the indicators of penetration (r = 0.137), which characterizes the structural and mechanical properties of beef, water-binding capacity (r = 0.093) and marbling of the medulla oblongata (r = 0.010) do not increase significantly. It is possible to change the water-binding capacity of meat and its structural and mechanical properties by finely grinding it **[28]**. The marbling class of beef increases with a simultaneous increase in fatty tissue under the skin **[29]**, although this was not found in some works **[19]**. Marbling is an important aspect of beef quality **[30]**. It is related to consumers' perception of meat and includes its physical (collagen tenderness and maturity) and chemical (moisture content, fatty acid composition, and antioxidant capacity) characteristics **[31]**. An increased level of marbling positively affects beef's tenderness, juiciness, aroma, and overall taste evaluation **[5]** when there are no off-flavours **[32]**. Between the evaluations of tenderness, juiciness, and taste of meat and fat content in the middle of the muscles, the curvilinear relationships are leveled at its value of 15-17% **[33]**.

With a better development of fatty fiber on the carcass, there is a tendency to worsen beef cooking and the general evaluation of the taste of cooked meat and its broth (Table 2).

Table 2 Correlation between the development of fat under the skin and organoleptic characteristics of beef.

Feature	r
Colour of muscular tissue	0.245
Colour of fat tissue	-0.543
Boiling	-0.144
Tasting broth	-0.328
Tasting Boiled meat	-0.288

The sensory properties of beef depend on marble fat located in the middle of the muscles **[34]**. When meat is fried or boiled, it melts, impregnating it. As a result, it becomes juicy and tender. The marbling of beef is influenced by the individual genetic characteristics of the cattle, their breed, sex, age at the time of slaughter, housing system, feeding level, and the temperature at which the meat is cooled and packed **[35]**. Intramuscular fat content in black wagyu cattle is about 30% **[36]**, and Ukrainian beef cattle is only 0.37-0.65% **[37]**. Among European breeds, the concentration of intramuscular fat is the highest in the beef of Aberdeen-Angus heifers

[38]. Meat marbling is better in steers **[39]** and in old cattle **[40]** when they are intensively fed concentrated feed with high energy content **[41]**, only after the "excess" fat accumulates in the middle of the belly, under the skin and between muscles **[42]**. The worst quality is the beef of uncastrated Bugai **[43]**. The morphology of marbling (coarse and small fat inclusions) especially affects beef's nutritional quality **[44]**. If the meat is coarsely marbled, it has a higher content of polyunsaturated fatty acids and aromatic compounds, and if it is fine-grained, the aroma and taste are better. However, consuming beef with high marbling does not benefit human health **[45]**.

Due to the increase in the development of fat under the skin, its color worsens and, accordingly, muscle tissue improves. Higher parameters of beef color and lower final pH for improving animal condition, which is affected by the content of subcutaneous fat **[46]**, were obtained **[47]** in crossbred (½ Angus x ½ Charolais) and purebred Charolais **[48]** animals. The color of beef is important for consumers to make decisions about its purchase **[49]**, as it is one of the first to be used to indicate the freshness and usefulness of meat **[50]**. The color of muscle tissue depends on myoglobin's concentration and chemical form **[35]**. Fresh meat contains deoxymyoglobin, oxymyoglobin, and metmyoglobin. Red pigmentation is given to it by deoxymyoglobin, which, in the presence of oxygen, is oxidized to oxymyoglobin and contributes to the manifestation of a bright pink-red color. When deoxymyoglobin and oxymyoglobin are oxidized to metmyoglobin, the meat turns brown. A decrease in the pH level, which leads to a better development of fat-irrigation, contributes to the formation of metmyoglobin. Metmyoglobin reductase reduces the concentration of an unwanted form of myoglobin in meat and stabilizes its color **[35]**. The color of beef is significantly influenced not only by its biochemistry, technological processing, and packaging but also by animal feeding **[51]**. Feeding animals on pastures with alfalfa, Bermuda grass, cowpeas, and pearl millet produces a richer meat color **[52]**. The color of meat products is improved **[53]** by using native lactic acid bacteria and gram-positive catalase-positive cocci.

The correlation between the visually assessed development of subcutaneous adipose tissue on the one hand and the chemical composition of beef on the other is moderate to low (Table 3). With better development of fatty tissue on the carcass, it practically did not change (r = 0.018) in m. longissimus dorsi moisture content, a large amount of which leads to rapid spoilage of beef **[54]**, and a tendency to increase protein content (r = 0.262) was observed.

Table 3 Correlation between the development of subcutaneous fat and the chemical composition of beef.

Feature	r
Moisture content	0.018
Solids content	-0.019
Protein	0.262
Fat	-0.262
Mineral substances	-0.089

Protein plays a key role in ATP production, energy exchange, oxidative stress, and redox processes in cells **[47]**. Under better conditions, the content of proteins related to energy metabolism increases in cattle. Under worse conditions, catabolic processes (glycolysis), oxidative stress, muscle structure, and contraction affect the meat's degree of marbling and color.

Using proteomics in combination with liquid chromatography and tandem mass spectrometry, 85 proteins were identified in Limousin cattle **[55]** that are correlated with tenderness, chewiness, hardness, and taste of meat. Predicted biomarkers were classified according to the interrelated biological directions of muscle contraction, energy metabolism of heat shock, oxidative stress, regulation of cellular processes, and binding.

According to our data, with better development of subcutaneous fat, the tendency to decrease (r = -0.262) the fat in meat is manifested in Bugai people. This does not confirm the probable connection between the investigated characteristics and does not allow for predicting the marbling of beef of 21-month-old Ukrainian dairy cows depending on the development of subcutaneous fat. The decrease in the content of fatty tissue in the muscles of cattle is the main reason for the negative effect of the better development of fat under the skin on the sensory properties of their meat. Beef, characterized by a low-fat muscle content, is darker in color, harder, and drier **[56]**. However, an increase in intramuscular fat with a higher class of subcutaneous adipose tissue development was observed in other **[19]** studies. The correlation coefficient between the development of subcutaneous fat, on the one hand, and the amount in m, on the other, is inverse and insignificant. longissimus dorsi of minerals that play an important role in human nutrition and health **[54]**.

Thus, based on the development of subcutaneous adipose tissue, assessed by the EUROP system in 21-month-old Ukrainian black-spotted dairy cattle, it is impossible to accurately predict the quality composition of

beef. Increasing the fat-watering on the carcass reduces its muscle content, does not give the beef more marbling, and worsens its tasting properties and the broth from it.

According to our data, improving the development of subcutaneous fat leads to increased water-binding capacity, which also characterizes juiciness, tenderness, and other technological properties of meat products. Due to this, the beef loses water during heat treatment, and the product becomes coarser. The quality of beef depends not only on the fat content in the middle of the muscles and their water-binding capacity but also on the interrelationship of the intramuscular connective tissue (general and insoluble collagen that surrounds the muscle fiber and their bundles and muscle as a whole) by types of muscle fibers and intramuscular fat **[57]**. The tenderness of meat depends on the amount of connective tissue, the diameter of muscle fibers, and the accumulation and distribution of fat in them. Bovine meat, characterized by a higher connective tissue content, is less tender and has greater losses during cooking **[35]**. Beef tenderness and subcutaneous fat thickness may be associated with the 526 T→ A mutation in the promoter region of exon 1 of the MyF – 5 gene **[58]**. A thin layer of fat on the carcasses and their rapid cooling are the reasons for increasing the stiffness of the beef, its drying, and the darker color of the muscle tissue **[59]**.

This shows that it is possible to preserve carcasses with sufficient subcutaneous adipose tissue. For 24-month-old bulls of British breeds and their crosses, a uniform adipose tissue thickness of 6.0 mm provides an adequate yield of edible beef in carcasses with high protein content and low fat concentration. It is a standard for consumers' quality of carcasses and meat products **[60]**. Today, they are interested in such properties of beef as nutritional value, sensory characteristics, and disease prevention **[61]**. In cattle, significantly developed fat under the skin is not desirable because it does not improve the quality characteristics of beef **[62]**, the number of scraps from carcasses during their stripping increases **[46]**, and the sexual precociousness of animals increases **[63]**.

Due to the accumulation of internal, subcutaneous, and intermuscular adipose tissue, which have a low value **[64]**, feed costs for their growth also increase **[65]**. Fat-watering is considered **[66]** to be a waste of beef production. Although there is already evidence **[67]** that external fat is richer in conjugated linoleic acid than other types. This could have important implications for human health and change how processors think about using fat trimmings from carcasses. It is necessary to reduce the deposition of subcutaneous adipose tissue for breeding animals obtained from inbreeding **[68]** and homozygous **[69]** and for a worse expression of meat forms **[46]**. Thus, there are many problems regarding the evaluation of carcasses of 21-month-old bulls of the Ukrainian black and mottled dairy breed for the best development of fat irrigation by the EUROP system, including high costs of feed for growth, live weight, deterioration of many quality characteristics of beef, disposal of raw trimmings or vice versa their use. Despite recent achievements in the world regarding regulating adipose tissue formation in cattle to improve the quality of beef from animals of breeds bred in Ukraine, this problem remains insufficiently resolved and calls for further research.

CONCLUSION

The assessment of cattle carcasses in Ukraine by DSTU 4673-2006, which provides for considering only live weight and carcass weight, differs significantly from EUROP requirements and does not take into account its conformation, thickness, and development of subcutaneous fat and the color and marbling of beef. The quality of carcasses of 21-month-old Ukrainian black-spotted dairy cows, classified by the development of subcutaneous adipose tissue by EUROP requirements, is not related to the quality of beef by sensory characteristics (evaluation in points of cooked meat and broth from it), and fat content in muscle tissue, its boiling. The better development of adipose tissue on the carcass is most negatively correlated with the area of the muscle eye, which indicates a decrease in the proportion of edible parts in the carcass. The development of adipose tissue under the skin practically does not correlate with the water-binding capacity, penetration, and marbling of beef. Additional research is needed on developing beef production technology with optimal development of inedible subcutaneous adipose tissue and appropriate levels of marbling to satisfy consumer preferences for meat quality and taste while preserving their health and supporting the economy of livestock farming.

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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.

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